

## REVIEW ARTICLE

## Astroglial activation: Current concepts and future directions

Paul Edison<sup>1,2</sup> 

<sup>1</sup>Division of Neurology, Department of Brain Sciences, Faculty of Medicine, Imperial College London, London, UK

<sup>2</sup>Division of Psychological medicine and clinical neurosciences, School of Medicine, Cardiff University, Wales, UK

**Correspondence**

Paul Edison, MD, PhD, MPhil, FRCP, FRCPI, Professor of Neuroscience, Imperial College London, Division of Neurology, Department of Brain Sciences, Faculty of Medicine, Imperial College Memory Research Centre, Level 2, Commonwealth Building, Hammersmith Campus, Du Cane Road, London, W12 0NN, UK.

Email: [paul.edison@imperial.ac.uk](mailto:paul.edison@imperial.ac.uk);  
[paul.edison@nhs.net](mailto:paul.edison@nhs.net)

**Abstract**

Astrocytes are abundantly and ubiquitously expressed cell types with diverse functions throughout the central nervous system. Astrocytes show remarkable plasticity and exhibit morphological, molecular, and functional remodeling in response to injury, disease, or infection of the central nervous system, as evident in neurodegenerative diseases. Astroglial mediated inflammation plays a prominent role in the pathogenesis of neurodegenerative diseases. This review focus on the role of astrocytes as essential players in neuroinflammation and discuss their morphological and functional heterogeneity in the normal central nervous system and explore the spatial and temporal variations in astroglial phenotypes observed under different disease conditions. This review discusses the intimate relationship of astrocytes to pathological hallmarks of neurodegenerative diseases. Finally, this review considers the putative therapeutic strategies that can be deployed to modulate the astroglial functions in neurodegenerative diseases.

**KEYWORDS**

Alzheimer's disease, astrocytes, cognitive impairment, glial activation

**Highlights**

- Astroglia mediated neuroinflammation plays a key role in the pathogenesis of neurodegenerative diseases.
- Activated astrocytes exhibit diverse phenotypes in a region-specific manner in brain and interact with  $\beta$ -amyloid, tau, and  $\alpha$ -synuclein species as well as with microglia and neuronal circuits.
- Activated astrocytes are likely to influence the trajectory of disease progression of neurodegenerative diseases, as determined by the stage of disease, individual susceptibility, and state of astroglial priming.
- Modulation of astroglial activation may be a therapeutic strategy at various stages in the trajectory of neurodegenerative diseases to modify the disease course.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Authors. *Alzheimer's & Dementia* published by Wiley Periodicals LLC on behalf of Alzheimer's Association.

## 1 | INTRODUCTION

Astrocytes are ubiquitously expressed throughout the central nervous system (CNS).<sup>1</sup> Astrocytes perform pleiotropic roles, including maintaining the blood-brain barrier (BBB); providing metabolic, trophic, and antioxidant support to neurons; regulating neurotransmitter levels; regulating synaptogenesis and synaptic transmission; and astrocytes are involved in the pathogenesis of neurodegenerative diseases.<sup>1</sup> Astrocytes play fundamental and active roles in neurodegenerative diseases. Significant loss of synaptic connectivity is the most proximal neurobiological event that accounts for the clinical features of dementia. Synaptic loss is consistently correlated with clinical features of dementia, while the association between hallmark lesions such as amyloid- $\beta$  and cognitive impairment in dementia is not linear.<sup>2</sup> Since astrocytes are integral in normal neuronal function, incorporating astrocytes as key criteria for Alzheimer's disease (AD) drug development is likely to yield fruition.

Since astrocytes are of paramount importance, this review focus on the emerging evidence for morphological and functional heterogeneity of astrocytes in the healthy CNS and neurodegenerative diseases. We explore the close association of astrocytes to pathological hallmarks of neurodegenerative diseases. Based on the evidence discussed—a hypothetical model is presented to explain the dynamic changes and heterogeneity in astroglial activation—and putative therapeutic strategies are suggested to modulate astroglial dysfunction in the context of neurodegenerative diseases.

## 2 | DEVELOPMENT AND COMPLEX MORPHOLOGY OF ASTROCYTES

During the late embryonic stages, the radial glia predominantly or the neural stem cells at the subventricular zone give rise to astrocytes during the first 2 weeks of postnatal life (Figure 1).<sup>3</sup>

Based on the morphological assessments of astrocytes detected by astrocyte-specific marker glial fibrillary acidic protein (GFAP)—they can be classified into four distinct classes: (1) interlaminar astrocytes in cortical layer I; (2) protoplasmic astrocytes in cortical layers II-VI (Figure 1); (3) varicose projection astrocytes in deep cortical layers V-VI, and (4) fibrous astrocytes in the white matter (Figure 1).<sup>4</sup> The interlaminar astrocytes and the varicose projection astrocytes are exclusively observed in humans and primates.

### 2.1 | Interlaminar astrocytes

The interlaminar astrocytes are characterized by tortuous and varicosity-free interlaminar projections originating from the soma in layer I and terminating in layers III or IV.<sup>4</sup> Interlaminar astrocytes display a multi-lamellar structure and mitochondria and may be involved in intra-cortical communication and hence neuronal support.<sup>4</sup>

### RESEARCH IN CONTEXT

1. **Systematic review:** Astroglial mediated neuroinflammation plays a prominent role in the pathogenesis of neurodegenerative diseases. In this review, evidence for morphological and functional heterogeneity of astrocytes in the healthy central nervous system and neurodegenerative diseases is discussed.
2. **Interpretation:** Activated astrocytes exhibit diverse phenotypes in a brain region-specific manner and interact with proteinopathy including  $\beta$ -amyloid, tau, and  $\alpha$ -synuclein species, microglia, and neuronal circuits. Activated astrocytes influence the trajectory of disease progression in Alzheimer's disease and other neurodegenerative diseases, as determined by the disease stage, individual susceptibilities, and state of astroglial priming.
3. **Future directions:** The review article argues for the inclusion of astrocyte reactivity in the research framework of Alzheimer's disease. It appears that modulation of astroglial activation may be an attractive therapeutic strategy at various stages in the trajectory of Alzheimer's disease and other neurodegenerative diseases to modify the disease course.

### 2.2 | Protoplasmic astrocytes

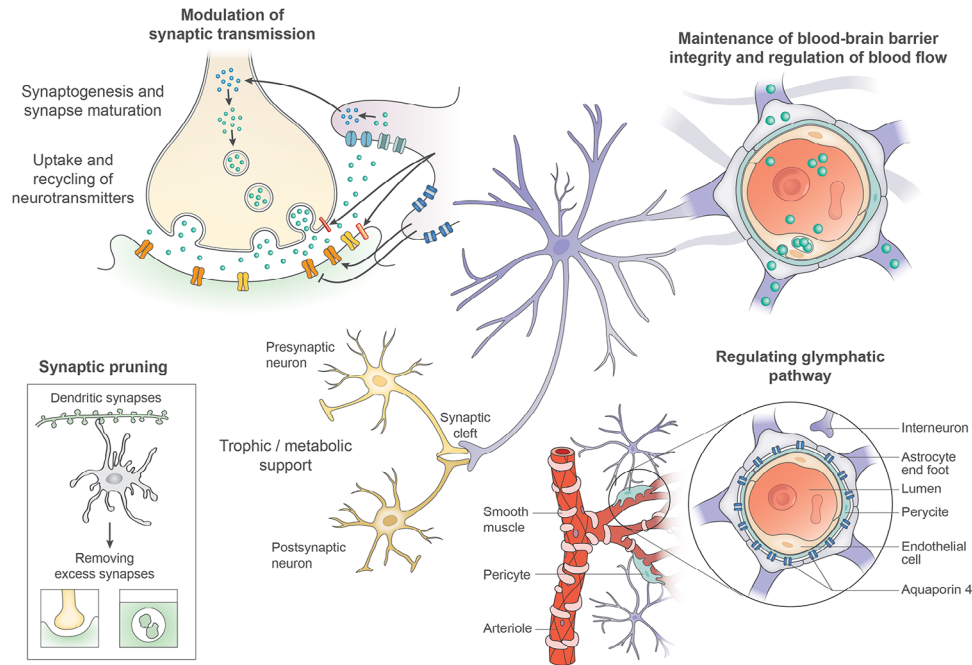
The protoplasmic astrocytes are the most abundant subtype and form most of the protoplasmic astrocytes endfeet forming neurovascular unit.<sup>4</sup> A single human protoplasmic astrocyte can provide coverage to 270,000–2,000,000 synapses,<sup>4</sup> while a rodent astrocyte can provide coverage to 20,000–120,000 synapses. This helps with exceptional computational power of the human brain and the ability of human protoplasmic astrocytes to assimilate information from multi-fold synapses to modulate efficient inter-neuronal communication.

### 2.3 | Varicose projection astrocytes

The third type of GFAP-positive astrocytes, varicose projection astroglia, are less branched than protoplasmic astroglia and make fewer synaptic contacts. The processes of varicose projection astroglia make direct contact with vasculature walls or capillaries and terminate in the neuropil or blood vessels.<sup>4</sup>

### 2.4 | Fibrous astrocytes

The fibrous astrocytes are primarily concerned with providing metabolic support as they contact the vasculature and support for the



**FIGURE 1** Physiological role of astrocytes. Astrocytes are involved in synaptogenesis, synaptic plasticity, neuroprotection, regulating glymphatic pathway and blood-brain barrier, among many physiological functions.

axons. The paucity of synapses in the white matter implies that fibrous astroglia do not modulate neuronal activity.

### 3 | ASTROCYTE MARKERS AND HETEROGENEITY

#### 3.1 | Astrocyte markers

Astrocytes populating the human CNS are unique and exhibit heterogeneity in the expression of proteins. In humans, GFAP has at least eight different isoforms owing to different splicing patterns,<sup>5</sup> which include  $\alpha$ ,  $\gamma$ ,  $\delta/\epsilon$ ,  $\kappa$ ,  $\Delta 135$ ,  $\Delta 164$ , and  $\Delta \text{exon}6$ . Although the functional significance of GFAP isoforms needs to be deciphered, they are invariably expressed in specific subset of astrocytes. Although GFAP has been used as a classical marker for mature astrocytes, it is not expressed by all astrocytes in the different brain regions under physiological conditions. Other astroglial markers, which include the glutamate transporters (GLT-1 excitatory amino acid transporter 2 (EAAT2) and GLAST EAAT1), have been used to stain astrocyte subtypes—Bergmann glia and Müller cells. Bergmann glia are the specific astrocytes found in the cerebellum that ensheath and control cerebellar synapses and are involved in granular cell migration. Müller cells are exclusively observed in the retina and involved in cell migration, neuronal regeneration, and synaptic control of retinal cells.

#### 3.2 | Astrocyte heterogeneity

There are morphological, functional, and molecular differences reported between different brain regions.<sup>6</sup> Rodent astrocytes in the

striatum occupy a larger territorial size and contact twice as many neuronal cell bodies compared with hippocampal astrocytes. However, hippocampal astrocyte territories contain  $\sim 95,200$  excitatory synapses compared with  $\sim 50,700$  in the striatum. Both hippocampal and striatal astrocytes displayed spontaneous calcium signals, with the latter heavily reliant on extracellular entry for basal calcium levels. The hippocampal astrocytes were mainly GFAP immunoreactive, while  $\mu$ -crystallin was exclusively expressed by striatal astrocytes.<sup>6</sup> Striatal astrocytes are altered in Huntington's disease (HD) and  $\mu$ -crystallin levels are attenuated in humans and mouse models of HD.

Differences in astrocytes derived from different mouse brain regions have been reported. Adult hippocampal neural stem cells differentiated into neurons when co-cultured with hippocampal astrocytes but were unable to do so when co-cultured with spinal cord astrocytes. The electrophysiological properties of rat hippocampal astrocytes vary depending on their localization in hippocampal subregions.<sup>7</sup> Astrocytes discriminate between neuron subtypes and differentially modulate their synaptic activity. Astrocyte subtypes have specific roles in the modulation of local and distant neuronal networks. Astrocytes regulate complex synaptic activity and the subsequent behavioral outputs in CNS regions. Astrocyte diversity is pivotal in the creation of highly specialized neuron-glia units that exhibit brain regional heterogeneity.

### 4 | THE NEURON TO GLIA RATIO

The ratio of neurons ( $86.06 \pm 8.12$  billion) to glia ( $84.61 \pm 9.83$  billion) is 1:1 in the whole brain but it is not uniform when considering specific brain regions.<sup>8</sup> In the cerebral cortex including the white and gray

matter, there are  $16.34 \pm 2.17$  billion neurons and  $60.84 \pm 7.02$  billion glia, which is a ratio of 1:3.76. In the cerebellum, there are  $69.03 \pm 6.65$  billion neurons to  $16.04 \pm 2.17$  billion glia. The ratio of neurons to glia in the cerebellum is almost reversed.<sup>8</sup> Of the glial population, Pelvig et al. reported 75% oligodendrocytes, 20% astrocytes, and 5% microglia in the human cerebral cortex. Some studies have reported the glial population to be composed of 45% oligodendrocytes, 45% astrocytes, 10% microglia in the human cortex.<sup>9,10</sup> Taking into consideration different studies, the neuronal cells constitute 50% of brain cells, while the remaining 50% is made up of astrocytes (23%), oligodendrocytes (23%), and microglia (4%). The varying distribution of glia across the brain regions suggests a region-specific function for the heterogeneous astrocytes.<sup>11,12</sup>

Astrocytes exhibit morphological heterogeneity comparable to that of neurons. Detailed mapping of gene expression and functional idiosyncrasies of astrocytes in different brain areas is of paramount importance. This task is not trivial as no universal marker of astroglial cells is yet to be discovered; neither the ideal astroglia-specific promoter allowing expression of reporter proteins has been identified. Astroglial syncytia with their anatomical segregation confer additional level of complexity providing anatomically predetermined communication channels. Most fascinating is the evolution of astrocytes in higher primates and in humans; these astrocytes are distinguished by their complexity, size, and specific cell types. The idiosyncrasies of human glia are cell autonomous and persist even after grafting human fetal astrocytes into a non-human host brain. Human cells outcompete astrocytes of the host, populate the brain, and confer improved cognitive performance.<sup>11</sup>

## 5 | ASTROCYTES REGULATE SYNAPTIC ACTIVITY

Astrocytes are dynamic and are chiefly involved in a plethora of physiological functions to maintain brain tissue homeostasis. Astrocyte play roles in regulating neurotransmitter and synaptic homeostasis, neuronal myelination, metabolic, and antioxidant status, and BBB function through neuron-glia-vascular units (Figure 1). First, astrocytes are integral components of synapses, since astroglial processes ensheath synapses to form a functional synaptic compartment<sup>13</sup> and maintain brain homeostasis and neuronal functioning. The astroglial processes sequester synaptically released neurotransmitters from the interstitial space, to avoid their accumulation in the extrasynaptic compartment and minimize their spillage to nearby synapses. For instance, astroglial processes are enriched with glutamate transporters (e.g., GLT1). These ensure that extrasynaptic glutamate excitotoxicity is avoided through uptake of excess glutamate, which is broken down by astrocytes into glutamine and shuttled back into neurons to bolster synaptic transmission. Astrocytes also regulate balance of synaptic ions such as potassium ions, which is critical for efficient synaptic transmission. Impaired astroglial functioning leads to tissue dyshomeostasis that contributes to neurodegeneration in neurodegenerative diseases, which renders astrocytes an attractive therapeutic target for modulating disease pathogenesis.

