Review

Astrocyte–neuron metabolic relationships: for better and for worse

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In recent years, previously unsuspected roles of astrocytes have been revealed, largely owing to the development of new tools enabling their selective study *in situ*. These exciting findings add to the large body of evidence demonstrating that astrocytes play a central role in brain homeostasis, in particular via the numerous cooperative metabolic processes they establish with neurons, such as the supply of energy metabolites and neurotransmitter recycling functions. Furthermore, impairments in astrocytic function are increasingly being recognized as an important contributor to neuronal dysfunction and, in particular, neurodegenerative processes. In this review, we discuss recent evidence supporting important roles for astrocytes in neuropathological conditions such as neuroinflammation, amyotrophic lateral sclerosis and Alzheimer's disease. We also explore the potential for neuroprotective therapeutics based on the modulation of astrocytic functions.

Introduction

Astrocytes have unique cytoarchitectural and phenotypic features that ideally position them to sense their surroundings and dynamically respond to changes in their microenvironment (Figure 1). They extend numerous processes (Figure 1a,b), forming highly organized anatomical domains with little overlap between adjacent cells (referred to as 'astrocytic domains'; Figure 1e) and are interconnected into functional networks via gap junctions (Figure 1f). Some astrocyte processes (which express a wide range of receptors and ion channels) closely ensheath synapses (Figure 1c), whereas others are in close contact with intraparenchymal blood vessels via specialized processes called endfeet (Figure 1d). In line with this, astrocytes have been shown to play an important role in neurovascular and neurometabolic coupling. Indeed, neuronal activity triggers the release of vasoactive substances by astrocytes, such as prostanoids, enabling the dynamic coupling of cerebral blood flow with the local energy demand [1,2]. At the metabolic level, as formulated by the astrocyte–neuron lactate shuttle model (ANLS), astrocytes respond to glutamatergic activation by increasing their rate of glucose utilization and the release of lactate in the extracellular space, which might, in turn, be used by neurons to sustain their energy demands [3,4] (Box 1; Figure 2, green pathway).

Several other homeostatic functions of astrocytes have been demonstrated, including glutamate, ion and water homeostasis, defense against oxidative stress, energy storage in the form of glycogen, scar formation and tissue repair, modulation of synaptic activity via the release of gliotransmitters, and synapse formation and remodeling (reviewed in [5–8]). Interestingly, recent evidence has shed light on previously unsuspected roles of astrocytes in higher brain functions, such as sleep homeostasis [9], memory consolidation [10] and in the regulation of breathing [11]. Some of the key homeostatic functions of astrocytes representative of their strong metabolic relationship with neurons are illustrated in Figure 2. In addition to these metabolic functions, astrocytes release several factors that sustain neuronal function and viability (Box 1). Interestingly, astrocytes are known to be a heterogenous cell population based on their morphology and the expression of different sets of receptors, transporters, ions channels and other proteins [12]. This raises the intriguing possibility that different subtypes of astrocyte are implicated in distinct metabolic/homeostatic functions.

Considering the pivotal role of astrocytes in brain homeostasis and the strong metabolic cooperation that exists between neurons and astrocytes, one would predict that astrocytic dysfunction might cause and/or contribute to neurodegenerative processes. In line with this, a growing body of evidence points to an important role of astrocytes in several pathologies, either through loss of normal function, or gain of defective functions. This review focuses on recent evidence highlighting the importance of astrocytes in selected pathologies and discusses how re-establishing or enhancing normal astrocytic functions might be a valuable strategy to promote neuroprotection in various central nervous system (CNS) disorders.

Primary astroglial diseases

Direct evidence for pivotal roles of astrocytes in maintaining normal brain function is provided by neurological disorders that are primarily caused by a dysfunction of these cells. Not surprisingly, these pathologies generally affect a wide range of cerebral processes owing to secondary malfunction of other cell types, including neurons, microglia and oligodendrocytes.

A striking example is Alexander disease (AXD), the first identified human neurological disorder unequivocally caused by a primary dysfunction of astrocytes (Table 1).

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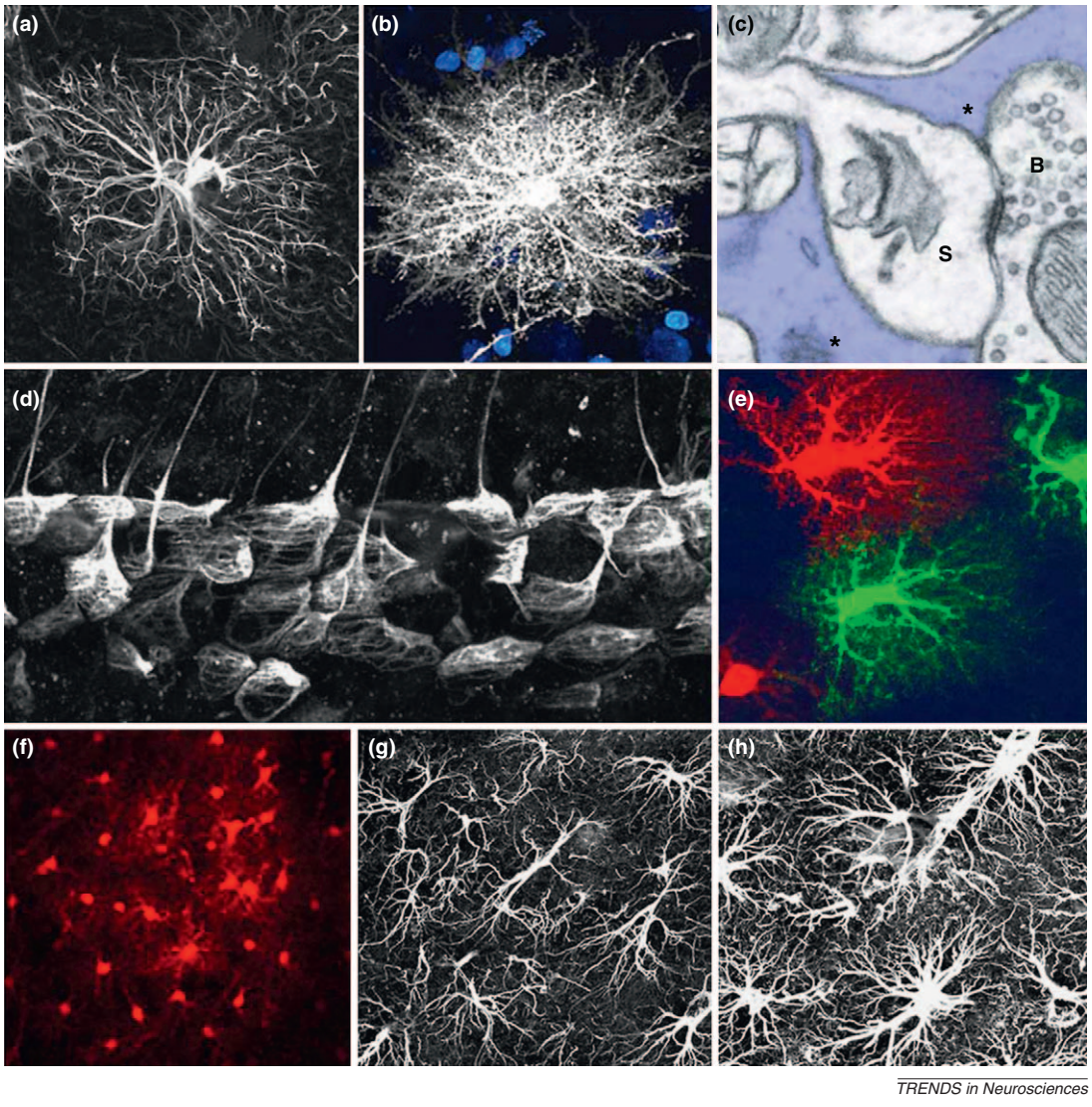


