The role of astroglia in neuroprotection

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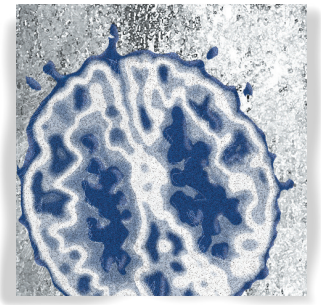

Astrocytes are the main neural cell type responsible for the maintenance of brain homeostasis. They form highly organized anatomical domains that are interconnected into extensive networks. These features, along with the expression of a wide array of receptors, transporters, and ion channels, ideally position them to sense and dynamically modulate neuronal activity. Astrocytes cooperate with neurons on several levels, including neurotransmitter trafficking and recycling, ion homeostasis, energy metabolism, and defense against oxidative stress. The critical dependence of neurons upon their constant support confers astrocytes with intrinsic neuroprotective properties which are discussed here. Conversely, pathogenic stimuli may disturb astrocytic function, thus compromising neuronal functionality and viability. Using neuroinflammation, Alzheimer's disease, and hepatic encephalopathy as examples, we discuss how astrocytic defense mechanisms may be overwhelmed in pathological conditions, contributing to disease progression.

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In the last two decades, intense research efforts aiming to provide a better understanding of astroglial cell function have revealed a number of previously unsuspected roles for these neural cells, which were long considered as relatively passive structural elements of the brain. It has now become quite clear that a plethora of cooperative metabolic processes and interdependencies exist between astrocytes and neurons. As a result of the growing appreciation of the role of astrocytes in both the normal and diseased brain, the traditional neuron-centric conception of the central nervous system (CNS) has been increasingly challenged.

Astrocytes are territorial cells: they extend several processes with little overlap between adjacent cells, forming highly organized anatomical domains[1-3] which are interconnected into functional syncytia via abundant gap junctions.[4] These astrocytic processes closely ensheath synapses and express a wide range of receptors for neurotransmitters, cytokines, and growth factors, as well as various transporters and ion channels.[5-11] In addition, astrocytes project specialized astrocytic endfeet which are in close contact with intraparenchymal blood vessels, almost entirely covering their surface.[12,13] Together, these cytoarchitectural and phenotypical features ideally position astrocytes to fulfill a pivotal role in brain homeostasis, allowing them not only to sense their surroundings but also to respond to—and consequently

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Selected abbreviations and acronyms

Aβ	*amyloid-beta*
AD	*Alzheimer's disease*
GSH	*glutathione*
MCT	*monocarboxylate transporter*
ROS	*reactive oxygen species*

modulate—changes in their microenvironment. Indeed, astrocytes can respond to neurotransmitters with transient increases in their intracellular Ca^{2+} levels, which can travel through the astrocytic syncytium in a wavelike fashion.[14,15] These Ca^{2+} signals can trigger the release of neuroactive molecules from astrocytes (or gliotransmitters), such as glutamate, D-serine, or adenosine triphosphate (ATP) which in turn modulate synaptic activity and neuronal excitability (see ref 16 for review). This process, for which the term "gliotransmission" has been coined, marks the emergence of an exciting new notion that information processing may not be a unique feature of neurons.

Remarkably, the phylogenetic evolution of the brain correlates with a steady increase of the astrocyte-to-neuron ratio—going from about 1/6 in nematodes to 1/3 in rodents, and reaching up to 1.65 astrocytes per neuron in the human cortex.[3,17] Importantly, more than simply outnumbering their rodent counterparts, human astrocytes are also strikingly more complex, both morphologically and functionally. In comparison, human neocortical astrocytes are 2.5 times larger, extend 10 times more processes, and display unique microanatomical features *(Figure 1)*.[2] In addition, they generate more robust intracellular Ca^{2+} responses to neurotransmitter receptor agonists and display a 4-fold increase in Ca^{2+} wave velocity.[2] In light of these evolution-driven modifications, it is tempting to hypothesize that the astrocytic contribution to the overall neural network complexity may in part provide the fine tuning necessary to take information processing to a higher level of competence, such as that seen in humans. At the very least, the evolutionary pressure exerted on astrocytes highlights the importance of this glial cell type in sustaining normal brain function as the brain itself becomes more complex. A continuously growing body of evidence demonstrates that astrocytes are essential sentinels and dynamic modulators of neuronal function. Considering the strong metabolic cooperation that exists between these two cell types, it is not surprising that alterations in astrocytic function have been shown to have potentially catastrophic consequences for neurons. In the present review we discuss the intrinsically protective role of astrocytes in the normal brain, and examine how these defense mechanisms may be overwhelmed in pathological conditions, contributing to disease progression.