## 6 | PHYSIOLOGICAL ROLE OF ASTROCYTES IN SYNAPTOGENESIS

Astrocytes play an essential role in synapse formation, maturation, and elimination (Figure 1).<sup>13</sup>

### 6.1 | SYNAPTIC PLASTICITY AND SYNAPTIC PRUNING

Astrocytes play an important role in hippocampal synaptic plasticity, which is required for learning and memory formation (Figure 1). Astrocytes release interleukin (IL)-1 family cytokine IL-33 acts on the neuronal IL-33 receptors to propagate excitatory synapse formation.<sup>14</sup> IL-33 is involved in synaptic localization of PSD-95 that in turn recruits AMPA receptors and subsequent strengthening of synaptic transmission. Neuron-derived IL-33 can also enhance excitatory synaptogenesis in the hippocampal dentate gyrus of adult mice via extracellular matrix clearance mediated by microglia. Hippocampal astrocytes eliminate unnecessary excitatory synaptic connections via multiple epidermal growth factor-like domains 10 (MEGF10). This astroglial function is pivotal for maintaining circuit connectivity and supporting cognitive function. Astroglia derived transforming growth factor (TGF)- $\beta$ 1 is implicated in the formation of structural and functional excitatory and inhibitory synapses.<sup>13</sup>

Astroglia release TGF- $\beta$  that induces neuronal complement protein C1q expression. C1q is tagged to unnecessary synapses which is detected by microglia expressing complement receptors, leading to phagocytosis of the unwanted synapses. Synaptic pruning is essential for neuronal remodeling, synaptic plasticity, and promoting optimal neural circuit connectivity.<sup>14</sup>

### 6.2 | ROLE OF ASTROCYTES IN THE TRIPARTITE SYNAPSE

The computational power of astrocytes enables them to control neuronal activity through their function in neurotransmitter uptake and are considered the guardians of neuronal excitability. Astrocytes in synergy with the presynaptic and postsynaptic terminals, form the "tripartite synapse".<sup>15</sup> In the "tripartite synapse" model, astrocyte terminals remove neurotransmitters and terminate their action via neurotransmitter transporters.<sup>15</sup> The perisynaptic astrocytic processes modulate the stabilization, dynamics, and maturation of dendritic synapses.<sup>16,17</sup> Astrocytes possess ionotropic and metabotropic membrane receptors, which are activated by neurotransmitters originating from the presynaptic cleft, to modulate synaptic activity.<sup>18</sup> Astrocytes exhibit a transient increase of intracellular calcium ion levels depending on the intensity of neuronal activity. This triggers selective release of gliotransmitters (e.g., adenosine triphosphate [ATP], glutamate, and D-serine) from the astrocytes to individual synaptic inputs, thus explaining the mechanism through which astrocytes control synaptic activity.<sup>18-23</sup> Moreover, the intrinsic spontaneous calcium

ion oscillations detected in a subpopulation of astrocytes is independent of neuronal activity and likely implicated in modulating neuronal activity.<sup>24</sup> The upregulation of astrocytic calcium ion influences (A) synapse via its effects on synaptic integrity and plasticity, and release of gliotransmitters; (B) circuit transmission via its effects on sensory plasticity and network synchronization.<sup>18</sup>

Astrocytes remove synaptic neurotransmitters such as excitatory glutamate and inhibitory gamma-aminobutyric acid (GABA), since their processes express glutamate and GABA transporters. The rapidity of removal of the neurotransmitters determines the intensity of post-synaptic activation, which further regulates signal transmission.<sup>25</sup> Astrocytes are involved in the spatial regulation of potassium ions to regulate the ionic environment of the neuropil as an alternative means to modulate neuronal signalling.<sup>26</sup> Astrocytes exert a long-term impact on synapses via releasing growth factors and related molecules, including brain derived neurotrophic factor (BDNF) and tumor necrosis factor (TNF)- $\alpha$ .<sup>27</sup>

Astrocytes in the tripartite synapses play crucial roles in the homeostasis of neurotransmitters.<sup>27</sup> The recycling of neurotransmitters by astrocytes exerts neuroprotection through maintenance of low levels of extrasynaptic glutamate to prevent excitotoxicity.<sup>28</sup> Further neuroprotection is conferred via the release of antioxidants including glutathione to protect neurons against oxidative stress.<sup>29,30</sup> Astrocytes provide metabolic support to neurons through the production, delivery, storage, and effective utilization of the brain energy including glucose, lactate, ketone bodies, glutamate, and pyruvate. Around a third of all astrocytes release bicarbonate ions to buffer extracellular positively charged hydrogen ions associated with increases in neuronal activity.<sup>31</sup> The underlying signaling mechanism involves activity-dependent release of ATP triggering secretion by astrocytes via activation of metabotropic P2Y1 receptors, recruitment of phospholipase C, release of calcium ions from the internal stores, and facilitated outward hydrogen carbonate ion transport by the electrogenic sodium bicarbonate cotransporter 1, NBCe1.<sup>31</sup> Astrocytes are the principal producers of neuronal cholesterol, which is an essential component of membranes and precursor for a plethora of signaling molecules.<sup>32</sup> Since astrocytes are prerequisites to ensuring neuronal/synaptic homeostasis and are metabolic housekeepers of neurons, synaptic dysfunction consistently observed in neurodegenerative diseases including AD renders astrocytes a prime target for therapeutic modulation.

## 7 | METABOLIC INTERACTIONS BETWEEN ASTROCYTES AND NEURONS

The relative oxygen consumption of the brain under basal conditions accounts to one fifth of the whole-body oxygen consumption.<sup>33</sup> During awake basal conditions, it was previously thought that the neurons consumed 70%–80% of the glucose oxidation rate, while astrocytes only consumed 20%–30%. Further examination revealed astroglial glucose oxidation rates exceed those of neurons.<sup>34</sup> This is because astrocytic energy demands were previously not accounted for; however, the com-

plexities of the astrocyte-neuron metabolic dialogue remain to be fully elucidated.

Lactate is a key substrate mediating the metabolic interplay between astrocytes and neurons.<sup>33,35</sup> Astrocytes specifically express glycolytic enzymes and utilize 80% of the glucose through glycolysis.<sup>36</sup> Astrocytes actively maintain synaptic homeostasis by providing neurons with intermediates for metabolism and neurotransmitter synthesis.<sup>37</sup> Astrocytes exhibit a more pronounced glycolytic profile while neurons rely on oxidative metabolism via mitochondrial oxidative phosphorylation (OXPHOS), which leads to formulation of the astrocyte-neuron lactate shuttle (ANLS) hypothesis.<sup>38</sup> The ANLS hypothesis posits that increases in extracellular glutamate during intense neuronal activity increase active astroglial glutamate uptake. This triggers sodium/potassium ATPase activation in astrocytes to maintain sodium/potassium ionic homeostasis and associated energy consumption coupled with attenuated ATP levels.<sup>39</sup> Astrocytes in response increase glucose uptake and glycolysis, secondarily augmenting lactate production and excretion, which is made available as an energy substrate for neurons for oxidative-derived ATP production. This concept is not consensual and alternative neuron-astrocyte lactate shuttle will likely be proposed.<sup>34</sup>

Electrical or mechanical stimulation causes calcium ion dependent release of glutamate, which is taken up by sodium ion-dependent glutamate transporters.<sup>40</sup> This triggers sodium ion “metabolic waves” in the astroglial network. The propagating sodium ion signal is correlated with increases in glucose uptake in a spatial-temporal manner, which is dependent on the activity of astroglial glutamate transporters. Metabolic waves can mediate the propagation of the metabolic response with the astroglial network distant from the site of activation. This allows for a concerted neurometabolic coupling within the areas of activation. Through coupling glucose utilization via astrocyte glycolysis to neuronal activity and oxidative phosphorylation, ANLS model supports the implication of a non-oxidative metabolic component during focal brain activation.<sup>41</sup> The ANLS model lends support to the viewpoint that glucose positron emission tomography (PET) signals may reflect glucose consumption in astrocytes rather than neurons.<sup>35</sup> This sparks a few questions regarding the interpretation of PET data demonstrating changes in glucose consumption (18-Fluoro-deoxyglucose [FDG] signal) as observed in AD. More studies are required to understand the contributions of neuronal or astroglial component to glucose metabolism.

Ageing process is associated with a 26% reduction of glucose metabolism from ages 18 to 78.<sup>42</sup> There is further deterioration in glucose metabolic rates in neurodegenerative diseases including AD and Parkinson's disease (PD).<sup>43</sup> In mild AD, [18F]FDG-PET demonstrates a characteristic reduction of glucose metabolism in the parietotemporal association cortices, posterior cingulate cortex, and the precuneus.<sup>42</sup> Aerobic glycolysis not coupled to oxidative phosphorylation is reduced in brain areas with high levels of tau deposition.<sup>44</sup> Changes in glucose metabolic rate occur in brains of patients with mild and moderate PD. Disease associated reduction in glucose metabolism and impaired astroglial functioning adversely affects brain function



through impaired neurotransmission, inefficient antioxidant defenses, and neurodegeneration.

Astrocytes are the major sites of glycogen storage in the brain, while neurons are nearly devoid of glycogen reserves. Neuronal activity regulates astroglial glycogen metabolism in conditions of adequate glucose supply, which suggests that glycogen not merely serves as an emergency energy reserve.<sup>45</sup> Astrocytes derive lactate from glycogen to provide neurons with energetic reserves during long-term memory consolidation.<sup>46</sup> This energetics is impaired in neurodegenerative diseases particularly AD. Inhibition of glycogen synthase leads to decreased glycogenesis owing to an overactivation of GSK-3 in AD.<sup>47</sup> The dynamics of glycogen metabolism are regulated by noradrenaline and insulin. The former stimulates glycogenolysis (mobilization of glycogen stores) while the latter promotes glycogenesis.<sup>48</sup> It is unsurprising that degeneration of the noradrenergic system in AD and the aberrant insulin signaling in AD and PD will further accentuate the metabolic impairment.

Astrocytes and neurons exhibit different dependencies on mitochondrial oxidative phosphorylation. The composition and organization of OXPHOS complexes in astrocytes is remarkably different from the neuronal respiratory chain structure.<sup>49</sup> Astrocytes contain a smaller percentage of complex I assembled into super complexes compared with neurons.<sup>50</sup> The differences in respiratory chain complex composition, distinctive transcriptional and molecular regulation of key metabolic enzymes and substrate transporters explains the higher glycolytic rates in astrocytes and their sophisticated antioxidant defense system.<sup>33,50</sup>

Mitochondria are implicated in intracellular calcium signaling in astrocytes, as metabolic demand and calcium signaling are closely associated.<sup>33,35</sup> Calcium release from mitochondria triggers spontaneous calcium ion oscillations in astroglial processes, which are propagated to other astrocytes and ultimately trigger neuronal activity. These calcium-dependent processes support synaptic integrity and regulate neurotransmission. Astrocytes actively respond to neurotransmitters through changes in calcium ion levels. Astrocytes react to glutamate, GABA, acetylcholine, ATP, and endocannabinoids by releasing glutamate, GABA, D-serine, and ATP to influence activity of neighboring neurons. Since reactive astrocytes exhibit alterations in calcium signaling, aberrant calcium signaling may be one of the key mechanisms contributing to the pathology of neurodegenerative diseases.<sup>33,35</sup> Astrocytes and neurons demonstrate remarkable cooperativity through release of neurotransmitters, dual sodium, and calcium signaling, which suggests that astroglial metabolism is dependent on the high metabolic demands of neurons and represents a viable therapeutic target.

## 8 | THE CALCIUM HYPOTHESIS: A BRIDGE BETWEEN VASCULAR FACTORS AND COGNITIVE IMPAIRMENT

In ageing and AD, astrocytes acquire both atrophic and reactive phenotypes in a region- and disease-stage-dependent manner.<sup>51</sup> Preva-

lence of atrophy over-reactivity, observed in certain brain regions and in terminal stages of the disease, facilitates the development of neurological deficits. Astrocytes exhibit ionic excitability mediated by changes in intracellular concentration of ions, most importantly of calcium ions and sodium ions, with intracellular ion dynamics triggered by the activity of neural networks. AD astrocytes associated with senile plaques demonstrate calcium hyperactivity in the form of aberrant calcium oscillations and pathological long-range calcium waves. Astroglial calcium ion signaling originating from calcium release from the endoplasmic reticulum is a key factor in initiating astroglial response; deficient calcium signaling toolkits observed in different cortices (e.g., entorhinal and prefrontal) of animal models of AD may account for vulnerability of respective regions to the pathology.<sup>51,52</sup>

The calcium hypothesis was formulated a few decades ago—originally by Landfield, Khachaturian and colleagues. It posits that those transient large increases of calcium (as observed in stroke or traumatic brain injury) result in neuronal damage owing to calcium dyshomeostasis.<sup>53–55</sup> It is predicted that the actual neurodegenerative process due to stroke is like other triggers producing a disruptive process. The theory postulates that small sustained and large rapid transient increases of calcium produce pathogenic conditions that contribute to age-related deficits and pathologies associated with neurodegeneration. The upstream events that may play essential roles include neuroinflammation, altered BBB permeability, apolipoprotein E (APOE) effects at the blood vessel, altered energy metabolism, impaired A $\beta$  clearance, imbalanced circuitry, and altered lymphatic flow. Each condition may involve different compensatory mechanisms and homeostatic responses. The net effect is a brain susceptible to dementia.<sup>53</sup>

Astrocytes are intimately involved in neurovascular coupling, which plays a key role in controlling cerebral blood flow, both physiologically and in disease. Neurovascular coupling at the capillary and arteriole levels differs mechanistically. Neurovascular coupling at the capillary level is largely dependent on astrocyte calcium signaling via the ionotropic ATP receptor P2  $\times$  1. Arachidonic acid synthesis via a PLD2-DAGL pathway, and downstream metabolism into prostaglandin E2 by COX1, are necessary for capillary dilation, with the required synthetic enzymes being expressed in astrocytes.<sup>56</sup>

The arteriole dilation is not mediated by astrocyte calcium signaling, but instead depends on N-methyl-D-aspartate (NMDA) receptor-mediated nitric oxide (NO) release. Activation of NMDA receptors, by calcium-dependent release of D-serine from astrocytes, generates NO from endothelial NO synthase (eNOS) in endothelial cells, and thus helps to dilate penetrating arterioles.<sup>56</sup> NMDA receptor activation controls astrocyte calcium indirectly through diffusible mediators such as NO released from post-synaptic neurons, because astrocyte-patch based NMDA inhibition with intracellular MK-801 preserves evoked calcium transients. Astrocyte calcium play a fundamental role in amplifying functional hyperemia—a phenomenon that is fundamental to fueling the brain—when neuronal activation is prolonged.<sup>57</sup> Astrocytes contribute to neurovascular coupling at the arteriole and capillary

levels could be specifically targeted therapeutically to increase blood flow in pathological conditions.