Figure 1. Morphological features of astrocytes. **(a)** GFAP immunostaining of the human brain shows that astrocytes extend numerous processes. **(b)** Dye labeling (white) of a human astrocyte reveals a complex network of fine processes. Nuclei are stained in blue (Dapi). **(c)** Astrocytic processes (in blue, marked by an asterisk) closely ensheathing a synapse, as revealed by electronic microscopy of mouse brain tissue (S, spine; and B, bouton). **(d)** Astrocytic endfeet are specialized astrocytic processes that are in close contact with blood vessels and cover most of their surface (GFAP staining in human brain tissue). **(e)** Dye-filling of two neighboring mouse hippocampal astrocytes with two different fluorochromes reveals that they occupy non-overlapping domains (astrocytic domains). **(f)** Dye-coupling using biocytin (red) injected into a single astrocyte in mouse brain slices results in the diffusion of the dye through the astrocytic network via gap junctions. **(g,h)** GFAP immunostaining in the molecular layer of the dentate gyrus of mice. Reactive astrocytes are hypertrophied and show thicker GFAP-positive processes following unilateral enthorinal cortex lesioning **(h)** compared with the non-reactive astrocytes in the contralateral side **(g)**. Reproduced, with permission, from [83] **(a,b,d)**, [84] **(c)**, [85] **(e,g,h)** and [86] **(f)**.

Mutations in the gene encoding glial fibrillary acidic protein (GFAP) have been identified in most AXD patients [13,14]. A characteristic feature of this fatal disorder is the widespread presence of intracellular protein aggregates in astrocytes, called Rosenthal fibers (RF), which contain mutant GFAP, heat shock protein 27 and αB-crystallin [15]. Postmortem brain tissue from AXD patients also presents other important anomalies, including abnormal myelination and neurodegeneration [16], suggesting that the failure of RF-bearing astrocytes to fulfil some of their key functions (e.g. the release of factors involved in myelin formation, maintenance and repair and/or in neuroprotection) contributes to the pathogenesis of AXD.

Another classic example of a primary astrogliopathy is provided by hepatic encephalopathy (HE), a neuropsychiatric syndrome occurring as a result of acute or chronic liver disease. In acute HE, high concentrations of ammonia derived from the periphery accumulate in the brain and are mainly detoxified in astrocytes by the glutamine synthetase (GS) enzyme (Figure 2), causing the intracellular accumulation of osmotically active glutamine and, in turn, astrocytic swelling [17,18]. The cytotoxic swelling of astrocytes is a key feature of acute HE, which can not only compromise their normal homeostatic functions, but also lead to increased intracranial pressure and brain herniation, a frequent cause of mortality in these patients. Several additional mechanisms contribute to the pathogenesis of acute HE and hyperammonemia, including changes in the expression of key astrocytic proteins [e.g. glutamate transporter 1 (GLT-1 in rodents, EAAT2 in humans), glucose transporter-1 (GLUT1), GFAP or aquaporin-4)], leading to alterations in the normal homeostatic functions of astrocytes [19].

Box 1. Examples of known astrocyte-derived factors acting on neuronal function and viability

Activity-dependent neuroprotective protein
Activity-dependent neuroprotective protein (ADNP) is a potent neuroprotective protein secreted by astrocytes in response to vasoactive intestinal peptide. NAP, a short active peptide derived from ADNP, has been used successfully to protect neurons against various CNS insults or pathologies [107].

Apolipoprotein E and cholesterol
Astrocytes are the main neural cell type responsible for the synthesis of apolipoprotein E (ApoE) and cholesterol. Release of cholesterol complexed to ApoE (in the form of high density lipoprotein-like particles) promotes axonal growth and synaptogenesis [108,109].

Ascorbic acid
Astrocytes recycle oxidized ascorbic acid (DHAA) back to ascorbic acid (AA), which can be exported to neurons [Figure 2 (purple pathway)]. AA can scavenge ROS directly and/or be used as a reducing equivalent for the recycling of vitamin E and GSH in astrocytes and neurons [110].

Cytokines
Various anti-inflammatory cytokines with known neuroprotective properties are released by astrocytes, in particular IL-6 and TGFβ [22–24]. However, their effects might be counterbalanced by the release of pro-inflammatory cytokines (see main text).

Glutathione
Astrocyte-derived glutathione (GSH) provides precursors for neuronal GSH synthesis [Figure 2 (pink pathway)] [89]. Enhancing GSH production and release from astrocytes via overexpression of the cystine/glutamate exchanger (xCT) [70] or Nrf2 [66] is neuroprotective.

Hydrogen sulfide
High levels of hydrogen sulfide (H_2S) are synthesized and released by astrocytes. H_2S has been shown to have neuromodulatory properties and protect neurons from oxidative stress via the stimulation of GSH synthesis [111,112].

Lactate
Astrocytes release large amounts of lactate in the extracellular space. Lactate can be used by neurons to meet their energy needs [Figure 2 (green pathway), main text] and has been shown to be a potent neuroprotective agent in various experimental paradigms [3,7,113]. For example, lactate infused in a single astrocyte can diffuse through the astrocytic network and rescue neuronal activity during glucose deprivation [114].

Neurotrophic factors
Astrocytes synthesize and release a wide range of neurotrophic and growth factors involved in neuronal survival and function, including NGF, BDNF, ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF), insulin growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), vascular endothelial growth factor (VEGF) and activity-dependent neurotrophic factor (ADNF) [22,23,115].

Thrombospondins
Thrombospondins are extracellular matrix proteins secreted by astrocytes. They have been shown to promote synaptogenesis during development as well as in experimental mouse models of stroke [82,116].