Astrocytes in the normal brain: maintenance of extracellular homeostasis

Despite the fact that the brain has a very high metabolic rate, neurons are by nature particularly sensitive to minute changes in their microenvironment. In this context, neuronal function and viability would rapidly be compromised without effective mechanisms for the supply of metabolic substrates and—equally as important—for the removal of waste products. In this respect, astrocytes play an essential role through a number of cellular processes; some of the most important are outlined in the following section.

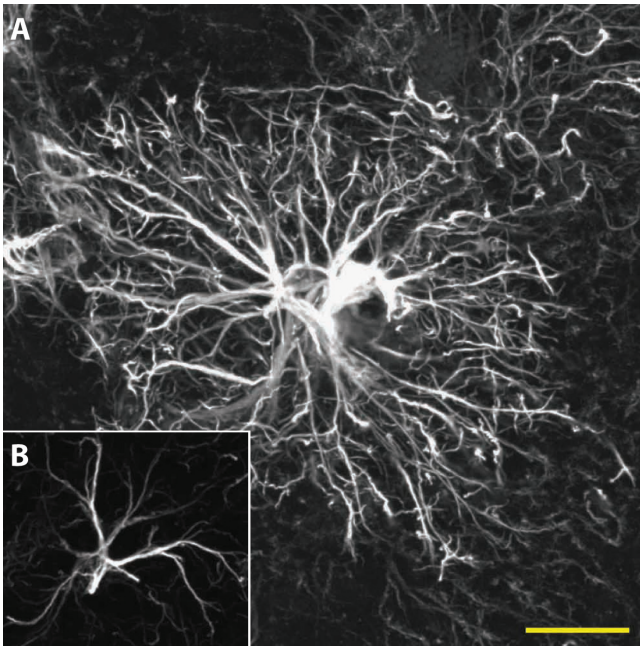

Figure 1. Human astrocytes are more complex then their rodent counterparts. Typical human (A) and mouse (B) protoplasmic astrocytes are shown at the same scale for comparison. Based on glial fibrillary acidic protein (GFAP) immunostaining, human protoplasmic astrocytes are 2.5-fold larger and project 10 times more main processes than mouse astrocytes. (GFAP, white. Scale bar, 20 μM).
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Glutamate uptake and recycling

Astrocytic processes surrounding synaptic elements express transporters for a variety of neurotransmitters and neuromodulators including glutamate, γ-aminobutyric acid (GABA), glycine, and histamine.[5-8] These transporters participate in the rapid removal of neurotransmitters released into the synaptic cleft, which is essential for the termination of synaptic transmission and maintenance of neuronal excitability. In the specific case of glutamate, its uptake by astrocytes is also crucial in protecting neurons against glutamate-induced excitotoxicity. Indeed, although glutamate is the primary excitatory neurotransmitter in the brain, overstimulation of glutamate receptors is highly toxic to neurons (reviewed in detail by Sattler and Tymianski).[18] While basal extracellular glutamate levels are maintained in the low micromolar range, they increase dramatically during glutamatergic neurotransmission, reaching up to 1 mM for a few milliseconds in the synaptic cleft.[19] This concentration of glutamate would cause extensive neuronal injury in the absence of highly efficient mechanisms for its removal at the synapse. This is primarily achieved by the astrocyte-specific sodium-dependent high-affinity glutamate transporters GLT-1 and GLAST (corresponding to human EAAT2 and EAAT1, respectively) and to a lesser extent by the neuronal glutamate transporters EAAC1 (human EAAT3) and EAAT4.[7] A number of in vitro and in vivo studies demonstrate the primary importance of astrocytic glutamate uptake in preventing glutamate-induced excitotoxicity.[20-23] A good example is provided by the phenotypical changes displayed by knockout mice for the various glutamate transporters. Indeed, knockout mice for GLT-1, considered the main astrocytic glutamate transporter, suffer lethal spontaneous seizures and selective hippocampal neuronal degeneration,[24] whereas knockout mice for the neuronal EAAC1 display no apparent neurodegeneration.[25] Interestingly, beta-lactam antibiotics have been shown to upregulate the expression of GLT-1 and to prevent neuronal loss both in vitro and in vivo in models involving excitotoxicity.[26] This suggests that modulation of the glutamate uptake capacity of astrocytes may be achievable in vivo with classical pharmacological tools, thus representing a promising therapeutic target for pathologies involving excitotoxicity.