In *post mortem* human AD brain specimens and rodent models of AD, reactive astrocytes exhibit signs of calcium dysregulation or hyperactivation of key signaling mediators including the calcium/calmodulin-dependent protein phosphatase calcineurin and the calcineurin-dependent transcription factor nuclear factor of activated T cells (NFAT). Drugs that target CN/NFAT activity including commercially available calcineurin inhibitors (e.g., tacrolimus), small chemical NFAT inhibitors (e.g., Q134R), or peptide-based NFAT inhibitors improve neuronal viability and/or function in animal models exhibiting AD-like pathology. Beneficial actions in brain are found even when calcineurin/NFAT inhibition is limited to astrocytes, suggesting that reactive astrocytes play a causative role in pathophysiology and cognitive decline in AD.<sup>58,59</sup>

Despite the indispensable role that astrocytes play in the neurovascular unit, the functional impact of astrocyte signaling in cognitive decline and dementia related to vascular pathology was explored in mice fed with a vitamin B deficient diet resulting in hyperhomocysteinemia (HHcy), cerebral vessel disease, and cognitive decline. Astrocyte-specific inhibition of the calcium-dependent transcription factor, NFAT, normalized cerebrovascular function in HHcy mice, ameliorated synaptic properties in brain slices, and stabilized cognition.<sup>58</sup>

## 9 | ASTROGLIAL MODULATION OF THE BBB AND GLYPHATIC SYSTEM

Astrocytes are strategically placed at the interface of capillaries and neurons, and form perivascular endfeet—which are astroglial terminal processes—at the BBB (Figure 1).<sup>60</sup> The astroglial endfeet contact the brain vasculature surface that is facing the endothelial cells and pericytes and enwrap neuronal synapses. This facilitates the modulation of neuronal activity and cerebral blood flow, via increases in intracellular calcium levels in the astroglial endfeet. Not only are astrocytes centrally involved in bolstering the BBB systems but are also required for maintaining transport pathways that constitute glymphatic system.<sup>60</sup> The glymphatic system is a recently identified glial-dependent waste clearance pathway in the brain to drain away soluble waste proteins and metabolic products. This pathway subserves the cerebrospinal fluid (CSF) flow into the brain along arterial perivascular spaces and into the brain interstitial tissue facilitated by Aqp4 water channels.<sup>61</sup> The vascular endfeet of astrocytes express the Aqp4 channels that allow two-way water transport between blood and CSF compartments, and brain parenchyma. It is the close interaction between the astroglial network, Aqp4 water channels, and perivascular spaces—which enables efficient exchange of solutes/ions and by-products of metabolism between CSF, interstitial fluid, and bulk flow of water. Impairment of the BBB and the glymphatic system leads to accumulation and misfolding of proteins to initiate and propagate disease pathogenicity in neurodegenerative diseases.

## 10 | ASTROGLIAL ROLE IN CEREBRAL BLOOD FLOW AND NEUROVASCULAR COUPLING

Astrocytes regulate an array of complex steps to co-regulate cerebral blood flow and neuronal activity referred to as neurovascular coupling.<sup>62</sup> The astrocyte participation in neurovascular coupling occurs primarily at the level of capillaries and less relevant in arterioles.<sup>62,63</sup> Astrocytes detect local rises in extracellular glutamate through metabotropic receptors, which activates a calcium ion dependent signaling pathway.<sup>64</sup> This pathway generates arachidonic acid and downstream vasodilating metabolites including prostaglandins and epoxyeicosatrienoic acids.<sup>65</sup> Arachidonic acid diffuses to the vascular endothelial cells where it is transformed to vasoconstricting molecules such as 20-HETE.<sup>66</sup> The divergent roles played by astroglial activation on cerebral hemodynamics that sustains brain's activity is heavily reliant on NO levels. NO levels regulate the routes of arachidonic acid conversion. The molecular orchestration underlying the neurovascular coupling is intricate and includes adaptation to hypoxic conditions.<sup>67</sup> Under pathological conditions as observed in neurodegenerative diseases, including AD and PD, disruption of normal astrocyte physiology compromises the regulation of blood flow, leading to cerebral hypoperfusion and impaired cerebral haemodynamics.<sup>33</sup> For example, A $\beta$  exhibits vasculotoxic and vasoactive properties that mediate cerebrovascular deficiency in AD.<sup>63</sup> Individuals carrying the APOE $\epsilon$ 4 risk variant demonstrate disrupted neurovascular coupling in preclinical stages of AD.<sup>68</sup> Impaired nutrient input, and accumulation of toxic metabolic products due to cerebral hypoperfusion are worsened by the loss of BBB integrity, a well-recognized feature of early AD, which occurs in PD.<sup>33,69</sup> Taken together, the disrupted BBB integrity contaminates the brain parenchyma with blood-borne immunogenic molecules and adversely affects the function of the glymphatic system.<sup>70</sup> This results in buildup of toxic protein aggregates in neurodegenerative diseases, further emphasizing the importance of therapeutic modulation of astrocytes.

## 11 | REACTIVE ASTROCYTES: POLARIZATION OF ASTROCYTES FROM RESTING (A0) TO ACTIVATED STATES (A1 AND A2) AND BEYOND

Astrocytes undergo morphological, molecular, and functional remodeling in the presence of injury and disease states, to become reactive astrocytes.<sup>71</sup> Reactive astrocytes exhibit beneficial and detrimental effects based on their reactivity profile. In mouse models of infection and stroke induced by lipopolysaccharide (LPS) induction and middle artery occlusion, respectively, resting astrocytes polarize to become reactive astrocytes.<sup>72</sup> Transcriptomics have been pivotal in profiling of reactive astrocytes into neurotoxic (A1) and neuroprotective (A2) forms.

A1 astroglial phenotype is induced by cytokines secreted by activated microglia, which include complement factors (C1q), TNF- $\alpha$ , and IL-1 $\alpha$ .<sup>71</sup> This leads to A1 astrocytes unable to promote neuronal

survival and outgrowth, synaptogenesis, and phagocytosis, and inducing the demise of neurons and oligodendrocytes. Detailed transcriptomic analyses have revealed that aged astrocytes adopt a phenotype of neuroinflammatory A1-like reactive astrocytes in a region-specific manner. Hippocampal and striatal astrocytes upregulated a greater number of reactive astroglial genes compared with cortical astrocytes. Aged brains form a plethora of A1 reactive astrocytes in response to LPS-induced neuroinflammation. Upregulation of reactive astroglial genes was minimized in mice lacking microglial-secreted cytokines—IL-1 $\alpha$ , TNF, and C1q—which are involved in inducing A1 reactive astroglial formation. The aging-induced up-regulation of reactive genes by astrocytes may contribute to cognitive decline in vulnerable brain regions and contribute to the greater vulnerability of the aged brain to injury as observed in neurodegenerative diseases. A1 astrocytes are found to be in abundance in various neurodegenerative diseases—AD, PD, HD, amyotrophic lateral sclerosis (ALS), and multiple sclerosis (MS). It is estimated that around 30–60% of astrocytes in brain regions specific to each neurodegenerative disease were A1 astrocytes and were hypothesized to be integral for disease initiation and progression.<sup>71</sup>

A2 astrocytes exhibit a neuroprotective astroglial phenotype which can be induced by microglia following traumatic brain injury.<sup>71</sup> A2 astrocytes can be modulated in an acute ischemic spinal cord injury model through silencing of miR-21. Nuclear factor IA (NFIA) has been identified as a molecular switch to induce human glial competency, which causes astroglial transitioning to adopt an A2 profile in engrafted adult mouse brain. A2 astrocytes increase expression of several neuroprotective factors to promote synaptic repair, growth, and survival of neurons. This contrasts with the upregulation of reactive oxygen species (ROS), IL-1 $\beta$ , and TNF $\alpha$  by the A1 astrocytes. Protective mediators such as prokineticin-2 (PK2), chitin-like 3, frizzled class receptor 1, Nrf2, pentraxin 3 (PTX3), sphingosine kinase 1, and transmembrane 4 L6 family member 1 are upregulated in A2 astrocytes.<sup>71</sup>

### 11.1 | Reactive astrocytes in AD

A1 astrocytes were higher than A2 astrocytes in *post mortem* AD.<sup>71</sup> This suggests that the neurotoxic astroglial phenotype predominates as 60% of GFAP-positive astrocytes expressed A1-specific markers and is an important pathological feature of AD.<sup>71</sup> In a mouse model recapitulating cerebral amyloid angiopathy involving early A $\beta$  deposition in the cerebral vasculature, reactive astrocytes exhibited A1 phenotype. A1 astrocytes that surround amyloid plaques secrete proinflammatory cytokines, for example, TNF- $\alpha$ , IL-1, and IL-6, promoting AD pathogenesis.<sup>1</sup> A1 astrocytes were activated by APOE $\epsilon$ 4 in a mouse model of tauopathy. Transcriptomics have unraveled astroglial phenotypes beyond the A1/A2 phenotypes. The analyses of single-nucleus transcriptome of the prefrontal cortex in AD and controls have identified transcriptionally diverse astroglial subpopulations that exhibit disease-specific changes.<sup>73</sup> Genes implicated in synaptic signaling, synaptogenesis, and neurotransmitter synthesis were downregulated—while genes suggestive of cellular stress and involved in instigating innate immune responses were upregulated in AD.<sup>73</sup> RNA

sequencing analysis of single-nucleus of the hippocampus in wild-type and transgenic mouse model of AD revealed a disease-specific reactive astroglial state observed in early disease phases and in the vicinity of A $\beta$  plaques.<sup>1</sup> The disease-specific astrocytes are observed during the early and advanced stages of AD pathogenesis.

The formation of A $\beta$  plaques, one of the major hallmarks of AD (alongside neurofibrillary tau tangles), is dependent on  $\beta$ - and  $\gamma$ -secretases mediated cleavage of the parent protein, APP. Low levels of APP and secretases have been observed in non-reactive astrocytes, but they are markedly upregulated in reactive astrocytes in the presence of inflammation and accrued cellular stress.<sup>74</sup> BACE1 is a  $\beta$ -secretase detected in reactive astrocytes in Tg2576 mouse model of AD. Exposure of primary mouse astroglial cultures to pro-inflammatory cytokines, TNF- $\alpha$  and IFN- $\gamma$ , leads to a robust increase in BACE1, APP, and subsequent A $\beta$  secretion.<sup>74</sup> Similarly, endogenous levels of APP and BACE1 in the astrocytes are increased in response to A $\beta$ 42 oligomers and fibrils, in Tg2576 mice.<sup>74</sup> Evidence suggests that in response to inflammation, reactive astrocytes contribute significant A $\beta$  plaque load to accentuate AD pathology.

The spectrum of A $\beta$  species mediating pathogenic changes in astrocytes is broad and complex. In PS1V97L-Tg mouse model of AD, nonameric and dodecameric A $\beta$  assemblies instigate neuronal injury and cognitive deficits.<sup>75</sup> Toxic A $\beta$  oligomeric forms were duplicated in a time-dependent manner specifically in astrocytes, and the BACE1 and APOE proteins were responsible for this self-replicating effect.<sup>75</sup> The addition of A $\beta$ 1-40 and A $\beta$ 1-42 oligomeric species augmented the levels of intracellular calcium in astrocytes but not in neurons. The rise in calcium increased the rate of production of ROS by nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) in both astrocytes and neurons. The increase in ROS then triggers caspase-3 activation, which leads to impairment of long-term potentiation (LTP; a cellular analogue of memory). Diverse APP fragments and N- and C-terminally truncated A $\beta$  isoforms confer neurotoxicity in AD. The findings discussed suggest an early and primary role for astrocytes in mediating neuronal damage in AD.

The astrocytes are involved in the uptake of neuron derived A $\beta$ . This is supported by *post mortem* studies showing colocalization of A $\beta$ 42 with the  $\alpha$ 7 neuronal nicotinic acetylcholine receptor and choline acetyltransferase in reactive astrocytes. Plaque-associated reactive astrocytes engulf, and digest presynaptic dystrophies in the hippocampus of APP/PS1 transgenic mice.<sup>76</sup> Microglia were not involved in this clearance. Phagocytic reactive astrocytes were evident in 35% and 67% of A $\beta$  plaques at 6 and 12 months of age, respectively. Only 7% of dystrophic neurites were engulfed by astrocytes, which is low. The beneficial astroglial phagocytic process may become less efficient due to accrual of dystrophic neurites during AD pathogenesis.

One of the important mechanisms underlying the uptake and clearance of A $\beta$  encompasses lipoprotein receptor-related protein 1 (LRP1). LRP1 regulates the endocytosis of soluble A $\beta$ —42 more efficiently than that of A $\beta$ —42 aggregates. LRP1 deletion in astrocytes leads to disturbed interstitial fluid (ISF) A $\beta$  clearance and elevates soluble and insoluble A $\beta$  levels in the brains of APP/PS1 mice.<sup>77</sup> Impaired LRP1-mediated A $\beta$  clearance may occur due to APOE-induced endosomal



dysfunction. APOE is predominantly expressed by astrocytes and microglia and is an LRP1 ligand. Three different APOE isoforms have been identified in humans:  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ . APOE $\epsilon 4$  is an established susceptibility risk locus in AD. In mice, astrocytes expressing the human APOE $\epsilon 4$  allele experience a downregulation of Na<sup>+</sup>/H<sup>+</sup> exchanger isoform 6, which results in over-acidification of the endosomes.<sup>78</sup> This impairs the endocytic recycling of cell surface proteins such as LRP1, with the receptor entrapped in intracellular compartments. The reduced LRP1 availability at the cell surface inhibits effective A $\beta$  uptake and hence astrocyte-mediated clearance.<sup>78</sup> The phagocytic capacity of astrocytes may be dependent on APOE isoforms. The role of LRP1 in mediating APOE-regulated astroglial phagocytic functioning in synaptic pruning and degradation of neuronal remnants/debris and A $\beta$  requires additional investigations.

APOE and clusterin (aka APOJ) null PDAPP mice had an earlier onset of disease and markedly increased A $\beta$  deposition in the parenchyma, CSF, and ISF.<sup>79</sup> The elimination half-life of ISF A $\beta$  measured by in vivo microdialysis was significantly diminished in APOE and clusterin knockout PDAPP mice.<sup>79</sup> Astrocytes are the predominant source of clusterin albeit neuronal subpopulations also express clusterin. Astrocytes synthesize and release clusterin into the extracellular in physiological conditions. Co-culture experiments conducted on rat hippocampal astrocytes and neurons demonstrated that clusterin incubation prevents A $\beta$ -induced astroglial calcium uptake, leading to attenuated ROS generation and caspase-3 activation. Clusterin blocked A $\beta$ -induced inhibition of LTP on hippocampal slices. In another study, incubation of clusterin with A $\beta$ -42 oligomers prior to their injection into the rat hippocampus prevented A $\beta$ -42-induced learning and memory impairments, glial inflammation, and neuronal degeneration. In pathological conditions including AD, the additive effects of APOE and clusterin involved in regulating A $\beta$  deposition are impaired, leading to increased A $\beta$  deposition.