Other pathologies involving astrocytes

In addition to the abovementioned primary astroglial disorders, disturbances of astrocytic functions have been shown to contribute, along with other neural cell types, to the development and progression of several other neuropathologies for which the primary cause might not have yet been identified (Table 1). Recent evidence highlighting the active role of astrocytes in neuroinflammation, Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS) are discussed below.

Neuroinflammation

Neuroinflammation is a normal defense mechanism aimed at protecting the CNS against insults such as infection, injury or disease. In most cases, it constitutes a beneficial process that resolves on its own once the threat has been eliminated and homeostasis has been restored [20]. In some instances, however, the insult can persist and/or the inflammatory process might get out of balance, resulting in chronic neuroinflammation, a deleterious process contributing to the pathogenesis of several neurological conditions [20,21]. Together with microglia (the resident immune cells in the brain), astrocytes are important players in neuroinflammatory processes. Similar to microglia, astrocytes become activated in response to various stimuli, from subtle changes in their microenvironment to massive tissue damage. This process, known as reactive astrogliosis, far from being an all-or-none event, is a finely graded and non-homogeneous response that varies according to the type, severity, time and duration of the insult [8]. Histopathologically, reactive astrocytes evolve through a spectrum of changes ranging from hypertrophy and increased expression of GFAP (Figure 1 g,h), to proliferation and overlapping of astrocytic domains, ultimately (but not necessarily) resulting in scar formation [8]. Reactive astrocytes release a wide array of mediators, including pro- and anti-inflammatory cytokines neurotrophic factors, chemokines, complement factors and reactive oxygen species (ROS), all of which potentially mediate neuroprotective and/or neurotoxic effects [8,22,23]. Cytokines are a striking example of the dual effect of neuroinflammatory mediators. Astrocyte-derived cytokines such as interleukin (IL)-1β and tumor necrosis factor-α (TNFα) are generally considered to promote neurotoxicity whereas others including IL-6 and transforming growth factor-β (TGFβ), have been implicated in neuroprotective processes [22–24].

In line with this, there has been considerable debate as to whether the contribution of reactive astrogliosis in neuroinflammatory processes should be regarded as beneficial or detrimental. The formation of a glial scar is a classic example of the complex interplay existing between the neuroprotective and neurotoxic processes associated with reactive gliosis. On the one hand, it is essential in the early stages to wall off the damaged or infected areas thus preserving the surrounding, relatively healthy brain parenchyma. On the other hand, at later stages it also inhibits neurite outgrowth, contributing to the poor regenerative capability of the brain [8,25]. Accordingly, dissecting out the precise contribution of the multiple factors involved has proven to be challenging. In recent years, the development of transgenic animals enabling the manipulation of defined inflammatory processes specifically in astrocytes has proved valuable in furthering our