Astrocytes also play a central role in the transfer of glutamate back to neurons following its uptake at the synapse. Failure to do so would result in the rapid depletion of the glutamate pool in presynaptic neurons and subsequent disruption of excitatory neurotransmission. This transfer is achieved by the well-described glutamate-glutamine cycle (*Figure 2*, pink box).[27,28] In short, glutamate is converted to glutamine by the astrocyte-specific enzyme glutamine synthetase (GS).[29] Glutamine is then transferred to neurons in a process most likely involving the amino acid transport systems N, L, and ASC in astrocytes and system A in neurons.[27] Glutamine is then converted back to glutamate via deamination by phosphate-activated glutaminase which is enriched in the neuronal compartment. The ammonia produced in the process is thought to be shuttled back to astrocytes following its incorporation into leucine and/or alanine.[27] It is important to note that glutamate can be metabolized in a number of different pathways in astrocytes and neurons, including oxidation in the tricarboxylic acid (TCA) cycle.[28] Astrocytes are responsible for the replenishment of brain glutamate, as they are the only neural cell type expressing pyruvate carboxylase, a key enzyme in the main anaplerotic pathway in the brain, effectively allowing them to synthesize glutamate from glucose.[30,31] This represents another level of cooperation between astrocytes and neurons.

K^+ buffering

Apart from the release of neurotransmitters which have to be rapidly removed from the synaptic cleft, neuronal activity and the resulting propagation of action potentials causes substantial local increases of extracellular potassium ions (K^+) in the restricted extracellular space. Without tight regulatory mechanisms, this could dramatically alter the neuronal membrane potential, leading to neuronal hyperexcitability and seriously compromising CNS function.[32] Such a scenario is prevented by the buffering of extracellular K^+ by glial cells[33,34] (*Figure 2*, orange box). Indeed, astrocytes have a strongly negative resting potential and express a number of potassium channels, resulting in a high membrane permeability to K^+.[35] These features, in conjunction with the action of the Na^+/K^+ ATPase, enable astrocytes to accumulate the excess extracellular K^+,[36] which can then travel in the astrocytic syncitium through gap junctions down its concentration gradient.[34,35] This allows for the spatial dispersion of K^+ from areas of high concentration to areas of lower concentration where it can be extruded either