The presence of tau inclusions in the astrocytes is attributed to the internalization of tau by astrocytes.<sup>80</sup> Astroglial tau pathologies have primarily been described based on six major distinct morphologies evident in primary tauopathies and age-related tau astrogliopathies. Age-related astrogliopathy is a general term, which includes the presence of granular-fuzzy astrocytes and thorn-shaped astrocytes.<sup>80</sup> Although these type of astrogliopathies are evident in the aged brain,<sup>80</sup> they have been implicated in neurodegenerative diseases including AD and parkinsonism-related disorders. Thorn-shaped astrocytes are defined as fibrillar astrocytes that are predominantly observed in subpial, subependymal or perivascular areas, and white matter. At advanced stages of AD, thorn-shaped astrocytes comprise of phosphorylated tau and tau exhibiting abnormal conformations. Thorn-shaped astrocytes—extracted from patients with age-related tau astrogliopathies and containing hyperphosphorylated tau— injected into the hippocampus of wild-type mice display hyperphosphorylated tau deposits in astrocytes, neurons, and oligodendrocytes. Hyperphosphorylated tau is transmissible from thorn-shaped astrocytes to neurons, which underscores the integral role played by astrocytes in the propagation of tau pathology. In addition, the accrual of tau oligomers in the astrocytes leads to calcium dyshomeostasis and subsequent synaptic dysfunction. Further

research is warranted to establish the exact mechanism which causes exacerbation of tau pathology in neurodegenerative diseases including AD.

Although a detrimental role for astrocytes has been defined in the context of propagating tau pathology; however, astrocytes exhibit beneficial properties by endocytosing tau through transcription factor EB (TFEB), to mediate clearance of excess tau.<sup>81</sup> TFEB is activated by lysosomal stress, which causes TFEB to translocate from the cytoplasm to the nucleus resulting in transcription of target genes to promote formation of autophagosome and biogenesis of lysosomes. The impairment of autophagy contributes to accumulation of protein aggregates in AD. Upregulation of TFEB expression has been observed in familial AD involving presenilin mutations and is considered a protective mechanism.

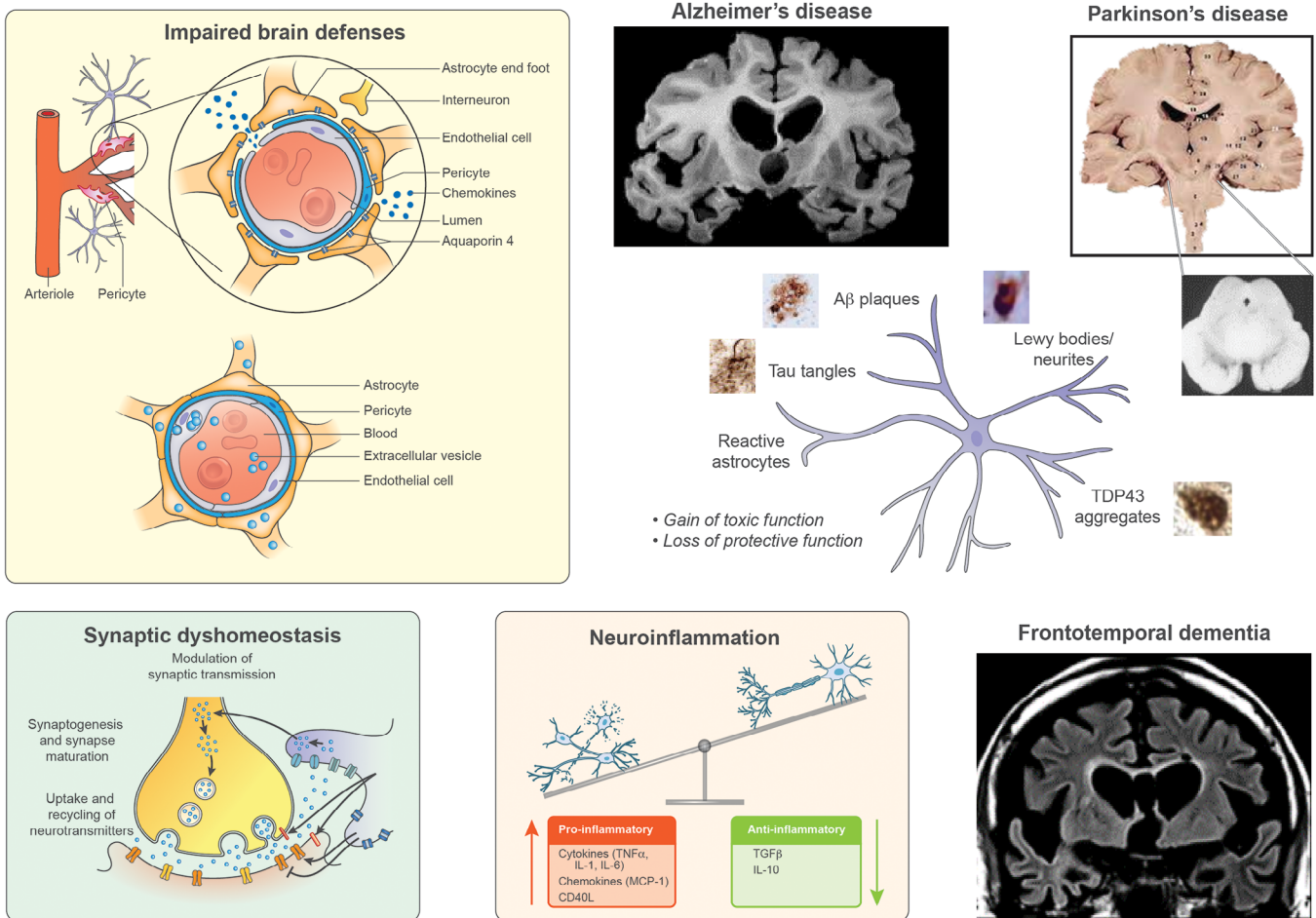
Recently, astroglial-sourced IL-3 was found to program microglia to ameliorate AD pathology.<sup>82</sup> Astrocytes constitutively produce IL-3 to elicit transcriptional, morphological, and functional programming of microglia, which stimulates microglia to induce an acute immune response, enhanced motility, and the capacity to clear aggregates of A $\beta$  and tau.<sup>82</sup> These molecular changes mediated by astrocytes limit AD pathology and cognitive decline, implying a beneficial role conferred by astrocytes.

Astrocytes play dual roles in tau pathology, where they may initially be involved in active clearance of pathological tau species. But with the accumulation of tau aggregates in astrocytes, astrocytes may lose their protective function (Figure 2) and instead may contribute to the spreading of tau pathology in AD and other tauopathies.

## 11.2 | Reactive astrocytes in PD

Pharmacological inhibition of microglial-induced polarization of astrocytes into the A1 phenotype is protective for dopaminergic neurons and ameliorates behavioral impairment. The overexpression of  $\alpha$ -synuclein induces A1 astrocytes to release chemokines (CCL2, CCL20, CXCL1, and CX3CL1) and pro-inflammatory cytokines (TNF- $\alpha$ , IL-1, and IL-6), which may contribute to dopaminergic dysfunction and neurodegeneration.<sup>83</sup> This suggests that A1 astrocytes contribute to neuronal toxicity in PD. The analysis of single-nucleus transcriptome of SN acquired from patients with PD reveal two astroglial subpopulations, with one expressing neuroinflammatory genes and the other expressing genes involved in repair.<sup>84</sup>

Protective upregulation of DJ-1 has been selectively observed in reactive astrocytes in PD. Overexpression of astroglial DJ-1 was protective against neurotoxicity in animal models of PD. Impaired DJ-1 functioning may impair neuroprotective properties and in turn accentuate PD pathology. Another astroglial protein, parkin, is selectively expressed under pathological conditions. Mutations in PARK2 gene that encodes parkin, tend to confer a loss of function and are associated with increased accumulation of  $\alpha$ -synuclein in *post mortem* brains of patients with PD.<sup>85</sup> Since parkin plays a role in astroglial proliferation, knockout of parkin leads to astroglial dysfunction and aggravated neuronal death in mice. Similarly, overexpression of Nrf2 in reactive



**FIGURE 2** Astrocytes in a pathological challenge. Reactive astrocytes lose their neuroprotective profile in favour of gain of toxic function leading to astroglial dyshomeostasis, contributing to neuroinflammation, neurodegeneration, impaired brain defenses, and propagating protein aggregation, in neurodegenerative diseases.

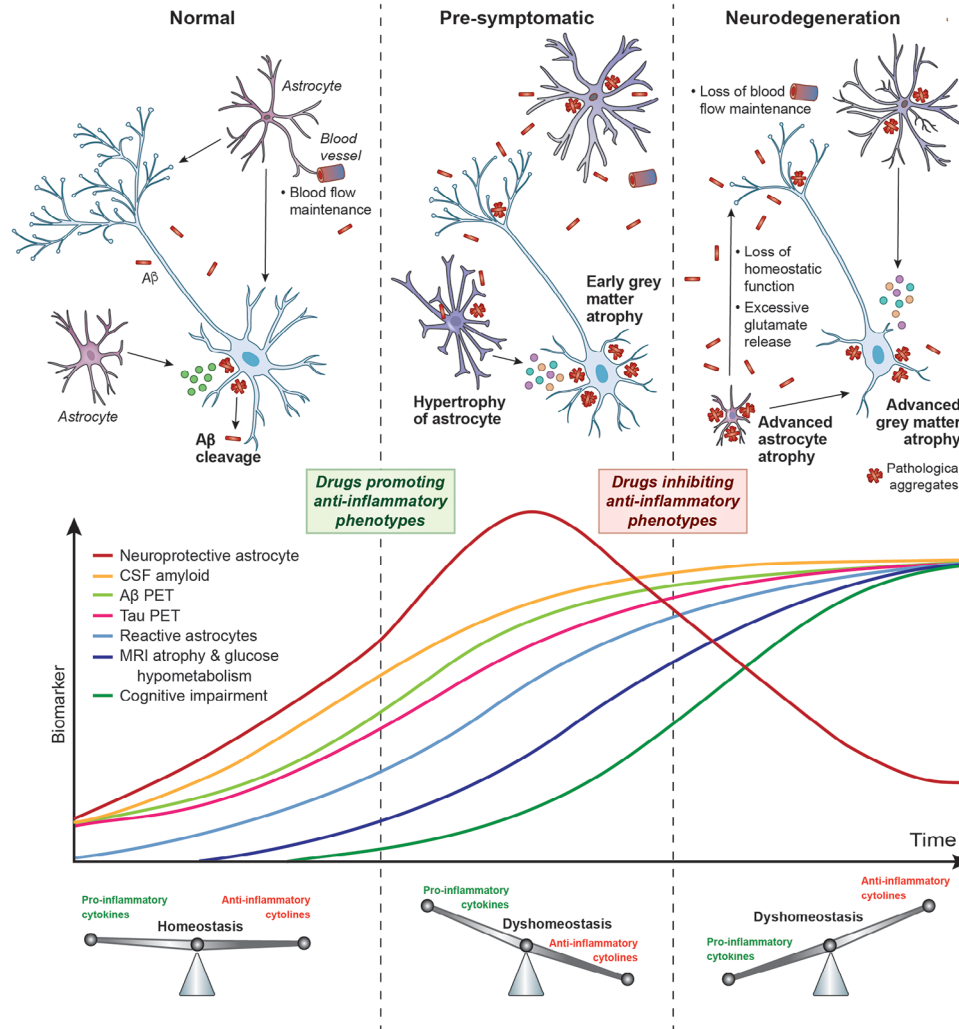
astrocytes conferred neuroprotection in 6-OHDA, MPTP, and A53T mouse models of PD. MPTP toxicity in mice accelerated dopaminergic neuron loss in connexin 30 (an astroglial gap junction protein) knockout mice.<sup>86</sup> Microarray analysis of striatum revealed attenuated expression of pan-reactive and A2 astroglial genes after induction of MPTP toxicity in connexin 30 knockout mice, with no change in the microglial gene expression. Moreover, MPTP toxicity in connexin 30 knockout mice reduced the number of striatal astrocytes co-expressing glial cell line derived neurotrophic factor (GDNF) mRNA and S100 $\beta$  protein or S100a10 mRNA and S100 $\beta$  protein.<sup>86</sup> These findings demonstrate that astrocytes play essential roles in regulating neuroinflammation in PD.

The accumulation of  $\alpha$ -synuclein, which is a hallmark of PD, has been observed in astrocytes. Astrocytes can mediate clearance of  $\alpha$ -synuclein via endocytosis of  $\alpha$ -synuclein released by axonal terminals, as suggested by the localization of  $\alpha$ -synuclein immunoreactive puncta in the lysosomal compartments of astrocytes.<sup>81</sup> However, increased uptake of  $\alpha$ -synuclein oligomers by astroglia overwhelms the lysosomal degradation pathway, which results in formation of  $\alpha$ -synuclein deposits and mitochondrial impairment. This suggests that pathological accumulation of  $\alpha$ -synuclein leads to diminished physiological

function of astrocytes as well as a gain of toxic function in astrocytes in PD (Figure 3).

## 12 | COMPLEX PHENOTYPIC DIVERSITY OF ASTROCYTES EXTENDS BEYOND A1 AND A2 PROFILING

Although reactive astrocytes are binarized into A1 and A2 astrocytes, only a subset of A1 and A2 genetic transcripts are upregulated in astrocytes in human AD or HD, or from numerous mouse models of acute and chronic CNS diseases.<sup>87</sup> Most importantly, the phenotypic diversity and its causal link to toxic or protective functions awaits experimental validation. The binary polarization of reactive astrocytes is simple as astrocytes exhibit profound phenotypic diversity, which is also complex and not well understood. This is highlighted by the numerous stage-dependent transcriptomic states in HD, AD, and MS that fail to comply with A1 and A2 profiles.<sup>87</sup> Advanced statistics performed on multidimensional data and co-clustering approaches have demonstrated that A1 and A2 transcriptomes represent only two of the many



**FIGURE 3** Dynamic changes in astroglial activation underlie progression of neurodegenerative diseases. In pre-symptomatic phases, astrocytes promote neuroprotection through homeostatic regulation of pro-inflammatory and anti-inflammatory cytokines. In the prodromal stages, when the proteins that are thought to be toxic in the respective neurodegenerative diseases accumulate, there is predominant increase (activation) of protective phenotype of astrocyte. However, as the toxic insult continues, there is a switch from neuroprotective profile to neurotoxic profile. During the neurodegenerative phases, the persistent accumulation of pathological aggregates and their ensuing toxicity causes astroglial dystrophy. This causes a loss of the homeostatic control through dysregulated increase in pro-inflammatory phenotype over anti-inflammatory phenotype, leading to disease pathogenesis (adapted from Livingstone et.al; 2022)<sup>93</sup>.

putative astroglial transcriptomes segregating along several latent variables. The analysis underscores the importance of multidimensional data to establish the distinctiveness of astroglial phenotypes, and to characterize the extensive and precise functional diversity of reactive astrocytes unveiled by transcriptomics.<sup>87</sup>

## 13 | USING PET IMAGING TO DETECT ASTROCYTES

Astrocytes and microglia mediate dynamic and multicellular processes of neuroinflammation. Acute inflammation is beneficial in response to CNS injury; however, chronic inflammation may cause more harm than benefit in neurodegenerative diseases. Since neuroinflammation is a key pathological driver of neurodegenerative disease, the development

of specific and sensitive tracers for PET studies are pivotal for tracking neuroinflammation in living patients and may aid in the diagnosis of neurodegenerative diseases.