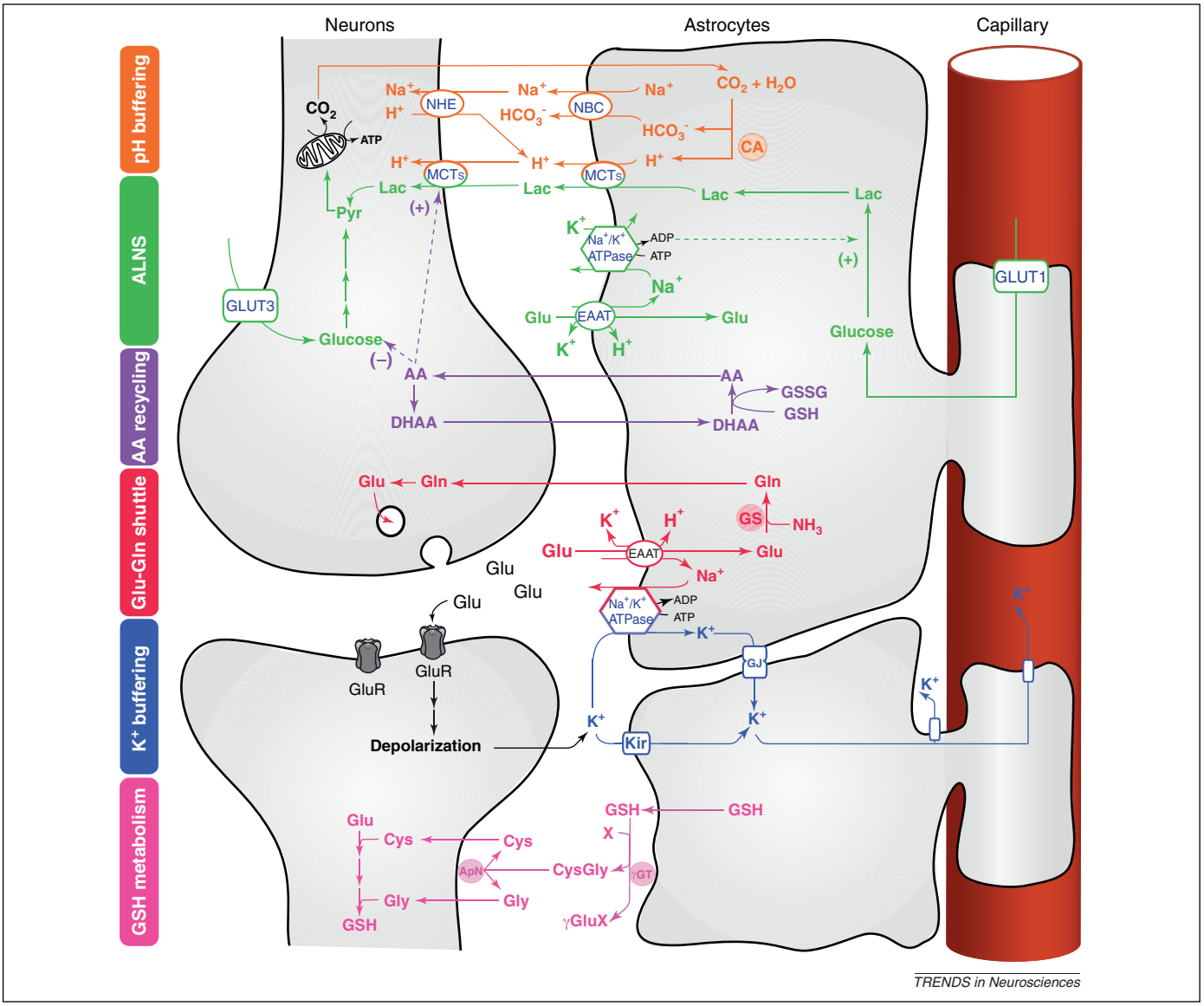


Figure 2. Astrocytes play a central role in brain homeostasis. **(i)** pH buffering (orange pathway). Abundant carbonic anhydrase (CA) in astrocytes converts CO_2 into H^+ and HCO_3^-. Two HCO_3^- are transported into the extracellular space along with one Na^+ via the Na^+-HCO_3^- co-transporter (NBC), thereby increasing the extracellular buffering power. Protons left in the glial compartment might drive the transport of lactate (Lac) outside of astrocytes and into neurons through MCTs. Excess H^+ in neurons is extruded via sodium-hydrogen exchange (NHE). **(ii)** ANLS (green pathway). Glutamate (Glu) uptake by astrocytes is accompanied by Na^+ entry, which is extruded by the action of the Na^+/K^+ ATPase. This triggers anaerobic glucose utilization in astrocytes and glucose uptake from the circulation through GLUT1. The lactate produced is shuttled to neurons through MCTs, where it can be used as an energy substrate after its conversion to pyruvate (Pyr). Neurons can also take up glucose via the neuronal GLUT3. **(iii)** Ascorbic acid (AA) recycling (purple pathway). Astrocytes recycle oxidized ascorbic acid [dehydroascorbic acid (DHAA)] back to AA using reduced glutathione (GSH) and forming oxidized glutathione (GSSG). AA can be used directly in astrocytes or released and taken up by neurons for ROS scavenging. AA might also act as a metabolic switch by inhibiting glucose consumption and stimulating lactate uptake in neurons [87]. **(iv)** Glu–glutamine cycle (red pathway). Glu released into the synaptic cleft activates ionotropic glutamatergic receptors (GluR), producing a postsynaptic depolarization. Astrocytic excitatory amino acid transporters (EAATs) are responsible for the uptake of a large fraction of Glu at the synapse. Glu is converted into glutamine (Gln) by GS and shuttled back to neurons for glutamate resynthesis. **(v)** K^+ buffering (blue pathway). Astrocytes buffer excess K^+ released into the extracellular space as a result of neuronal activity [e.g. through inwardly rectifying K^+ channels (Kir)]. K^+ ions travel through the astrocytic network via gap junctions (GJ) down their concentration gradient and are released in sites of lower concentration. **(vi)** GSH metabolism (pink pathway). Astrocytes release GSH in the extracellular space, where it is cleaved by the astrocytic ectoenzyme γ-glutamyl transpeptidase (γGT). The resulting dipeptide, CysGly, is cleaved by the neuronal ectopeptidase aminopeptidase N (ApN), forming cysteine (Cys) and glycine (Gly), which serve as precursors for neuronal GSH synthesis [88]. X represents an acceptor for the γ-glutamyl moiety (γGlu) in the reaction catalysed by γGT. These astrocytic functions are reviewed in further detail in [3,7,8,89].

understanding of the overall impact of reactive astrogliosis *in vivo*. In particular, the use of a transgenic mouse model allowing for the conditional ablation of dividing reactive astrocytes has demonstrated that scar-forming astrocytes are essential for spatially and temporally restricting inflammation, promoting blood–brain barrier (BBB) repair, limiting neuronal injury and improving the outcome in various models of CNS injury or disease, such as traumatic brain injury (TBI), spinal cord injury (SCI) and experimental autoimmune encephalitis (EAE) [8,25–27].

Another useful approach has been the development of conditional knockout mice for specific inflammatory pathways in astrocytes using the Cre–loxP recombination system. Astrocyte-specific deletion of signal transducer and activator of transcription 3 (STAT3), an important signaling molecule for several cytokines and growth factors, resulted in impaired reactive astrogliosis as well as increased inflammation and tissue damage, altogether leading to impaired motor recovery after SCI [28,29]. Similarly, in a setting of CNS infection in mice,

Table 1. Neuropathologies in which astrocytic dysfunction has been demonstrated to be involved[a]

Disease	Evidence/mechanisms	Disease model/experimental system			Refs
		Human	Animal model	*In vitro* model	
AXD	• Intracellular accumulation of Rosenthal fibers in astrocytes owing to mutations in the gene encoding GFAP	X	X		[15,16]
AD	• Reactive astrocytes are present at the site of Aβ plaques	X	X		[90]
	• Intracellular accumulation of Aβ in astrocytes	X		X	[38,41–43]
	• Cultured astrocytes exposed to Aβ induce degeneration of surrounding neurons			X	[43,49–51]
ALS	• Decreased expression of EAAT2 (GLT-1), which might lead to excitotoxicity	X	X		[54,55]
	• Mutant SOD1 in astrocytes contributes to disease progression		X		[52,55]
	• Expression of mutant SOD1 in cultured astrocytes is sufficient to induce neurodegeneration			X	[61–64]
Depression	• Reduction in the density of astrocytes is observed in the prefrontal cortex	X			[91]
EAST/SeSAME syndrome	• Mutations in the gene encoding Kir4.1 (highly expressed in astrocytes) result in symptoms such as ataxia and epilepsy, likely due to impaired K^+ buffering by astrocytes	X	X		[92,93]
Epilepsy	• Morphological and molecular alterations in astrocytes (e.g. massive astrogliosis, upregulation of glutamate dehydrogenase and downregulation of GS) are observed	X			[94]
	• Alterations in astrocytic functions, such as K^+ buffering, calcium signaling and glutamate and water homeostasis, have been reported		X		[94,95]
HE	• Ammonia detoxification by GS leads to cytotoxic swelling of astrocytes	X	X	X	[17,19]
	• Alterations of astrocytic homeostatic functions owing to changes in the expression of key astrocytic proteins (e.g. GLT-1, GLUT1, GFAP and aquaporin-4)	X	X	X	[19]
HD	• Mutant huntingtin and EAAT2 (GLT-1) downregulation are observed in astrocytes, suggesting a role for excitotoxicity in the associated neurodegeneration	X	X		[96,97]
	• Selective expression of mutant huntingtin in astrocytes results in age-dependent neurological symptoms and decreased lifespan, associated with decreased expression and activity of glutamate transporters		X		[96,98]
	• Expression of mutant huntingtin in cultured astrocytes decreases the expression and activity of glutamate transporters. Neurons co-cultured with these astrocytes show decreased viability and increased vulnerability to excitotoxicity			X	[97]
Ischemia/stroke	• Decreased expression of astrocytic glutamate transporters contributes to excitotoxic neuronal damage. Downregulation of astrocytic glutamate transporters increases ischemia-induced neurodegeneration, whereas selective upregulation of GLAST in astrocytes is neuroprotective		X	X	[99–101]
	• Downregulation or pharmacological inhibition of the astrocytic connexin 43 affects tissue damage associated with stroke		X		[99]
	• Inhibition of astroglial NF-κB protects retinal neurons following ischemia reperfusion		X		[33]
Multiple sclerosis	• Astrocytes participate in the inflammatory response and in the formation of a glial scar that, although essential to limit inflammation, might also impede tissue repair		X		[8,102]
	• Inhibition of astroglial NF-κB is beneficial in a mouse EAE model		X		[34]
PD	• Astrocytic MAO-B is responsible for the conversion of MPTP to the PD-inducing toxin MPP^+. MPP^+ is released to the extracellular space by the organic cation transporter 3, which is selectively expressed in astrocytes		X	X	[103]
	• Overexpression of Nrf2 selectively in astrocytes protects from neurodegeneration in a MPTP mouse model of PD		X		[75]
	• Expression of mutant α-synuclein selectively in astrocytes induces widespread glial activation, neurodegeneration and paralysis in mice		X		[104]
Schizophrenia	• Changes in the density of astrocytes in various brain areas have been reported (either increases or decreases), but this issue remains controversial	X			[105]
	• The expression of several genes is altered in astrocytes, including those encoding GFAP, GS, S100β and MAO-B	X			[105]
Spinocerebellar ataxia	• Bergmann glia-specific expression of mutant ataxin-7 is sufficient to produce ataxia and neurodegeneration		X		[55]
TBI	• Expression of glutamate transporters in astrocytes is decreased	X	X		[106]
	• Conditional ablation of dividing reactive astrocytes results in increased inflammation, impaired BBB repair and exacerbates neuronal injury		X		[26,27]