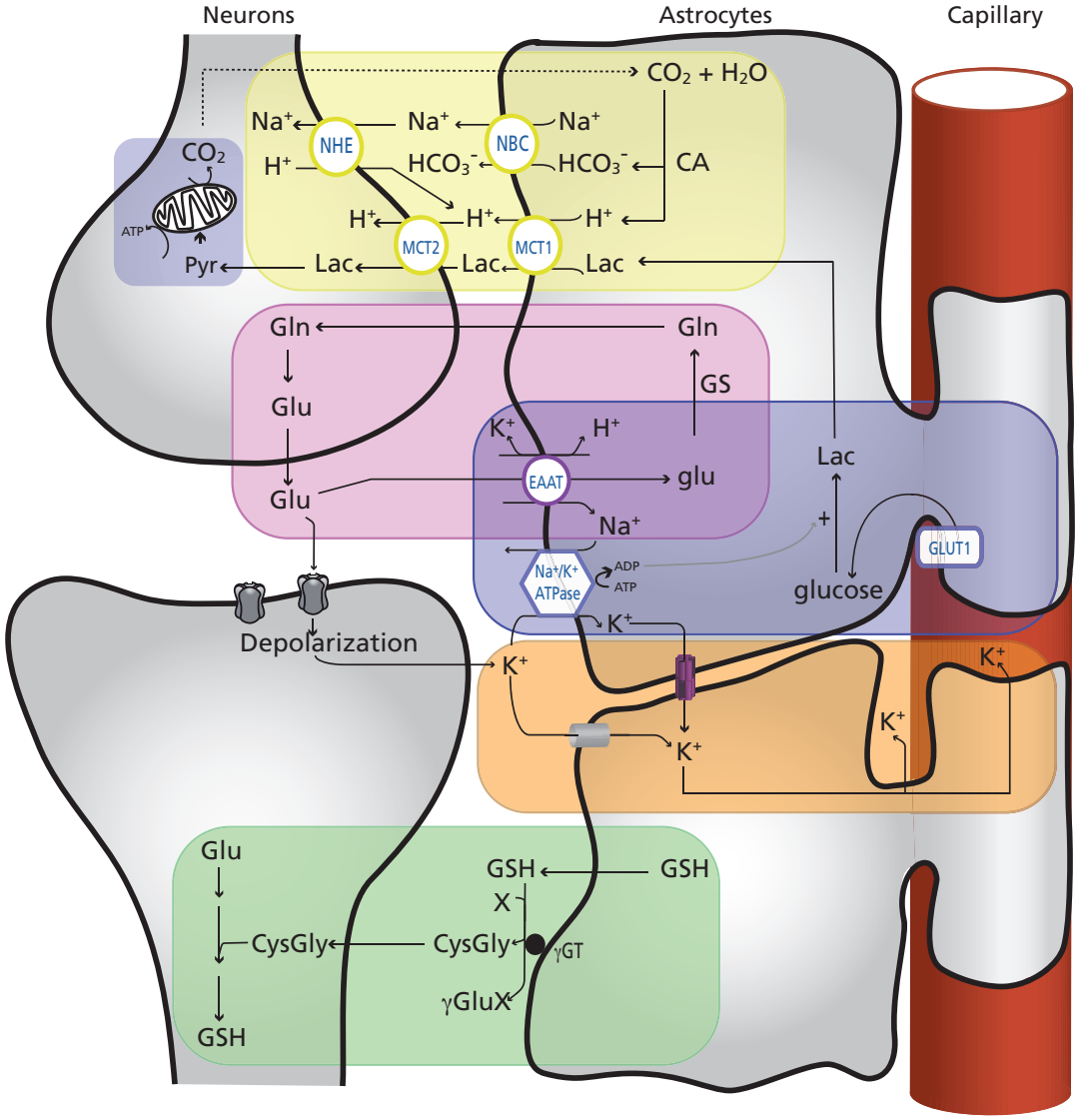

Figure 2. Simplified representation of the main roles of astrocytes in brain homeostasis. *Pink box: glutamate-glutamine cycle*. Astrocytic excitatory amino acid transporters (EAATs) are responsible for the uptake of a large fraction of glutamate at the synapse. Glutamate is converted into glutamine by glutamine synthetase (GS) and shuttled back to neurons for glutamate resynthesis. *Blue boxes: Lactate shuttle*. Glutamate uptake by astrocytes is accompanied by Na^+ entry which is counteracted by the action of the Na^+/K^+ ATPase. The resulting increase in ADP/ATP ratio triggers anaerobic glucose utilization in astrocytes and glucose uptake from the circulation through the glucose transporter GLUT1. The lactate produced is shuttled to neurons through monocarboxylate transporters (mainly MCT-1 in astrocytes and MCT-2 in neurons), where it can be used as an energy substrate after its conversion to pyruvate. *Yellow box: pH buffering*. 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^- cotransporter (NBC), thereby increasing the extracellular buffering power. Protons left in the glial compartment may drive the transport of lactate outside of astrocytes and into neurons through MCTs. Excess H^+ in neurons is extruded via sodium-hydrogen exchange (NHE). *Orange box: K^+ buffering*. Astrocytes buffer excess K^+ released into the extracellular space as a result of neuronal activity. Potassium ions travel through the astrocytic syncitium down their concentration gradient and are released in sites of lower concentration. *Green box: Glutathione metabolism*. Astrocytes release glutathione (GSH) in the extracellular space where it is cleaved by the astrocytic ectoenzyme γ-glutamyl transpeptidase (γGT). The resulting CysGly serves as a precursor for neuronal GSH synthesis. X represents an acceptor for the γ-glutamyl moiety in the reaction catalyzed by γGT.

into the extracellular space or the circulation, thus maintaining the overall extracellular K^+ concentration within the physiological range. In addition to spatial buffering, other mechanisms such as the transient storage of K^+ ions appear to contribute to the potassium-buffering capacity of astrocytes.[32]

Supply of energy substrates