### 13.1 | Astroglial-specific PET tracers

Various PET tracers have been developed that non-selectively detect microglia and astrocytes.<sup>88</sup> [11C]-deuterium-L-deprenyl ([11C]DED) binds to monoamine oxidase-B (MAO-B), which is highly abundant in the astrocytes. As a proof of concept, strong correlation has been noted for [11C]DED and MAO-B activity in the autopsy brain tissue for both non-demented and AD cases. [11C]DED has been useful for visualizing reactive astrogliosis in chronic brain diseases including Creutzfeldt-Jakob disease, ALS, and epilepsy. Most of the work with <sup>11</sup>C-DED has

been done in patients with AD. A high degree of reactive astrogliosis as revealed by [11C]DED has been demonstrated in cortical and subcortical regions in A $\beta$ -positive MCI relative to controls, A $\beta$ -negative MCI, and AD patients, and in pre-symptomatic autosomal-dominant AD. An initially high and then declining astrogliosis is observed in prodromal AD, in contrast with the increasing A $\beta$  plaque deposition during disease progression, implicating astroglial activation in early AD pathology.

Previous studies demonstrate negative correlation between reactive astrogliosis and A $\beta$  plaque load. A positive correlation between [11C]DED and [18F]FDG uptake has been reported in pre-symptomatic autosomal-dominant AD, but not in non-carriers.<sup>89</sup> MAO-B represent attenuated glucose demand by astrocytes owing to astrodegeneration. This term defines astroglial phenotype closely associated with progression of neurodegenerative disease and thereby reduced glucose utilization or lactate availability for neurons in the vicinity. Astrocytes may contribute to glucose metabolism. In ALS, similar correspondence between [11C]DED and [18F]FDG uptake was observed where increased MAO-B was evident in the pons and white matter and found hypermetabolism in similar brain regions.<sup>90</sup> This change in glucose metabolism was attributed to neuroinflammation. Similarly, a late-phase decline in MAO-B expression in autosomal-dominant AD could potentially be considered a downstream effect of early MAO-B upregulation and a reflection of chronic neuroinflammation due to excess glutamate, leading to impaired astroglial functioning and atrophy.<sup>89</sup>

Autoradiography experiments using <sup>3</sup>H-PIB and <sup>3</sup>H-DED demonstrate that astrocytosis exhibit a distinct regional pattern in AD brain compared to fibrillar A $\beta$ .<sup>91</sup> This may be due to different types of astrocytes associated with pathophysiological processes in AD. A clear lamination pattern was evident with high <sup>3</sup>H-PIB and <sup>3</sup>H-DED binding in the superficial layers of the frontal cortex.<sup>91</sup> However, <sup>3</sup>H-PIB demonstrated lower binding to fibrillar A $\beta$  while <sup>3</sup>H-DED showed high binding to activated astrocytes throughout the hippocampus. A similar discordance in the laminar distribution of these two modalities was prevalent in the medial temporal gyrus and insular cortex. Immunohistochemical detection of GFAP-positive astrocytes were observed in the vicinity and surrounding A $\beta$  neuritic plaques in the frontal cortex and the hippocampus.<sup>91</sup> Consistent with autoradiography, fewer A $\beta$  plaques were present in the hippocampus, but some hippocampal GFAP immunoreactive astrocytes contained A $\beta$  positive granules within their somata.<sup>91</sup> Soluble A $\beta$  oligomers rather than A $\beta$  plaques per se are the real culprits mediating neuronal damage. It is not known whether concentration of A $\beta$  oligomers or intracellular A $\beta$  are significantly greater in the hippocampus relative to cortical regions. Studies have not characterized the numerous A $\beta$  oligomeric assemblies in AD postmortem brain, instead used cortical brain extracts. Non-fibrillar A $\beta$  aggregates are not detected by amyloid PET tracers. Hence, soluble assemblies may underlie the increased astrogliosis observed in the hippocampus.

Similar laminar brain distribution of tau deposits and activated astrocytes has been observed, suggestive of an intimate pathological interconnection. <sup>3</sup>H-THK5117 tau tracer showed a distinct laminar cortical binding akin to <sup>3</sup>H-DED autoradiography, with an extensive

binding in the superficial and deep layers of the temporal neocortices, whereas the middle frontal gyrus showed an even binding throughout the layers.<sup>88</sup> A positive correlation was evident between activated astrocytes and tau loads for carriers of Arctic mutation in the amyloid- $\beta$  precursor protein (A $\beta$ PP<sub>Arctic</sub>), but not in presenilin 1 mutation carrier (PSEN1 $\Delta$ E9). The differences in APP and PSEN1 mutation yields major differences in A $\beta$  and tau deposition, which may differentially affect astroglial activity.

A few caveats concerning the use of [11C]DED urge caution when interpreting findings. [11C]DED is not a marker for astroglial function per se; it is rather a measure of MAO-B expression and not astroglial glucose or glutamate uptake. MAO-B is not exclusive to astrocytes but is also expressed by microglia, non-reactive astrocytes, serotonergic, histaminergic, and cholinergic neurons. Moreover, it suffers from a limitation in that it is not elevated at late disease stages when A $\beta$  load is high.

[11C]BU99008—a PET ligand targeting imidazoline-2 binding sites (I<sub>2</sub>BS) exclusively expressed on astrocytes and involved in the regulation of GFAP expression—was found to detect reactive astrocytes with good selectivity and sensitivity in animals and humans.<sup>88</sup> Human studies have demonstrated a favorable biodistribution and dosimetry profile for [11C]BU99008. Regional binding studies with BU99008 demonstrated significantly higher astrogliosis (indicated by higher BU99008 uptake) in the hippocampus and frontal cortex homogenates derived from patients with AD compared with cognitively normal. Comparative autoradiography studies strengthened these findings, showing higher specific binding for <sup>3</sup>H-BU99008 than <sup>3</sup>H-DED in sporadic AD brain compared to CN, suggesting BU99008 tracer binds to a different target site than DED tracer.

We have recently demonstrated that [11C]BU99008 can track astrocytosis in people with late-life cognitive impairment.<sup>92</sup> In A $\beta$ -positive cognitively impaired, [11C]BU99008 was particularly pronounced in frontal, temporal, medial temporal, and occipital lobes, compared with individuals who were cognitively normal. Using biological parametric mapping, we found a positive voxel-wise neuroanatomical correlation between <sup>3</sup>H-BU99008 and [18F]florbetaben.<sup>92,93</sup> This suggests that astrocyte reactivity is found particularly in cortical regions with A $\beta$  overload and is consistent with animal and human studies showing astroglial reactivity in the vicinity of A $\beta$  plaques. Astrocytes are key mediators of A $\beta$ -induced neurotoxicity and tau phosphorylation in primary culture, through augmented expression of neurotoxic substances such as NO and TNF- $\alpha$ . The activation of astrocytes prior to the development of AD as observed in late-onset cognitive impairment implies that astroglial activation is an early event in the disease pathogenesis.<sup>92</sup> Indeed, increased [11C]DED binding in autosomal dominant AD patients and A $\beta$ -positive MCI subjects has been reported and discussed in the preceding sections. [11C]BU99008 uptake was observed in A $\beta$ -negative cognitively impaired individuals, which suggests that A $\beta$  plaques detected by [18F]florbetaben are not necessary for astroglial reactivity.<sup>92</sup> Astroglial reactivity is capable of accelerating A $\beta$  production via increased expression of APP and its cleavage enzymes,  $\beta$ -secretase and  $\gamma$ -secretase. The lysis of dead phagocytic astrocytes that have engulfed A $\beta$  peptides may even contribute to the



genesis of the consolidated A $\beta$  plaques detected by PET A $\beta$  imaging in vivo.

Astroglial reactivity is not confined to AD pathology and may represent a common theme of neurodegeneration prevalent across neurodegenerative diseases. The greatest increase in astrocytosis revealed by [11C]BU99008 was observed in early PD, while a loss of BU99008 uptake was seen in moderate/severe PD.<sup>94</sup> The studies discussed suggest that astrocytes are involved in the initiation and progression of neurodegenerative diseases, such as PD and AD.<sup>92,94</sup> Reactive astrocytes may be observed early and could reflect a neuroprotective compensatory mechanism and a pro-inflammatory upregulation in response to A $\beta$  or  $\alpha$ -synuclein pathology. However, with the advancing disease progression, astroglial function may be impaired and their degeneration is a contributory factor to disease pathogenesis.

Astrocytes regulate homeostasis of brain glucose metabolism and contribute to [18F]FDG uptake in the brain. The regional decline in glucose metabolism observed in PET studies may reflect astroglial atrophy and degeneration, which means loss of astroglial support to neurons leading to neuronal demise and hypometabolism.

### 13.2 | [18F]FDG-PET

Astrocytes outnumber neurons by a ratio of 4-5 fold particularly in the cerebral cortex, which make astrocytes a strong contender for [18F]FDG-PET uptake. Astrocytes couple neuronal activity to glucose utilization. In response to glutamate released by active neurons, glucose is predominantly taken up by astrocytes; glucose is then metabolized to lactate, which provides a preferred energy substrate for neurons.<sup>95</sup> More specifically, astroglial glutamate transport through GLT-1 or GLAST has been demonstrated to act as trigger to signal glucose uptake by astrocytes. GLT-1 or GLAST knockout mice display reduced [<sup>14</sup>C]-2-deoxyglucose uptake in the somatosensory cortex following whisker stimulation. These findings reinforce the view that [18F]FDG PET signal is largely representative of glucose metabolism by astrocytes rather than neurons. The first in vivo PET evidence for astroglial glutamate transport via GLT-1 triggering glucose uptake has been provided in a rat brain.<sup>96</sup> [18F]FDG PET signal is substantially affected by the activation of astroglial glutamate transport.

Longitudinal decline in astroglial function as measured using [11C]DED PET was significantly associated with progressive hypometabolism ([18F]FDG PET uptake) in mutation carriers; however, no significant association was observed in noncarriers.<sup>89</sup> If astrogliosis is an early event, some studies demonstrating cortical hypermetabolism on [18F]FDG PET particularly in subjects with MCI may be reflective of increased compensatory astroglial activity. Once patients develop considerable A $\beta$  and tau pathology, the astroglial functionality may be impaired and is expressed as glucose hypometabolism on [18F]FDG PET. An inverse association between high A $\beta$  load and decreased astrocyte uptake ([11C]BU99008) in the temporal cortex.<sup>93</sup> The decreased astrocyte uptake was associated with greater relative progression of A $\beta$ -associated neuropathology, that is, glucose hypometabolism and grey matter atrophy. The

reduced astrocyte uptake in the temporal cortex reflects astrocyte dystrophy, which leads to astroglial and neuronal atrophy, and glucose hypometabolism ([18F]FDG PET). Regional reductions in [11C]DED and [18F]FDG PET signals have been reported with regionally more advanced [11C]PIB PET in a longitudinal study involving patients with autosomal dominant MCI or AD. A regionally dynamic relationship between astroglial reactivity and A $\beta$  uptake has been reported, where increased A $\beta$  load in primary motor and sensory areas (in which A $\beta$  deposition occurs later) was associated with increased astroglial uptake.<sup>93</sup> These findings demonstrate the widespread heterogeneity in the astrocytes and their region-dependent association with A $\beta$  deposition and glucose hypometabolism. It appears that astroglial reactivity may be triggered by early A $\beta$ -deposition; however, the sustained pro-inflammatory astroglial reactivity with greater A $\beta$  deposition may lead to astroglial dystrophy and A $\beta$ -associated neuropathology. Future research should aim to dissect the exact determinants of [18F]FDG PET in longitudinal studies, with a renewed focus on particularly astrocytes in shaping glucose metabolism.

## 14 | THERAPEUTIC IMPLICATIONS

Astroglial modulation may be a viable therapeutic approach to consider in neurodegenerative diseases. We hypothesize that early on in neurodegenerative diseases, astrocytes become activated as part of a compensatory response to counteract the accruing disease pathology. However, this beneficial activation is impaired yielding a defective protective response. In later stages of the disease pathology, chronically activated astrocytes revert to a proinflammatory phenotype mounting an exaggerated detrimental response, further aggravating disease pathology. Understanding the role of astrocytes in the trajectory of neurodegenerative diseases allows us to intervene in multiple ways. Different treatments may be required to either (1) stimulate anti-inflammatory properties of astrocytes while suppressing the pro-inflammatory effects, and/or (2) targeting astroglial-mediated functions, depending on the stage of neurodegenerative disease.

### 14.1 | Modulating astroglial-mediated neuroinflammation

Astrocytes play important roles in maintaining neuronal functions by producing various cytokines, chemokines, and growth factors, which serve as molecular coordinators of neuron-glia interactions. At the sites of neuroinflammation, astrocyte-derived cytokines and chemokines play both neuroprotective and neurotoxic roles in brain lesions of human neurological diseases. Non-stimulated human astrocytes in vitro were found to express eight cytokines, including G-CSF, GM-CSF, GRO $\alpha$  (CXCL1), IL-6, IL-8 (CXCL8), MCP-1 (CCL2), MIF, and Serpin E1. Following stimulation with IL-1 $\beta$  and TNF- $\alpha$ , activated astrocytes newly produced IL-1 $\beta$ , IL-1ra, TNF- $\alpha$ , IP-10 (CXCL10), MIP-1 $\alpha$  (CCL3), and RANTES (CCL5), in addition to the induction of sICAM-1 and complement component 5.<sup>97</sup> Most of cytokines and chemokines



produced by non-stimulated and activated astrocytes are direct targets of the transcription factor NF- $\kappa$ B. Cultured human astrocytes express a distinct set of NF- $\kappa$ B-target cytokines and chemokines in resting and activated conditions, suggesting that the NF- $\kappa$ B signaling pathway differentially regulates gene expression of cytokines and chemokines in human astrocytes under physiological and inflammatory conditions. Although the precise mechanisms remain to be deciphered, A $\beta$ /NF- $\kappa$ B interaction in astrocytes may play an important role in AD pathophysiology.<sup>98,99</sup> Exposure to A $\beta$  activates astroglial NF- $\kappa$ B and C3 release. High levels of C3 expression are observed in brain tissue from AD patients and APP transgenic mice.<sup>100</sup> This pathway may provide a potential entry point for therapeutic intervention in AD because short-term C3a receptor inhibition rescued multiple cognitive deficits in APP transgenic mice.

Myeloid differentiation primary response 88 (MyD88), an adapter protein, participates in inflammatory responses driven by TLRs (except TLR3) and receptors of the IL-1 family of cytokines. While astrocytes express these receptors constitutively, their levels are upregulated in AD. MyD88 acts as a bridge between these receptors and IL-1 receptor-associated kinases, amplifying inflammatory signals via the activation of transcriptional factors NF- $\kappa$ B and activator protein-1.<sup>101</sup> TLRs and IL-1 family of cytokine signaling have been associated with AD pathogenesis, with A $\beta$  directly activating some of these receptors. Pharmacological inhibition of the interaction between TLR2 and MyD88 ameliorated inflammation and AD-like pathology in the 5XFAD mouse model.<sup>102</sup>

MyD88 are enriched in astrocytes near A $\beta$  plaques and neurofibrillary tau tangles. Deletion of astrocytic MyD88 in mice conferred protection against A $\beta$ -induced acute synaptic toxicity and cognitive impairment. Loss of astrocytic MyD88 ameliorated astrocytic reactivity, lowered levels of proteins involved in inflammation, and increased expression of synaptic-related proteins.<sup>103</sup> Since MyD88 play a pivotal role in the regulation of astrocytes in response to AD pathology, they may serve as a molecular marker of AD and an attractive therapeutic target.