[a]Abbreviations: MPP^+, 1-methyl-4-phenylpyridinium; MAO-B, monoamine oxidase-B; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; GLAST, GLutamate-ASpartate Transporter.

conditional astrocytic deletion of gp130, a cytokine receptor upstream of STAT3, resulted in deficient astrogliosis, widespread inflammation, loss of astrocytes and increased mortality [30].

In contrast to the abovementioned studies (which, as a whole, suggest a beneficial role of reactive astrogliosis) astrocyte-specific suppression of a key pathway regulating inflammation [via the targeted inhibition of nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB)], has been shown to be beneficial in various models of CNS disease or injury. Inhibition of astroglial NF-κB significantly limited tissue injury and improved functional recovery following contusive SCI [31], possibly as a result of increased sparing and sprouting of spinal tracts axons [32]. Subsequent studies using the same transgenic model reported increased neuronal survival in the retinal ganglion cell layer following ischemia–reperfusion injury [33] and reduced disease incidence and severity accompanied by significant functional recovery in murine EAE [34].

If the results obtained with different transgenic models might at first seem contradictory, they in fact simply underscore the complex effects of the multiple pathways activated in astrocytes in the context of neuroinflammation. These studies suggest that, as a whole, reactive astrogliosis is an essential process for brain defense and repair and that its complete suppression probably has little therapeutic interest. However, careful manipulation of specific pathways (such as STAT3 or NF-κB) to promote the beneficial aspects and/or reduce the detrimental effects of reactive astrogliosis might prove to be a valid therapeutic target for a variety of disorders involving a neuroinflammatory component.

Alzheimer's disease

AD is the most prevalent neurodegenerative disorder. It exists in familial and sporadic forms, both of which are manifested by cognitive deficits, such as learning and memory impairments. At the histological level, AD is characterized by extracellular deposits of fibrillar β-amyloid (Aβ) (also known as neuritic plaques), vascular amyloidosis, neurofibrillary tangles, and severe neuronal and synaptic loss [35]. The exact contribution of each of these components in the progression of the disease remains controversial; however, a central role for Aβ production and accumulation in the pathogenesis of AD is now well recognized [36,37].

A first element to suggest the involvement of astrocytes in AD pathology is the observation of activated astrocytes at the site of Aβ deposits in both human AD postmortem brain tissue and in the brain tissue derived from animal models of AD [38–40]. In human AD brains, large amounts of Aβ were observed in activated astrocytes (Figure 3a), suggesting that they have an important role in Aβ clearance [38]. In support of this, different studies have shown that astrocytes can indeed internalize Aβ both *in vitro* and *ex vivo* (Figure 3b) [41–43]. For instance, when plated on Aβ-bearing brain sections from a mouse model of AD, astrocytes associate with the Aβ deposits resulting in a reduction in Aβ levels [41,42]. More recently, the capacity of astrocytes to migrate to and internalize deposited Aβ has been demonstrated *in vivo* by monitoring transplanted enhanced green fluorescent protein (eGFP)-expressing astrocytes in a transgenic mouse model of AD [44]. Interestingly, the extent of reactive gliosis, as well as the amount of accumulated Aβ in astrocytes, have been shown to correlate with the severity of AD-associated tissue damage, strengthening the view that astrocytes play an important role in AD pathology [38,39,45].

Reactive astrocytes located near Aβ plaques in the brains of a mouse model of AD showed clear modifications in their functions with regard to calcium homeostasis [46] and connectivity [47,48], raising the important question of the putative impact of Aβ-associated astrogliosis on neurons. Evidence obtained in astrocyte-neuron mixed cultures or co-culture experiments argues in favor of a deleterious effect of astrocyte–Aβ interactions on neuronal viability [43,49–51]. Aβ has been shown to induce cellular oxidative stress in mixed cultures, which is coupled to glutathione depletion and neuronal death through an astrocyte-dependent mechanism involving the specific induction of intracellular astrocytic calcium concentration oscillations [50,51]. Internalization of the aggregated form of Aβ by astrocytes has also been observed to affect their metabolic phenotype profoundly, both in terms of energy metabolism and oxidative stress status [43]. Exposure of neurons to these 'Aβ-filled' astrocytes resulted in neurotoxicity in a co-culture model, in the absence of any direct contact between both cell types, demonstrating that diffusible factors are important in mediating this phenomenon (Figure 3c) [43].

Finally, recent evidence suggests that astrogliosis related to Aβ deposits are not the only astrocytic reaction occurring in the AD brain. In addition to the hypertrophic astrocytes typically observed at neuritic plaques, astroglial atrophy was also observed distal from neuritic plaques in various brain regions in a transgenic mouse model of AD [40].

Amyotrophic lateral sclerosis

ALS is a fatal neurodegenerative disease principally characterized by the loss of motor neurons in the brain stem and spinal cord, leading to paralysis and muscle atrophy. Genetic inheritance accounts for 5–10% of cases, among which approximately 20% are caused by mutations of the gene encoding superoxide dismutase 1 (SOD1), leading to a toxic gain-of-function of this enzyme [52–54]. Despite their different etiologies, familial and sporadic cases of ALS present a similar clinical picture, which can be mimicked by the overexpression of mutant forms of human SOD1 in rodents [55]. These transgenic animals are currently the best available experimental models of ALS.