Although the brain represents only 2% of the body weight, it is responsible for the consumption of an estimated 25% of all glucose in the body.[37] This disproportionate energy need compared with other organs can be largely explained by the energetic cost of maintaining the steep ion gradients necessary for the transmission of action potentials.[38] For this reason, neurons in particular have very high energy requirements, and are therefore highly dependent upon a tight regulation of energy substrate supply in order to sustain their normal function and cellular integrity.

As mentioned previously, the morphological features of astrocytes ideally position them to sense neuronal activity at the synapse and respond with the appropriate metabolic supply via their astrocytic endfeet which almost entirely enwrap the intracerebral blood vessels *(Figure 3)*. In line with this, an increasing body of evidence suggests that astrocytes play a key role in the spatiotemporal coupling between neuronal activity and cerebral blood flow (known as functional hyperemia) in a process that involves transient neurotransmitter-induced increases of $[Ca^{2+}]_i$ in astrocytes, the subsequent propagation of Ca^{2+} waves through the astrocytic syncitium and the release of vasoactive substances (such as arachidonic acid metabolites or ATP) by astrocytic endfeet.[13] Importantly, the role of astrocytes in functional hyperemia does not preclude a concerted contribution of neurons via the release of vasoactive substances such as neurotransmitters, nitric oxide, H^+, and K^+ to name a few.[39]

Although neurons can import glucose directly from the extracellular space, astrocytes have been proposed to play an instrumental role in coupling neuronal activity and brain glucose uptake through a mechanism referred to as the astrocyte–neuron lactate shuttle (ANLS) (*Figure 2*, blue boxes).[40,41] In brief, according to the ANLS, glutamate uptake into astrocytes following synaptic release causes a stimulation of anaerobic glycolysis and glucose uptake from the circulation via GLUT1, a glucose transporter expressed specifically by glial and capillary endothelial cells in the brain.[42] Lactate produced by astrocytes as an end result of glycolysis is released into the extracellular space and taken up by neurons via monocarboxylate transporters (MCTs) expressed on astrocytes and neurons.[42] Once into neurons, lactate can be used as an energy substrate via its conversion to pyruvate by the action of lactate