### 14.1.1 | Therapeutic modulation of astrocytes by minocycline

Since astrocytes along with microglia and peripheral immune cells, function as key regulators of inflammation in the CNS, modulation of these cells may prevent pro-inflammatory responses.<sup>104</sup> Minocycline, an antibiotic with anti-inflammatory properties, reduced the number of activated astrocytes in the cortex of young human tau transgenic mice.<sup>105</sup> Minocycline treatment significantly reduced global levels of many pro-inflammatory factors: GM-CSF, I-309, eotaxin, IL-6, IL-10, M-CSF, MCP-1 (CCL2), MCP-5 (CCL12), and TREM-1.<sup>106</sup>

### 14.1.2 | Therapeutic modulation of astrocytes by non-steroidal anti-inflammatory drugs

Another drug class with potential to modulate inflammation in AD and induce intriguing effects on astrocyte function is non-steroidal

anti-inflammatory drugs (NSAIDs).<sup>107</sup> Reactive astrocytes neglect their neuronal supportive functions, thus rendering neurons vulnerable to neurotoxins including pro-inflammatory cytokines and ROS. NSAIDs may interact with astrocytes by modulating their activation and migration. A short-term treatment with ibuprofen of adult APP-transgenic mice resulted in a significant reduction in the number of reactive astrocytes in the hippocampus and cortex.<sup>108</sup> The effects of NSAIDs on number of reactive astrocytes may be due to its inhibitory effect on A $\beta$  deposition or a direct modulating effect on astrocytes. In rat primary cultures, ibuprofen produced profound stellation of astrocytes and altered their migration. In mouse and human astrocyte cell culture ibuprofen reduced  $\alpha$ 1-antichymotrypsin release induced by LPS or IL-1 $\beta$ .<sup>109</sup> Ibuprofen significantly reduced NMDA-induced neuronal cell death in mixed cortical cultures containing mice neuronal and glial cells but not in near-pure neuronal cultures containing less than 5% astrocytes. NSAIDs may protect against AD via direct and indirect interaction with reactive astrocytes.<sup>107</sup>

### 14.1.3 | Therapeutic modulation of astrocytes by $\gamma$ -secretase modulator

Moreover, chronic treatment with CHF5074 (a novel  $\gamma$ -secretase modulator) in Tg2576 mice increased localization of reactive astrocytes around A $\beta$  plaques.<sup>106,110</sup> These changes indicate cytoskeletal reorganization to promote migration to areas of injury, enabling increased astrocyte-mediated neuroprotection (i.e., phagocytosis of A $\beta$  plaques).

## 14.2 | Targeting astroglial-mediated functions

### 14.2.1 | Cell's intrinsic antioxidant system

No treatments exist that formally target astrocytes per se in neurodegenerative diseases. Clinical trials involving N-acetylcysteine demonstrated improved cognitive performance in a subset of patients with AD ( $n = 47$ ).<sup>111</sup> N-acetylcysteine is a precursor to the amino acid cysteine, which is astroglial derived and essential for neuronal glutathione synthesis, for maintenance of antioxidant status of cells to combat oxidative stress. The presence of astroglial cells has been demonstrated to increase the content of glutathione in neurons, which supports the notion of a metabolic interaction between astrocytes and neurons mediating glutathione metabolism. It has been suggested that astroglial cells generate the precursor of neuronal glutathione through their ectoenzyme  $\gamma$ -glutamyl transpeptidase from the glutathione that they release.

A randomized, double-blind, pilot evaluation of intravenous glutathione in PD was conducted ( $n = 21$ ).<sup>112</sup> Although glutathione was well tolerated, no clinical benefit for patients with PD was observed due to its low permeability across the BBB.<sup>112</sup> Zonisamide, an epileptic drug, was found to increase GSH production by astrocytes and reduce  $\alpha$ -synuclein neurotoxicity and protected dopaminergic neurons in a mouse model of PD.<sup>113</sup> Daily administration of zonisamide was found

to significantly improve motor function in patients with DLB and PD and was well tolerated in phase 3 clinical trials. Moreover, zonisamide improved wearing-off without increasing dyskinesia in patients with PD in phase 3 clinical trials.

The activation of astroglial serotonin 1A receptor by agonist 8-hydroxy-2-(dipropylamino)tetralin hydrobromide induced astroglial expression of the antioxidant metallothionein-1/2, which protected dopaminergic neurons in rodent models of parkinsonism.<sup>114</sup> One such serotonin 1A receptor agonist, pardoprunox, led to improved motor symptoms in patients with early PD.

FDA-approved ceftriaxone, a  $\beta$ -lactam antibiotic, was repositioned for use in ALS, as it increased astroglial expression of GLT-1 and induced neuroprotective action in ALS mouse model. In a combined phase 1, 2, and 3 trials, ceftriaxone significantly delayed functional decline in patients with ALS during stages 1 and 2. However, this ceftriaxone-mediated benefit was not translated in stage 3 and no differences were observed in patients receiving ceftriaxone ( $n = 156$ ) compared with placebo ( $n = 71$ ).<sup>115</sup>

### 14.2.2 | Astroglial metabolism

Liraglutide, a glucagon-like peptide-1 (GLP-1) analogue, which is currently approved for type 2 diabetes and obesity is being trialed in a phase 3 study in patients with mild AD.<sup>116,117</sup> GLP-1 is a glycoprotein excreted by gut entero-endocrine cells and exerts neuroprotective effects by reducing the levels of  $A\beta$  oligomers in transgenic models of AD, normalizing synaptic plasticity in APP/PS1 mice, and attenuating the decline in cerebral glucose metabolism in human AD, and increasing the proliferation of neuronal progenitor cells in APP/PS1 and littermate control mice. GLP-1 improves the supportive ability of astrocytes to neurons by promoting aerobic glycolysis in 5xFAD mice and astroglial cell models, and subsequently reducing brain oxidative phosphorylation levels and oxidative stress. GLP-1 alleviated the  $A\beta$ -induced glycolysis decreases in astrocytes resulting in reduced oxidative phosphorylation levels and ROS production. At behavioral level, GLP-1 mediated biochemical effects were observed in the form of improved cognitive performance. The mechanism underlying the neuroprotective effects of GLP-1 was activation of PI3K/Akt pathway, intimately involved in energy metabolism and cellular survival. The increased astroglial glycolysis ameliorated astroglial support of neurons and promoted neuronal survival and axonal growth. The modulation of the energetic phenotype of astrocytes may be a promising therapeutic target and could prove to be an effective way of delaying the progression of AD.

### 14.2.3 | Astroglial calcium signaling

Intracellular calcium ion homeostasis is essential for neuronal function, and impairment of calcium homeostasis might be a major mechanism through which  $A\beta$  and tau exert their neurotoxicity. The voltage-gated calcium channels allow calcium influx following neuronal depolariza-

tion and receptor-operated calcium channels (e.g., NMDA receptors). Treating calcium hikes represents an important therapeutic target. Toxic  $A\beta$  augments cytosolic calcium that may affect enzymes including proteases or phosphatases, resulting in cytoskeletal modifications, free radical production, and apoptosis.  $A\beta$  may cause excessive activation of calcium channels and form pores in the cytosolic plasma membrane. This allows a substantial influx of calcium ions from the extracellular space into the cytosol, impairing cellular function.  $A\beta$  has been found to potentiate calcium influx through the voltage gated calcium channels, particularly the L-type. Nimodipine is an L-type voltage gated calcium channel inhibitor that was able to block excessive calcium influx in  $A\beta$ -treated cultured neurons.<sup>118</sup> However, this phenomenon was not observed in brain slices from mouse models of AD.<sup>119</sup> In a NILD-VARD multicentre trial, L-type voltage gated calcium channel inhibitor attenuated  $A\beta$  levels but failed to ameliorate cognitive decline.<sup>120</sup> Tau aggregates increase cytosolic calcium and ROS generation via NADPH. This effect was prevented by nifedipine and verapamil, both L-type voltage gated calcium channel inhibitors. Tau fibrils activate voltage gated calcium channels leading to neuronal dysfunction.<sup>121</sup>

NMDA receptors are permeable to calcium and often implicated in neuronal pathophysiology.<sup>118</sup> Memantine is an approved NMDA receptor antagonist for the treatment of AD, which provides modest memory and cognitive improvements in moderate to severe AD. Memantine works by restricting excessive calcium influx, reducing neuronal excitability while preserving basal NMDA receptor function. Memantine exerts neuroprotective effects against oxidative stress, neuroinflammation, and tau phosphorylation. Galantamine, rivastigmine, and donepezil are the anti-cholinesterase approved drugs to treat AD. These treatments work by increasing acetylcholine levels, which delay progression of AD through calcium-dependent mechanisms.<sup>118</sup> In addition, anti-cholinesterase drugs administered with memantine may provide improved behavioral, cognitive, and global outcomes in patients affected with AD. Aducanumab is a controversial FDA approved treatment for MCI due to AD and mild AD in US, which selectively binds to aggregated  $A\beta$  fibrils and soluble oligomers (and not monomers) and was found to ameliorate calcium dysregulation in AD.<sup>122</sup> Systemic administration of aducanumab rescued calcium overload in neurons.

Mitochondria buffer calcium and regulate calcium levels required for synaptic functioning.<sup>123</sup> Mitochondrial calcium uptake activates some dehydrogenases at the electron transport chain, activating mitochondrial respiration and ATP production. Lysosomes are acidic organelles that participate in the endolysosomal system. They are important for autophagy and intracellular calcium storage (with comparable calcium levels to those of the ER). Lysosomal calcium efflux has been linked to changes in lysosomal pH.<sup>124</sup> Impaired calcium signaling due to altered mitochondrial and lysosomal functioning is observed in AD and represent attractive therapeutic options. Mitochondria-targeted protective compounds have shown favorable outcomes in AD. These include antioxidants vitamin E and C, coenzyme Q10, mitoQ, and phenylpropanoids including resveratrol, quercetin, and curcumin.<sup>118</sup> The Szeto-Schiller (SS) tetrapeptides, an alternative type of antioxidants that target mitochondria, have been tried.<sup>125</sup> These are small

molecules that can reach the mitochondrial matrix and act as antioxidants. SS31 combined with mitochondrial division inhibitor 1 showed favorable results in vitro suggesting warranting further exploration of mitochondria-targeted antioxidants in AD.<sup>126</sup>

Astrocytes do not generate action potentials but exhibit transient fluctuations in calcium followed by release of gliotransmitters in response to neurotransmitters.<sup>118</sup> Since astrocytes are the most abundant cells and ensure neuronal homeostasis, targeting calcium signaling is likely to modulate astroglial responses with potentially beneficial outcomes in AD. Reactive astrocytes in the vicinity A $\beta$  plaques express augmented levels of mGluR5, which induces calcium release from the intracellular stores. Astrocytes exposed to A $\beta$  in vitro lead to increased basal intracellular calcium levels owing to extracellular calcium entry, release from mGluR5 and IP<sub>3</sub>R, and induced calcium oscillations.<sup>127</sup> Pharmacological inhibition of ER calcium release inhibits A $\beta$ -induced astrogliosis, suggestive of aberrant astrocytic calcium causing neurotoxicity.<sup>128</sup> APOE $\epsilon$ 4 has been found to dysregulate calcium excitability in astrocytes by altering membrane lipid composition.<sup>129</sup> In primary cortical co-cultures of neurons and astrocytes, astrocytes exposed to insoluble tau aggregates failed to elicit a calcium response.<sup>121</sup> Further research is required to investigate the effects of tau on astroglial calcium, which remains an area of research in its infancy.

In physiological conditions, astrocytes demonstrate sporadic calcium ion transients as a signature of astroglial activity.<sup>130</sup> In the presence of A $\beta$ , astrocytes exhibit higher resting calcium levels particularly in the areas of A $\beta$  deposits.<sup>131</sup> Future studies are warranted to determine the exact A $\beta$  species mediating the calcium dyshomeostasis. Calcium hyperactivity in astrocytes is associated with abnormal purinergic signaling, perhaps owing to the excessive amounts of ATP released by reactive astrocytes. This subsequently activates P2Y purinoceptors mediating the cytosolic calcium signalling.<sup>132</sup> Altered extracellular calcium levels have been implicated in astroglial hyperactivity. During augmented neuronal activity, extracellular calcium decreases following activation of ionotropic glutamate receptor and voltage gated calcium channels. Astrocytes sense and release ATP in the extracellular medium.<sup>133</sup> The elevated levels of extracellular ATP trigger astroglial calcium transients and may contribute to AD-associated astroglial hyperactivity. This further underscores the importance of astrocytes as therapeutic targets in AD.

Calcineurin is a calcium/calmodulin-dependent protein phosphatase with high abundance in the CNS. Calcineurin can become strongly induced in subsets of activated astrocytes under different pathological conditions where it interacts extensively with the NFATs. Blockade of the astrocytic CN/NFAT pathway in a rat model of TBI using adeno-associated virus (AAV) vectors expressing the astrocyte-specific promoter Gfa2 and the NFAT-inhibitory peptide VIVIT ameliorated hippocampal synaptic functioning and plasticity.<sup>59</sup> VIVIT treatment in 5xFAD mice led to increased expression of the astrocytic glutamate transporter GLT-1 and to attenuated changes in dendrite morphology, synaptic strength, and NMDAR-dependent responses.<sup>134</sup> Acute treatment of Tg2576 mice with the calcineurin inhibitor FK506 improved memory function.<sup>135</sup> The rationale for the

clinical development of calcineurin inhibition as a viable treatment for AD emerges from the real-world showing individuals treated with calcineurin inhibitors have a lower incidence of AD compared with the general population.<sup>136,137</sup>

Astrocytes can sense numerous extracellular signals including cytokines, growth factors, nucleotides, endothelins, or ephrins, that activate various intracellular signaling pathways, such as the mitogen-activated protein kinase, NF- $\kappa$ B, and the Janus kinase/signal transducer and activator of transcription (JAK/STAT) pathways. The JAK/STAT3 pathway is likely a central player in the induction of astrocyte reactivity and is activated by cytokines and growth factors that signal through the gp130 receptor. Activation of the JAK/STAT3 pathway has been observed in reactive astrocytes in several conditions of acute injury and in patients, mouse models of ALS, and in several mouse and nonhuman primate models of AD and HD. Overexpression of suppressor of cytokine signaling 3, the endogenous inhibitor of the JAK/STAT3 pathway in astrocytes in vivo, inhibited this pathway and prevented astrocyte reactivity. JAK/STAT3 inhibitor decreased microglial activation and increased the formation of mutant huntingtin (Htt) aggregates but did not affect neuronal death.<sup>138</sup> Using cell type-specific approaches in vivo, JAK2-STAT3 pathway was necessary and sufficient for the induction and maintenance of astrocyte reactivity in mouse models of AD. Modulation of JAK2-STAT3 pathway by viral gene transfer in mouse astrocytes controlled several morphological and molecular features of reactivity, resulting in reduced A $\beta$  deposition, improved spatial learning, and restoration of synaptic deficits.<sup>139</sup> JAK2-STAT3 cascade appears to be a master regulator of astrocyte reactivity and its modulation offers new therapeutic venues for neurodegenerative diseases including AD.