There is a growing appreciation for the contribution of non-neuronal cells, in particular astrocytes and microglia, in the pathophysiological mechanisms responsible for ALS. Early evidence pointing to a role of astrocytes was provided by reports showing decreased expression of EAAT2 (GLT-1) in a large proportion of patients with sporadic or familial forms of ALS, as well as in mutant SOD1 mice [54]. This suggested that failure of astrocytes to clear glutamate at the synapse contributes to the degeneration of motor neurons via excitotoxic mechanisms.

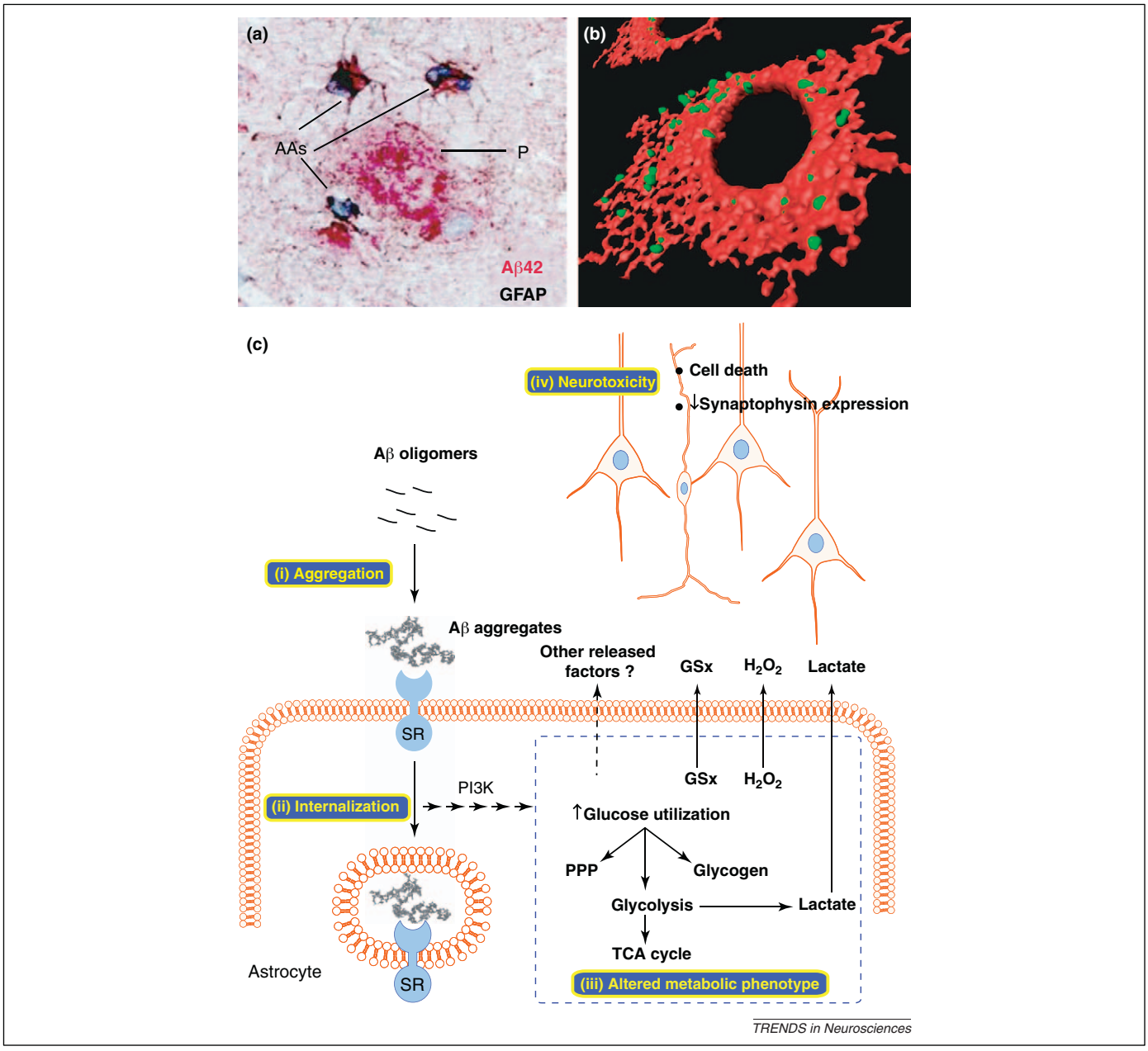


Figure 3. Accumulation of Aβ-positive material in astrocytes is potentially deleterious for neurons. **(a)** Double immunolabeling with GFAP (black)- and $Aβ_{1–42}$ (Aβ42; red)-specific antibodies demonstrates the presence of intracellular $Aβ_{1–42}$ in activated astrocytes (AAs) positioned at the boundary of plaques (P) in the entorhinal cortex of human postmortem AD brains. **(b)** Three-dimensional reconstruction surface imaging of a single cultured mouse cortical astrocyte demonstrating that exogenously added FITC-labelled $Aβ_{1–42}$ (green) was internalized into astrocytes (stained in red using a fluorescent dye). **(c)** Model diagram illustrating that the internalization of aggregated Aβ into astrocytes leads to a modification of their phenotype and negatively impacts neuronal viability *in vitro* (based on the experimental results in [43]). Aβ in its aggregated form (but not as oligomers), profoundly affects astrocyte metabolism with regard to glucose metabolism and oxidative stress status. For instance, Aβ stimulated glucose utilization and its storage as glycogen, increased glucose metabolism through glycolysis coupled to increased lactate release, tricarboxylic acid (TCA) cycle and pentose phosphate pathway (PPP) fluxes. In parallel, Aβ increased production and release of GSx (total glutathione) and H_2O_2. The effects of Aβ were shown to be dependent upon its internalization through scavenger receptors (SR) and the activation of the phosphoinositide 3-kinase (PI3K) pathway. In a neuron-astrocyte co-culture model in which both cell types were physically separated, Aβ pre-treated astrocytes significantly decreased neuronal viability and function. These astrocyte-mediated neurotoxic effects were mimicked by scavenger receptor agonists and were dependent upon the activation of the PI3K pathway in astrocytes. As a whole, these observations reveal a deleterious cascade triggered by **i**) Aβ aggregation; **ii**) its internalization by scavenger receptors; **iii**) modifications of the metabolic profile of astrocytes; and **iv**) the release of neurotoxic factors (eg. H_2O_2) by astrocytes. Reprinted, with permission, from [38] (**a**); modified, with permission, from [43] (**b**).