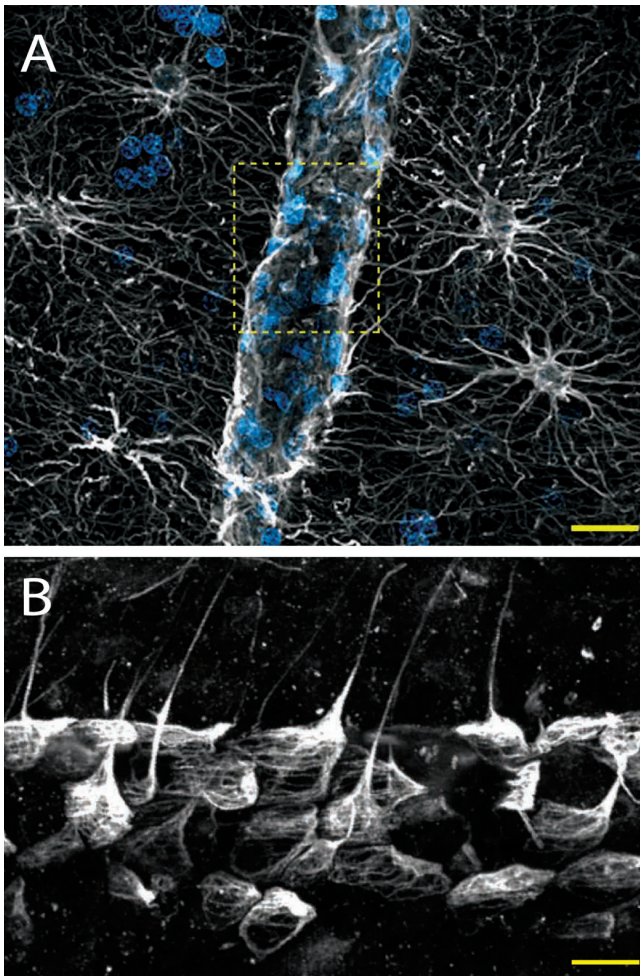

Figure 3. Astrocytic endfeet in humans. **(A)** Protoplasmic astrocytes project specialized processes towards the intraparenchymal vasculature (part of a blood vessel is highlighted in the yellow box) (glial fibrillary acidic protein – (GFAP), white; nuclei (4',6-diamidino-2-phenylindole - DAPI), blue. Scale bar, 20 μM). **(B)** Astrocytic endfeet are in close contact with blood vessels and almost entirely cover their surface (GFAP, white. Scale bar, 20 μM).

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dehydrogenase and subsequent oxidation in the mitochondrial TCA cycle. The existence of a lactate shuttle between astrocytes and neurons is supported by a number of experimental studies (reviewed in ref 41). For instance, in an elegant study by Rouach and colleagues,[43] it was recently demonstrated that 2-NBDG (a fluorescent glucose analogue) injected into a single astrocyte in hippocampal slices traffics through the astrocytic network as a function of neuronal activity. The diffusion of 2-NBGD across the astrocytic syncitium was indeed reduced when spontaneous neuronal activity was inhibited with tetrodotoxin, whereas increasing neuronal activity by means of epileptiform bursts or stimulation of the Schaffer collaterals resulted in the trafficking of 2-NBDG to a larger number of astrocytes.[43] They next went on to show that during glucose deprivation which resulted in a 50% depression of synaptic transmission in hippocampal slices, glucose delivery into a single astrocyte and its subsequent (and necessary) diffusion through the astrocytic syncitium could rescue neuronal activity. This effect was mimicked by lactate but was abolished in the presence of the MCT inhibitor α-cyano-4-hydroxycinnamic acid (4-CIN), demonstrating that glucose present in the astrocytic network is metabolized to lactate, transported out of astrocytes, and used by neurons to sustain their activity.[43] Interestingly, lactate has also been shown to preserve neuronal function in experimental models of excitotoxicity,[44] posthypoxic recovery,[45,46] cerebral ischemia,[47] and energy deprivation,[48] highlighting the importance of astrocyte-derived lactate for neuronal function and viability.