The current evidence discussed generally supports a role for astrocytes throughout the neurodegenerative disease continuum (Figure 3). In the normal homeostatic stage there is an equilibrium between the pro and anti-inflammatory phenotypes of astrocytes. Accumulation of proteins thought to be toxic, for example, A $\beta$ ,  $\alpha$ -synuclein, tau may provide the stimulus for astroglial activation. In the prodromal stage, reactive and neuroprotective phenotypes for astrocytes, may be observed with a shift in equilibrium towards an initial increase in anti-inflammatory cytokines. With the advancing pathology as observed in neurodegenerative diseases, astroglial dysregulation results in loss of the neuroprotective function and a gain of toxic function by astrocytes resulting an increase in proinflammatory cytokines. Therapeutic modulation to promote the astroglial anti-inflammatory phenotype may be required during the prodromal stages of the disease, while drugs suppressing the astroglial pro-inflammatory phenotype may be required towards later stages of the disease.

## 15 | CONCLUSION

The evidence reviewed suggests that astroglial-mediated inflammation demonstrate both beneficial and detrimental properties. Classification of A1 and A2 is over simplistic as their function extends beyond pro- and anti-inflammatory properties. The astrocytes are

heterogeneous and dynamic, and their activation varies based on different pathological contexts and during disease trajectory. The astroglial response is dependent on individual susceptibility and influenced by prior astroglial priming. Selective modulation (promotion) of anti-inflammatory phenotype in the early stage and selective modulation (inhibition) of proinflammatory phenotype may need to be deployed in the early and late-stage neurodegenerative diseases. This may have to be in combination with strategies targeting neuroinflammation in addition to inhibiting the aggregation and pathogenicity of  $\beta$ -pleated sheet structures.

## ACKNOWLEDGMENTS

PE was funded by the Medical Research Council and now by Higher Education Funding Council for England (HEFCE). He has also received grants from Alzheimer's Research, UK, Alzheimer's Drug Discovery Foundation, Alzheimer's Society, UK, Alzheimer's association, US, Medical Research Council, UK, Novo Nordisk, Piramal Life Sciences and GE Healthcare. P.E. is a consultant to Roche, Pfizer and Novo Nordisk. He has received speaker fees from Novo Nordisk, Pfizer, Nordea, Piramal Life Science. He has received educational and research grants from GE Healthcare, Novo Nordisk, Piramal Life Science/Life Molecular Imaging, Avid Radiopharmaceuticals and Eli Lilly. He is an external consultant to Novo Nordisk and Cytodyn and is a member of their Scientific Advisory Board. There was no specific grant funding for this manuscript.

## CONFLICT OF INTEREST STATEMENT

None declared. Author disclosures are available in the [supporting information](#).

## ORCID

Paul Edison  <https://orcid.org/0000-0002-6551-2002>

## REFERENCES

- Habib N, McCabe C, Medina S, et al. Disease-associated astrocytes in Alzheimer's disease and aging. *Nat Neurosci*. 2020;23:701-706.
- Terry RD, Masliah E, Salmon DP, et al. Physical basis of cognitive alterations in Alzheimer's disease: synapse loss is the major correlate of cognitive impairment. *Ann Neurol*. 1991;30:572-580.
- Magavi S, Friedmann D, Banks G, Stolfi A, Lois C. Coincident generation of pyramidal neurons and protoplasmic astrocytes in neocortical columns. *J Neurosci*. 2012;32:4762-4772.
- Oberheim NA, Takano T, Han X, et al. Uniquely hominid features of adult human astrocytes. *J Neurosci*. 2009;29:3276-3287.
- Middeldorp J, Hol EM. GFAP in health and disease. *Prog Neurobiol*. 2011;93:421-443.
- Chai H, Diaz-Castro B, Shigetomi E, et al. Neural circuit-specialized astrocytes: transcriptomic, proteomic, morphological, and functional evidence. *Neuron*. 2017;95:531-549 e9.
- D'Ambrosio R, Wenzel J, Schwartzkroin PA, McKhann GM 2nd, Janigro D. Functional specialization and topographic segregation of hippocampal astrocytes. *J Neurosci*. 1998;18:4425-4438.
- Azevedo FA, Carvalho LR, Grinberg LT, et al. Equal numbers of neuronal and nonneuronal cells make the human brain an isometrically scaled-up primate brain. *J Comp Neurol*. 2009;513:532-541.
- Pelvig DP, Pakkenberg H, Stark AK, Pakkenberg B. Neocortical glial cell numbers in human brains. *Neurobiol Aging*. 2008;29:1754-1762.
- Pelvig DP, Pakkenberg H, Regeur L, Oster S, Pakkenberg B. Neocortical glial cell numbers in Alzheimer's disease. A stereological study. *Dement Geriatr Cogn Disord*. 2003;16:212-219.
- Verkhatsky A, Nedergaard M. Physiology of astroglia. *Physiol Rev*. 2018;98:239-389.
- Blinkow S, Glezer I. The neuroglia. In: Blinkow S, Glezer I, eds. *The Human Brain in Figures and Tables; A Quantitative Handbook*. Plenum Press; 1968.
- Allen NJ, Eroglu C. Cell biology of astrocyte-synapse interactions. *Neuron*. 2017;96:697-708.
- Wang Y, Fu WY, Cheung K, et al. Astrocyte-secreted IL-33 mediates homeostatic synaptic plasticity in the adult hippocampus. *Proc Natl Acad Sci U S A*. 2021;118(1):e2020810118.
- Parpura V, Basarsky TA, Liu F, Jęftinija K, Jęftinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature*. 1994;369:744-747.
- Ullian EM, Sapperstein SK, Christopherson KS, Barres BA. Control of synapse number by glia. *Science*. 2001;291:657-661.
- Haber M, Zhou L, Murai KK. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. *J Neurosci*. 2006;26:8881-8891.
- Liu CY, Yang Y, Ju WN, Wang X, Zhang HL. Emerging roles of astrocytes in neuro-vascular unit and the tripartite synapse with emphasis on reactive gliosis in the context of Alzheimer's disease. *Front Cell Neurosci*. 2018;12:193.
- Dani JW, Chernjavsky A, Smith SJ. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron*. 1992;8:429-440.
- Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science*. 1994;263:1768-1771.
- Araque A, Sanzgiri RP, Parpura V, Haydon PG. Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *J Neurosci*. 1998;18:6822-6829.
- Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron*. 2004;43:729-743.
- Rusakov DA. Disentangling calcium-driven astrocyte physiology. *Nat Rev Neurosci*. 2015;16:226-233.
- Nett WJ, Oloff SH, McCarthy KD. Hippocampal astrocytes in situ exhibit calcium oscillations that occur independent of neuronal activity. *J Neurophysiol*. 2002;87:528-537.
- Arnth-Jensen N, Jabaudon D, Scanziani M. Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nat Neurosci*. 2002;5:325-331.
- Halassa MM, Haydon PG. Integrated brain circuits: astrocytic networks modulate neuronal activity and behavior. *Annu Rev Physiol*. 2010;72:335-355.
- Sofroniew MV, Vinters HV. Astrocytes: biology and pathology. *Acta Neuropathol*. 2010;119:7-35.
- Schousboe A, Sarup A, Bak LK, Waagepetersen HS, Larsson OM. Role of astrocytic transport processes in glutamatergic and GABAergic neurotransmission. *Neurochem Int*. 2004;45:521-527.
- Chen Y, Vartiainen NE, Ying W, Chan PH, Koistinaho J, Swanson RA. Astrocytes protect neurons from nitric oxide toxicity by a glutathione-dependent mechanism. *J Neurochem*. 2001;77:1601-1610.
- Shih AY, Johnson DA, Wong G, et al. Coordinate regulation of glutathione biosynthesis and release by Nrf2-expressing glia potently protects neurons from oxidative stress. *J Neurosci*. 2003;23:3394-3406.
- Theparambil SM, Hosford PS, Ruminot I, et al. Astrocytes regulate brain extracellular pH via a neuronal activity-dependent bicarbonate shuttle. *Nat Commun*. 2020;11:5073.
- Pfriegeer FW, Ungerer N. Cholesterol metabolism in neurons and astrocytes. *Prog Lipid Res*. 2011;50:357-371.



33. Mulica P, Grunewald A, Pereira SL. Astrocyte-neuron metabolic crosstalk in neurodegeneration: a mitochondrial perspective. *Front Endocrinol (Lausanne)*. 2021;12:668517.
34. Diemel GA, Rothman DL. Reevaluation of astrocyte-neuron energy metabolism with astrocyte volume fraction correction: impact on cellular glucose oxidation rates, glutamate-glutamine cycle energetics, glycogen levels and utilization rates vs. exercising muscle, and Na(+)/K(+) pumping rates. *Neurochem Res*. 2020;45:2607-2630.
35. Belanger M, Allaman I, Magistretti PJ. Brain energy metabolism: focus on astrocyte-neuron metabolic cooperation. *Cell Metab*. 2011;14:724-738.
36. Beard E, Lengacher S, Dias S, Magistretti PJ, Finsterwald C. Astrocytes as key regulators of brain energy metabolism: new therapeutic perspectives. *Front Physiol*. 2021;12:825816.
37. Muller MS, Fouyssac M, Taylor CW. Effective glucose uptake by human astrocytes requires its sequestration in the endoplasmic reticulum by glucose-6-phosphatase-beta. *Curr Biol*. 2018;28:3481-3486 e4.
38. Pellerin L, Magistretti PJ. Glutamate uptake into astrocytes stimulates aerobic glycolysis: a mechanism coupling neuronal activity to glucose utilization. *Proc Natl Acad Sci U S A*. 1994;91:10625-10629.
39. Magistretti PJ, Chatton JY. Relationship between L-glutamate-regulated intracellular Na+ dynamics and ATP hydrolysis in astrocytes. *J Neural Transm (Vienna)*. 2005;112:77-85.
40. Bernardinelli Y, Magistretti PJ, Chatton JY. Astrocytes generate Na+-mediated metabolic waves. *Proc Natl Acad Sci U S A*. 2004;101:14937-14942.
41. Fox PT, Raichle ME, Mintun MA, Dence C. Nonoxidative glucose consumption during focal physiologic neural activity. *Science*. 1988;241:462-464.
42. Marcus C, Mena E, Subramaniam RM. Brain PET in the diagnosis of Alzheimer's disease. *Clin Nucl Med*. 2014;39:e413-e422. quiz e23-6.
43. Camandola S, Mattson MP. Brain metabolism in health, aging, and neurodegeneration. *EMBO J*. 2017;36:1474-1492.
44. Vlassenko AG, Gordon BA, Goyal MS, et al. Aerobic glycolysis and tau deposition in preclinical Alzheimer's disease. *Neurobiol Aging*. 2018;67:95-98.
45. Rahman B, Kussmaul L, Hamprecht B, Dringen R. Glycogen is mobilized during the disposal of peroxides by cultured astroglial cells from rat brain. *Neurosci Lett*. 2000;290:169-172.
46. Suzuki A, Stern SA, Bozdagi O, et al. Astrocyte-neuron lactate transport is required for long-term memory formation. *Cell*. 2011;144:810-823.
47. Bak LK, Walls AB, Schousboe A, Waagepetersen HS. Astrocytic glycogen metabolism in the healthy and diseased brain. *J Biol Chem*. 2018;293:7108-7116.
48. Morita M, Ikeshima-Kataoka H, Kreft M, Vardjan N, Zorec R, Noda M. Metabolic plasticity of astrocytes and aging of the brain. *Int J Mol Sci*. 2019;20(4):941.
49. Stewart VC, Land JM, Clark JB, Heales SJ. Comparison of mitochondrial respiratory chain enzyme activities in rodent astrocytes and neurones and a human astrocytoma cell line. *Neurosci Lett*. 1998;247:201-203.
50. Lopez-Fabuel I, Le Douce J, Logan A, et al. Complex I assembly into supercomplexes determines differential mitochondrial ROS production in neurons and astrocytes. *Proc Natl Acad Sci U S A*. 2016;113:13063-13068.
51. Verkhatsky A. Astroglial calcium signaling in aging and Alzheimer's disease. *Cold Spring Harb Perspect Biol*. 2019;11(7):a035188.
52. Verkhatsky A, Untiet V, Rose CR. Ionic signalling in astroglia beyond calcium. *J Physiol*. 2020;598:1655-1670.
53. Hachinski V, Einhaupl K, Ganten D, et al. Preventing dementia by preventing stroke: the Berlin Manifesto. *Alzheimers Dement*. 2019;15:961-984.
54. Landfield PW. Increased calcium-current' hypothesis of brain aging. *Neurobiol Aging*. 1987;8:346-347.
55. Khachaturian ZS. The role of calcium regulation in brain aging: reexamination of a hypothesis. *Aging (Milano)*. 1989;1:17-34.
56. Mishra A, Reynolds JP, Chen Y, Gourine AV, Rusakov DA, Attwell D. Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. *Nat Neurosci*. 2016;19:1619-1627.
57. Institoris A, Vandal M, Peringod G, et al. Astrocytes amplify neurovascular coupling to sustained activation of neocortex in awake mice. *Nat Commun*. 2022;13:7872.
58. Sompol P, Gollihue JL, Weiss BE, et al. Targeting astrocyte signaling alleviates cerebrovascular and synaptic function deficits in a diet-based mouse model of small cerebral vessel disease. *J Neurosci*. 2023;43:1797-1813.
59. Furman JL, Sompol P, Kraner SD, et al. Blockade of astrocytic calcineurin/NFAT signaling helps to normalize hippocampal synaptic function and plasticity in a rat model of traumatic brain injury. *J Neurosci*. 2016;36:1502-1515.
60. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci*. 2006;7:41-53.
61. Rasmussen MK, Mestre H, Nedergaard M. The glymphatic pathway in neurological disorders. *Lancet Neurol*. 2018;17:1016-1024.
62. Iadecola C. The neurovascular unit coming of age: a journey through neurovascular coupling in health and disease. *Neuron*. 2017;96:17-42.
63. Love S, Miners JS. Cerebrovascular disease in ageing and Alzheimer's disease. *Acta Neuropathol*. 2016;131:645-658.
64. Kisler K, Nelson AR, Montagne A, Zlokovic BV. Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease. *Nat Rev Neurosci*. 2017;18:419-434.
65. Zonta M, Angulo MC, Gobbo S, et al. Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. *Nat Neurosci*. 2003;6:43-50.
66. MacVicar BA, Newman EA. Astrocyte regulation of blood flow in the brain. *Cold Spring Harb Perspect Biol*. 2015;7(5):a020388.
67. Carmignoto G, Gomez-Gonzalo M. The contribution of astrocyte signalling to neurovascular coupling. *Brain Res Rev*. 2010;63:138-148.
68. Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G. Apolipoprotein E and Alzheimer disease: risk, mechanisms and therapy. *Nat Rev Neurol*. 2013;9:106-118.
69. Carvey PM, Zhao CH, Hendey B, et al. 6-Hydroxydopamine-induced alterations in blood-brain barrier permeability. *Eur J Neurosci*. 2005;22:1158-1168.
70. Reeves BC, Karimy JK, Kundishora AJ, et al. Glymphatic system impairment in Alzheimer's disease and idiopathic normal pressure hydrocephalus. *Trends Mol Med*. 2020;26:285-295.
71. Liddelow SA, Guttenplan KA, Clarke LE, et al. Neurotoxic reactive astrocytes are induced by activated microglia. *Nature*. 2017;541:481-487.
72. Zamanian JL, Xu L, Foo LC, et al. Genomic analysis of reactive astrogliosis. *J Neurosci*. 2012;32:6391-6410.
73. Lau SF, Cao H, Fu AKY, Ip NY. Single-nucleus transcriptome analysis reveals dysregulation of angiogenic endothelial cells and neuroprotective glia in Alzheimer's disease. *Proc Natl Acad Sci U S A*. 2020;117:25800-25809.
74. Zhao J, O'Connor T, Vassar R. The contribution of activated astrocytes to Abeta production: implications for Alzheimer's disease pathogenesis. *J Neuroinflammation*. 2011;8:150.
75. Wang W, Hou TT, Jia LF, Wu QQ, Quan MN, Jia JP. Toxic amyloid-beta oligomers induced self-replication in astrocytes triggering neuronal injury. *EBioMedicine*. 2019;42:174-187.
76. Gomez-Arboledas A, Davila JC, Sanchez-Mejias E, et al. Phagocytic clearance of presynaptic dystrophies by reactive astrocytes in Alzheimer's disease. *Glia*. 2018;66:637-653.