Perhaps the most convincing evidence for a contribution of non-cell-autonomous toxicity in ALS (indicating that cell subtypes other than neurons might serve distinct and required roles in neurodegeneration) arose in recent years from the use of transgenic animals allowing cell type-specific manipulations of mutant SOD1 expression. For instance, unlike the classic SOD1 mouse models [56], which display ubiquitous expression of mutant SOD1, selective expression of mutant SOD1 only in neurons [57], or in astrocytes [58], was not sufficient to cause ALS-like pathology in mice [52,55]. Subsequent studies have used the Cre–loxP recombination system to achieve cell-specific deletion of mutant SOD1 in mice otherwise ubiquitously expressing mutant SOD1. Reduction of mutant SOD1 expression specifically in neurons significantly delayed disease onset, but had little effect on disease

progression thereafter [59,60]. By contrast, selective reduction of mutant SOD1 expression in microglia [59], or in astrocytes [60], had no incidence on disease onset, but significantly delayed disease progression. Together, these studies suggest that SOD1 expression in neurons is necessary to initiate the disease but that other cell types, including astrocytes and microglia, have a role in driving disease progression (reviewed in [52]).

The involvement of astrocytes is further corroborated by *in vitro* studies that have demonstrated that mouse [61,62] or human stem cell-derived [63,64] motor neurons carrying wild-type SOD1 undergo degeneration when cultured in the presence of astrocytes carrying mutant SOD1. Remarkably, the survival of other types of neuron, including interneurons or dorsal root ganglion neurons, was not affected by the presence of mutant SOD astrocytes [62–64]. Moreover, co-culture of motor neurons with other cell types expressing mutant SOD1, including fibroblasts, microglia, cortical neurons and myocytes, did not induce neurodegeneration, demonstrating the existence of astrocyte-specific mechanisms contributing to mutant SOD1-induced motor neuron degeneration [62–64].

Exactly how mutant SOD1 astrocytes contribute to motor neuron degeneration *in vitro* and *in vivo* is not completely understood. Several non-mutually exclusive mechanisms have been proposed, such as oxidative stress, increased release of nitric oxide or nerve growth factor (NGF), increased microglial activation and/or excitotoxicity [52,54,55,60,64–66].

Consistent with the important role of astrocytes in the pathophysiology of ALS, recent studies in animal models of ALS demonstrate that targeting astrocytic functions could be a promising therapeutic avenue. Indeed, administration of β-lactam antibiotics in a mouse model of ALS was shown to prevent partially the loss of GLT-1 in the spinal cord and could significantly extend survival [54]. This approach is the object of an ongoing clinical trial (Identifier NCT00349622, http://www.clinicaltrials.gov). Other studies using cell-replacement approaches have shown that mesenchymal stem cells transplanted in the cisterna

Box 2. Technologies enabling the selective study of astrocytes *in situ*

Transgenic animals

Several promoters have been used to achieve astrocyte-specific gene manipulations, including GFAP, S100β, glutamate-aspartate transporter (GLAST), GLT-1, connexin-30, ApoE and aquaporin-4 [117,118]. None of these are completely satisfactory (in terms of specificity and efficacy) in part because astroglial populations are heterogeneous and differentially express these astrocytic markers. Nevertheless, useful transgenic animals have been produced using the aforementioned promoters, in particular to drive the expression of: (i) reporter genes for the identification and/or isolation of astrocytes [119] (see also Figure I); (ii) Cre recombinase for astrocyte-specific gene deletion using the Cre–LoxP system [118]; and (iii) genes of interest to study their impact on brain functions [66,71]. Interestingly, aldehyde dehydrogenase-1L1 (Aldh1L1), a promising new marker, has recently been reported to be expressed in most astrocytes [120]. This widely expressed astrocytic marker might be useful for the future development of new transgenic lines targeting astrocytes.

Viruses

Viruses have proven to be an invaluable tool for gene delivery. Different types and serotypes of viruses have been explored to achieve astrocyte-selective tropism, with some of them showing encouraging results [121,122]. Complementary to viral tropism, cell specificity can be enhanced using astrocyte-specific promoters [118] or miRNA-based strategies to eliminate expression in other cell types [123].

***In vivo* imaging**

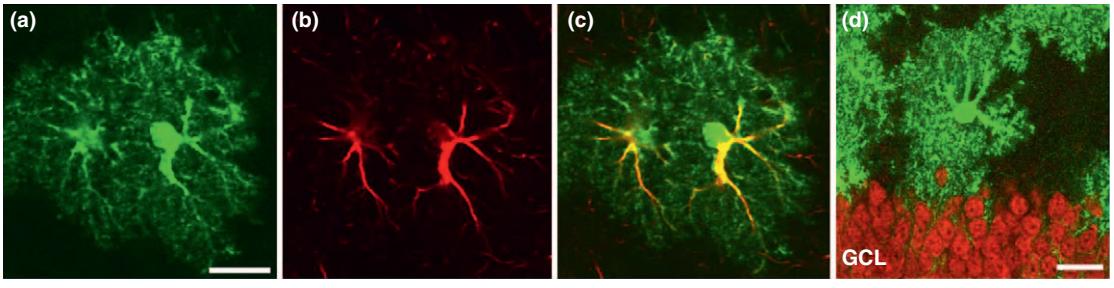
Sulforhodamine-101 is a red fluorescent dye that specifically labels astrocytes [124]. It can be used in 2-photon imaging along with various cell-permeant fluorescent indicators to monitor different cellular processes specifically in astrocytes *in vivo*, such as calcium oscillations [125] or GSH variations [126]. Recently, astrocyte-specific calcium signals have also been studied using a genetically encoded fluorescent calcium indicator expressed under the control of an astrocytic promoter [127]. Finally, noninvasive whole-body *in vivo* bioluminescence imaging provides a method for monitoring glial responses in animals expressing luciferase under the control of the GFAP promoter [128].

Toxins

Fluorocitrate, and its precursor fluoroacetate, are inhibitors of the TCA cycle that are preferentially taken up by astrocytes. Although they have classically been used to study the effects of the selective blockade of the astrocytic TCA cycle, a recent report proposed 2-^{18}F-fluoroacetate as a useful tool for the noninvasive study of reactive astrogliosis using positron emission tomography (PET) [129,130].

Nuclear magnetic resonance spectroscopy

In vivo metabolic interactions between astrocytes and neurons can be monitored using nuclear magnetic resonance (NMR) spectroscopy, in particular by using ^{13}C-labelled acetate, which is preferentially metabolized by astrocytes compared with neurons [131].



TRENDS in Neurosciences

Figure I. Transgenic animals expressing eGFP under the control of the mouse GFAP promoter enable the detailed characterization of astrocytic morphology. Brain sections were immunostained for eGFP **(a)** or for the astrocytic marker GFAP **(b)**. The merged image **(c)** shows eGFP immunoreactivity throughout hippocampal astrocytes, including in their fine processes, as compared with GFAP immunoreactivity, which is restricted to the main processes. Astrocyte-specific localization of eGFP is shown in **(d)**. Brain sections were immunostained for eGFP (green) and for the neuronal marker NeuN (red), showing the absence of eGFP in neurons of the granule cell layer (GCL) of the hippocampus. Scale bars = 20 μm. Reprinted, with permission, from [132].

magna [65], or glial-restricted precursor cells injected directly into the spinal cord [67], of mutant SOD1 rats migrated to the damaged areas of the spinal cord, where they largely differentiated into astrocytes and reduced microglial activation. This resulted in a preservation of motor neurons, delayed the decline of motor function and extended survival.