77. Liu CC, Hu J, Zhao N, et al. Astrocytic LRP1 mediates brain abeta clearance and impacts amyloid deposition. *J Neurosci*. 2017;37:4023-4031.
78. Prasad H, Rao R. Amyloid clearance defect in ApoE4 astrocytes is reversed by epigenetic correction of endosomal pH. *Proc Natl Acad Sci U S A*. 2018;115:E6640-E6649.
79. DeMattos RB, Cirrito JR, Parsadanian M, et al. ApoE and clusterin cooperatively suppress Abeta levels and deposition: evidence that ApoE regulates extracellular Abeta metabolism in vivo. *Neuron*. 2004;41:193-202.
80. Ferrer I, Zelaya MV, Aguilo Garcia M, et al. Relevance of host tau in tau seeding and spreading in tauopathies. *Brain Pathol*. 2020;30:298-318.
81. Acioglu C, Li L, Elkabes S. Contribution of astrocytes to neuropathology of neurodegenerative diseases. *Brain Res*. 2021;1758:147291.
82. McAlpine CS, Park J, Griuciu A, et al. Astrocytic interleukin-3 programs microglia and limits Alzheimer's disease. *Nature*. 2021;595(7869):701-706.
83. Lee HJ, Suk JE, Patrick C, et al. Direct transfer of alpha-synuclein from neuron to astroglia causes inflammatory responses in synucleinopathies. *J Biol Chem*. 2010;285:9262-9272.
84. Agarwal D, Sandor C, Volpato V, et al. A single-cell atlas of the human substantia nigra reveals cell-specific pathways associated with neurological disorders. *Nat Commun*. 2020;11:4183.
85. Hayashi S, Wakabayashi K, Ishikawa A, et al. An autopsy case of autosomal-recessive juvenile parkinsonism with a homozygous exon 4 deletion in the parkin gene. *Mov Disord*. 2000;15:884-888.
86. Fujita A, Yamaguchi H, Yamasaki R, et al. Connexin 30 deficiency attenuates A2 astrocyte responses and induces severe neurodegeneration in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine hydrochloride Parkinson's disease animal model. *J Neuroinflammation*. 2018;15:227.
87. Escartin C, Galea E, Lakatos A, et al. Reactive astrocyte nomenclature, definitions, and future directions. *Nat Neurosci*. 2021;24:312-325.
88. Narayanaswami V, Dahl K, Bernard-Gauthier V, Josephson L, Cumming P, Vasdev N. Emerging PET radiotracers and targets for imaging of neuroinflammation in neurodegenerative diseases: outlook beyond TSPO. *Mol Imaging*. 2018;17:1536012118792317.
89. Carter SF, Chiotis K, Nordberg A, Rodriguez-Vieitez E. Longitudinal association between astrocyte function and glucose metabolism in autosomal dominant Alzheimer's disease. *Eur J Nucl Med Mol Imaging*. 2019;46:348-356.
90. Chio A, Pagani M, Agosta F, Calvo A, Cistaro A, Filippi M. Neuroimaging in amyotrophic lateral sclerosis: insights into structural and functional changes. *Lancet Neurol*. 2014;13:1228-1240.
91. Marutle A, Gillberg PG, Bergfors A, et al. (3)H-deprenyl and (3)H-PIB autoradiography show different laminar distributions of astroglia and fibrillar beta-amyloid in Alzheimer brain. *J Neuroinflammation*. 2013;10:90.
92. Calsolaro V, Matthews PM, Donat CK, et al. Astrocyte reactivity with late-onset cognitive impairment assessed in vivo using (11)C-BU99008 PET and its relationship with amyloid load. *Mol Psychiatry*. 2021;26(10):5848-5855.
93. Livingston NR, Calsolaro V, Hinz R, et al. Relationship between astrocyte reactivity, using novel (11)C-BU99008 PET, and glucose metabolism, grey matter volume and amyloid load in cognitively impaired individuals. *Mol Psychiatry*. 2022;27(4):2019-2029.
94. Wilson H, Dervenoulas G, Pagano G, et al. Imidazoline 2 binding sites reflecting astroglia pathology in Parkinson's disease: an in vivo (11)C-BU99008 PET study. *Brain*. 2019;142:3116-3128.
95. Magistretti PJ, Pellerin L. The contribution of astrocytes to the 18F-2-deoxyglucose signal in PET activation studies. *Mol Psychiatry*. 1996;1:445-452.
96. Zimmer ER, Parent MJ, Souza DG, et al. [(18)F]FDG PET signal is driven by astroglial glutamate transport. *Nat Neurosci*. 2017;20:393-395.
97. Choi SS, Lee HJ, Lim I, Satoh J, Kim SU. Human astrocytes: secretome profiles of cytokines and chemokines. *PLoS One*. 2014;9:e92325.
98. Shi ZM, Han YW, Han XH, et al. Upstream regulators and downstream effectors of NF-kappaB in Alzheimer's disease. *J Neurol Sci*. 2016;366:127-134.
99. Gonzalez-Reyes RE, Nava-Mesa MO, Vargas-Sanchez K, Ariza-Salamanca D, Mora-Munoz L. Involvement of astrocytes in Alzheimer's disease from a neuroinflammatory and oxidative stress perspective. *Front Mol Neurosci*. 2017;10:427.
100. Lian H, Yang L, Cole A, et al. NFkappaB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's disease. *Neuron*. 2015;85:101-115.
101. Deguine J, Barton GM. MyD88: a central player in innate immune signaling. *F1000Prime Rep*. 2014;6:97.
102. Rangasamy SB, Jana M, Roy A, et al. Selective disruption of TLR2-MyD88 interaction inhibits inflammation and attenuates Alzheimer's pathology. *J Clin Invest*. 2018;128:4297-4312.
103. Jong Huat T, Onraet T, Camats-Perna J, et al. Deletion of MyD88 in astrocytes prevents beta-amyloid-induced neuropathology in mice. *Glia*. 2023;71:431-449.
104. Anderson MA, Burda JE, Ren Y, et al. Astrocyte scar formation aids central nervous system axon regeneration. *Nature*. 2016;532:195-200.
105. Garwood CJ, Cooper JD, Hanger DP, Noble W. Anti-inflammatory impact of minocycline in a mouse model of tauopathy. *Front Psychiatry*. 2010;1:136.
106. Sadick JS, Liddelow SA. Don't forget astrocytes when targeting Alzheimer's disease. *Br J Pharmacol*. 2019;176:3585-3598.
107. Imbimbo BP, Solfrizzi V, Panza F. Are NSAIDs useful to treat Alzheimer's disease or mild cognitive impairment? *Front Aging Neurosci*. 2010;2:19.
108. Heneka MT, Sastre M, Dumitrescu-Ozimek L, et al. Acute treatment with the PPARgamma agonist pioglitazone and ibuprofen reduces glial inflammation and Abeta1-42 levels in APPV7171 transgenic mice. *Brain*. 2005;128:1442-1453.
109. Morigara T, Teter B, Yang F, et al. Ibuprofen suppresses interleukin-1beta induction of pro-amyloidogenic alpha1-antichymotrypsin to ameliorate beta-amyloid (Abeta) pathology in Alzheimer's models. *Neuropsychopharmacology*. 2005;30:1111-1120.
110. Imbimbo BP, Giardino L, Sivilia S, et al. CHF5074, a novel gamma-secretase modulator, restores hippocampal neurogenesis potential and reverses contextual memory deficit in a transgenic mouse model of Alzheimer's disease. *J Alzheimers Dis*. 2010;20:159-173.
111. Adair JC, Knoefel JE, Morgan N. Controlled trial of N-acetylcysteine for patients with probable Alzheimer's disease. *Neurology*. 2001;57:1515-1517.
112. Hauser RA, Lyons KE, McClain T, Carter S, Perlmutter D. Randomized, double-blind, pilot evaluation of intravenous glutathione in Parkinson's disease. *Mov Disord*. 2009;24:979-983.
113. Asanuma M, Miyazaki I, Diaz-Corrales FJ, et al. Neuroprotective effects of zonisamide target astrocyte. *Ann Neurol*. 2010;67:239-249.
114. Miyazaki I, Asanuma M, Murakami S, et al. Targeting 5-HT(1A) receptors in astrocytes to protect dopaminergic neurons in Parkinsonian models. *Neurobiol Dis*. 2013;59:244-256.
115. Cudkovic ME, Titus S, Kearney M, et al. Safety and efficacy of ceftriaxone for amyotrophic lateral sclerosis: a multi-stage, randomised, double-blind, placebo-controlled trial. *Lancet Neurol*. 2014;13:1083-1091.
116. Femminella GD, Frangou E, Love SB, et al. Evaluating the effects of the novel GLP-1 analogue liraglutide in Alzheimer's disease: study protocol for a randomised controlled trial (ELAD study). *Trials*. 2019;20:191.

117. Davies MJ, Bergenstal R, Bode B, et al. Efficacy of liraglutide for weight loss among patients with type 2 diabetes: the SCALE diabetes randomized clinical trial. *JAMA*. 2015;314:687-699.
118. Calvo-Rodriguez M, Kharitonova EK, Bacskai BJ. Therapeutic strategies to target calcium dysregulation in Alzheimer's. *Disease Cells*. 2020;9(11):2513.
119. Stutzmann GE, Smith I, Caccamo A, Oddo S, Laferla FM, Parker I. Enhanced ryanodine receptor recruitment contributes to Ca<sup>2+</sup> disruptions in young, adult, and aged Alzheimer's disease mice. *J Neurosci*. 2006;26:5180-5189.
120. Lawlor B, Segurado R, Kennelly S, et al. Nilvadipine in mild to moderate Alzheimer disease: a randomised controlled trial. *PLoS Med*. 2018;15:e1002660.
121. Esteras N, Kundel F, Amodeo GF, Pavlov EV, Klenerman D, Abramov AY. Insoluble tau aggregates induce neuronal death through modification of membrane ion conductance, activation of voltage-gated calcium channels and NADPH oxidase. *FEBS J*. 2021;288:127-141.
122. Kastanenka KV, Bussiere T, Shakerdge N, et al. Immunotherapy with Aducanumab Restores Calcium Homeostasis in Tg2576 Mice. *J Neurosci*. 2016;36:12549-12558.
123. Billups B, Forsythe ID. Presynaptic mitochondrial calcium sequestration influences transmission at mammalian central synapses. *J Neurosci*. 2002;22:5840-5847.
124. Lloyd-Evans E, Platt FM. Lysosomal Ca(2+) homeostasis: role in pathogenesis of lysosomal storage diseases. *Cell Calcium*. 2011;50:200-205.
125. Birk AV, Chao WM, Bracken C, Warren JD, Szeto HH. Targeting mitochondrial cardiolipin and the cytochrome c/cardiolipin complex to promote electron transport and optimize mitochondrial ATP synthesis. *Br J Pharmacol*. 2014;171:2017-2028.
126. Reddy PH, Manczak M, Yin X, Reddy AP. Synergistic protective effects of mitochondrial division inhibitor 1 and mitochondria-targeted small peptide SS31 in Alzheimer's disease. *J Alzheimers Dis*. 2018;62:1549-1565.
127. Lim D, Iyer A, Ronco V, et al. Amyloid beta deregulates astroglial mGluR5-mediated calcium signaling via calcineurin and Nf-kB. *Glia*. 2013;61:1134-1145.
128. Alberdi E, Wyssenbach A, Alberdi M, et al. Ca(2+) -dependent endoplasmic reticulum stress correlates with astrogliosis in oligomeric amyloid beta-treated astrocytes and in a model of Alzheimer's disease. *Aging Cell*. 2013;12:292-302.
129. Larramona-Arcas R, Gonzalez-Arias C, Perea G, et al. Sex-dependent calcium hyperactivity due to lysosomal-related dysfunction in astrocytes from APOE4 versus APOE3 gene targeted replacement mice. *Mol Neurodegener*. 2020;15:35.
130. Hirase H, Qian L, Bartho P, Buzsaki G. Calcium dynamics of cortical astrocytic networks in vivo. *PLoS Biol*. 2004;2:E96.
131. Kuchibhotla KV, Lattarulo CR, Hyman BT, Bacskai BJ. Synchronous hyperactivity and intercellular calcium waves in astrocytes in Alzheimer mice. *Science*. 2009;323:1211-1215.
132. Delekate A, Fuchtemeier M, Schumacher T, Ulbrich C, Foddiss M, Petzold GC. Metabotropic P2Y1 receptor signalling mediates astrocytic hyperactivity in vivo in an Alzheimer's disease mouse model. *Nat Commun*. 2014;5:5422.
133. Torres A, Wang F, Xu Q, et al. Extracellular Ca(2)(+) acts as a mediator of communication from neurons to glia. *Sci Signal*. 2012; 5:ra8.
134. Sompol P, Furman JL, Pleiss MM, et al. Calcineurin/NFAT signaling in activated astrocytes drives network hyperexcitability in abeta-bearing mice. *J Neurosci*. 2017;37:6132-6148.
135. Dineley KT, Hogan D, Zhang WR, Taglialatela G. Acute inhibition of calcineurin restores associative learning and memory in Tg2576 APP transgenic mice. *Neurobiol Learn Mem*. 2007;88:217-224.
136. Taglialatela G, Rastellini C, Cicalese L. Reduced incidence of dementia in solid organ transplant patients treated with calcineurin inhibitors. *J Alzheimers Dis*. 2015;47:329-333.
137. Silva JD, Taglialatela G, Jupiter DC. Reduced prevalence of dementia in patients prescribed tacrolimus, sirolimus, or cyclosporine. *J Alzheimers Dis*. 2023;95:585-597.
138. Ben Haim L, Ceyzeriat K, Carrillo-de Sauvage MA, et al. The JAK/STAT3 pathway is a common inducer of astrocyte reactivity in Alzheimer's and Huntington's diseases. *J Neurosci*. 2015;35:2817-2829.
139. Ceyzeriat K, Ben Haim L, Denizot A, et al. Modulation of astrocyte reactivity improves functional deficits in mouse models of Alzheimer's disease. *Acta Neuropathol Commun*. 2018;6:104.

#### SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Edison P. Astroglial activation: Current concepts and future directions. *Alzheimer's Dement*. 2024;1-20. <https://doi.org/10.1002/alz.13678>