Perspectives

As described above, considering the numerous cellular interactions between astrocytes and neurons (Figure 2, Box 1), it is evident that defects of astrocytic functions and/or alterations of astrocyte–neuron cooperativity might lead to neuronal damage. Consistent with this, it has become obvious that enhancing or re-establishing astrocytic functions could represent a valuable strategy for neuroprotection. *In vitro* evidence of such neuroprotective actions has been obtained by enhancing the exchange of energy substrates between astrocytes and neurons [68], increasing astrocytic energy reserves in the form of glycogen [69] or increasing the oxidative stress defense capacity of astrocytes [66,70]. In particular, an *in vitro* gene therapy approach to enhance lactate shuttling between neurons and astrocytes via the overexpression of GLUT1 and of the monocarboxylate transporter 2 (MCT2) in astrocytes and neurons, respectively (Figure 2, Box 1), resulted in increased protection against neuronal excitotoxicity [68].

For decades, most of the knowledge about astrocytic roles in physiological and pathological processes, as well as their potential neuroprotective properties, has been restricted to *in vitro* studies. With the development of new technologies, it is now possible to study and modify the physiology and functions of astrocytes directly and selectively *in vivo* (Box 2). These tools not only further our knowledge of astroglial functions and/or dysfunctions, but also facilitate the *in vivo* demonstration that targeting astrocytes is a valuable and innovative strategy for neuroprotection. Representative examples of such strategies applied in the context of ALS or neuroinflammation have been described above, but they are neither unique nor restricted to these situations. Here, we outline three different and possibly complementary approaches that have been used successfully, opening new therapeutic perspectives for several CNS disorders that often share similar pathophysiological mechanisms.

Cell grafting is one example of a strategy that affords beneficial effects in different animal models of neurodegenerative pathologies, such as ALS [65,67], Huntington's disease (HD) [71] and Parkinson's disease (PD) [72]. For example, in an experimental mouse model of HD, the transplantation of astrocytes that were genetically modified to express brain-derived neurotrophic factor (BDNF) under the control of a GFAP promoter into the striatum provided enhanced neuroprotective effects in these mice compared with mice that were transplanted with wild-type astrocytes [71]. This suggests that astrocytes engineered to release BDNF (or potentially other neurotrophic factors) constitute an interesting therapeutic approach for HD and potentially other neurodegenerative diseases.

A second approach for modifying astrocytic functions has been to target a more global and coordinated cellular response, rather than a specific single gene. Such an approach is possible because several related cellular functions are often under the control of a specific signaling pathway and/or transcription factor acting as a 'master switch'. This strategy is exemplified by studies manipulating STAT3 or NF-κB to modulate inflammation processes linked to reactive astrogliosis [28,29,31–34]. Using a similar approach, it is also possible to enhance defense mechanisms against oxidative stress in astrocytes [and the release of glutathione precursors for neurons (Figure 2)] by increasing the activity of nuclear factor erythroid 2-related factor 2 (Nrf2), which regulates a wide range of antioxidant-related proteins [73]. In disorders associated with oxidative stress, such as PD, ALS and HD, overexpression of Nrf2 selectively in astrocytes has proven to be an effective approach to preserve neuronal viability in mice [66,74,75].

Finally, a third approach concerns the use of pharmacological agents. In the CNS, pharmacological approaches have traditionally been limited by poor BBB permeability or lack of specificity [76]. Nevertheless, some compounds have demonstrated promising results. Ceftriaxone, a β-lactam antibiotic, and GPI-1046, a synthetic derivative of the macrolide immunosuppressive drug FK506, have been shown to prolong survival of transgenic ALS mice through the induction of the glutamate transporter GLT-1 specifically in astrocytes [54,77]. Pharmacological tools targeting inflammatory processes have also been used efficiently to preserve neuronal viability. These include MW01-5-188WH and ONO-2506 (also known as arundic acid), two selective inhibitors of inflammation in glia, which have shown promising neuroprotective properties in experimental models of AD and PD [78–80]. Finally, increasing astrocytic glycogen levels by using an inhibitor of glycogen phosphorylase, which permits glycogen mobilization when glucose levels are low, sustained neuronal activity and markedly reduced neuronal death during hypoglycemia in rats [81].

Box 3. Outstanding questions

- Are all neurodegenerative processes associated with defects in astrocytic functions?
- Are there pathophysiological mechanisms common to multiple disorders that result from impaired astrocytic functions? Could these mechanisms thus represent a valid broad-spectrum therapeutic target?
- Because astrocytes are a heterogeneous cell population [12], do the different subtypes contribute equally (or not) to neurodegenerative processes? Can the different astrocyte subpopulations be targeted selectively and specifically?
- Could combined therapeutic approaches, such as targeting multiple pathways within astrocytes or multiple astrocytic subtypes, provide additional neuroprotection?
- What are the potential side effects of manipulating astrocytic functions as a therapeutic approach? Could they be limited by restricting the therapeutic delivery system to a specific time window?
- Could astrocytic dysfunction be detected and monitored before the onset of clinical symptoms to intervene before irreversible neurodegeneration occurs?
- There is some evidence indicating that astrocytes are involved in psychiatric disorders, such as depression and schizophrenia [91,105]. Which animal models would be most helpful to further explore the putative role of astrocytes in these disorders?

Concluding remarks

As discussed in this review, the study of the role of astrocytes in physiological and pathophysiological processes is a rapidly expanding field of research. Such studies largely rely on the development of new technologies that enable the contribution of specific cell types to various brain functions to be dissected. Although compelling evidence points to a role of astrocytes in various pathologies, it must be emphasized that, in many cases, the primary cause of the disease has not been clearly identified and that other cell types are likely to be involved. Nevertheless, considering the crucial role of astrocytes in brain homeostasis, these cells represent an interesting therapeutic target (e.g. by re-establishing their normal function or enhancing neuroprotective pathways). Although several questions remain (Box 3), a solid body of evidence has now established that harnessing the natural capacity of astrocytes to protect neurons is a promising therapeutic strategy. As Ben Barres fittingly wrote: 'Quite possibly saving astrocytes from dying in neurological disease would be a far more effective strategy than trying to save neurons (glia already know how to save neurons, whereas neuroscientists still have no clue)' [82].

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