Another key feature of astrocytes is their capacity to store glucose in the form of glycogen. Indeed, in the CNS glycogen is almost exclusively present in astrocytes and virtually constitutes the only energy reserve.[37,49] Interestingly, it has recently been demonstrated that neurons also possess the enzymatic machinery to synthesize glycogen, but that it normally is tightly suppressed.[50] Failure to do so results in neuronal apoptosis, suggesting that intracellular glycogen is actually toxic to neurons.[50] In astrocytes, glycogen can be rapidly mobilized in response to neuronal activity.[51,52] The glycosyl units resulting from glycogen breakdown are fed into the glycolytic pathway of astrocytes, and released into the extracellular space in the form of lactate which can be used to face the transiently elevated energy requirements associated with neuronal activation.[49,52-54] Storage of energy in the form of glycogen is also essential for the preservation of neuronal viability in situations where glucose becomes scarce. For example, it has been demonstrated that brain glycogen levels are increased following mild hypoxic preconditioning in vivo, resulting in significant protection from brain damage as a result of subsequent cerebral hypoxic-ischemic injury.[55]

Beyond lactate, it is of interest to note that astrocytes may also transfer other energy substrates to neurons. Indeed, evidence suggests that in certain conditions, astrocytes may be able to metabolize fatty acids or leucine to produce ketone bodies which are know to be readily used by neurons as an energy substrate.[56-58] It has been suggested that this pathway may also serve a neuroprotective purpose by scavenging nonesterified phospholipids which can lead to the production of proapoptotic sphingolipids.[58,59]

pH buffering

Another instrumental function of astrocytes in supporting proper neuronal function is their contribution to pH regulation of the brain microenvironment (*Figure 2*, yellow box).[60-62] Several neuronal processes are strongly affected by relatively small shifts in pH, including energy metabolism, membrane conductance, neuronal excitability, synaptic transmission, and gap junction communication.[60,62] The main feature of glial cells, endowing them with a high pH buffering capacity, is their enriched expression of carbonic anhydrase (CA) which converts CO_2 into H^+ and HCO_3^-—effectively allowing them to act as a CO_2 sink. Indeed, CA is preferentially expressed in astrocytes and oligodendrocytes,[63,64] although low activity levels are also observed in neurons and in the extracellular space.[62] A coupling mechanism which integrates synaptic transmission, pH regulation, and energy supply between neurons and glia has been proposed by J. W. Deiter.[61,65] According to this model, during periods of high neuronal activity, the CO_2 produced by elevated (mostly neuronal) oxidative metabolism diffuses into glial cells and is converted to H^+ and HCO_3^- by the action of glial CA. Two HCO_3^- can then be transported into the extracellular space along with one Na^+ via the Na^+- HCO_3^- cotransporter (NBC), thereby increasing the extracellular buffering power. The protons left in the glial compartment could be used to drive the transport of lactate outside of astrocytes through MCT-1 and -4 and its subsequent transport by MCT-2 into neurons, since MCTs exploit proton gradients for the transport of

lactate.[41,61] As previously discussed, according to the ANLS hypothesis, this lactate can then be used as an energy substrate by neurons.[40,41] Alternatively, protons released into the extracellular space may also be reconverted to CO_2 and water by the action of extracellular CA at the expense of one HCO_3^-.[61] This model suggests that pH buffering taking place in glial cells during neuronal activation may also act cooperatively to: i) contribute, via the Na^+- HCO_3^- cotransporter, to the extrusion against its concentration gradient of the excess intracellular Na^+ resulting from glutamate uptake in astrocytes, thereby alleviating the metabolic burden on the glial Na^+/K^+ ATPase; and ii) drive the efflux of lactate which is produced in response to glutamate uptake in astrocytes, thus providing an energy substrate for the neuronal TCA cycle.[61,65]

Defense against oxidative stress

Oxidative stress occurs as a result of an imbalance between the production of reactive oxygen species (ROS) and antioxidant processes. It is known to be involved in a number of neuropathological conditions, including neurodegenerative diseases, traumatic brain injury, and stroke,[66] suggesting that the CNS is particularly vulnerable to oxidative injury. This can be explained by the brain's high rate of oxidative energy metabolism (which inevitably generates ROS), combined with a relatively low intrinsic antioxidant capacity.[67] Compared with neurons, astrocytes display a much more effective artillery against ROS. Accordingly, cooperative astrocyte-neuron defense mechanisms against oxidative stress seem to be essential for neuronal viability.[68] This is supported by a number of studies demonstrating that when cultured in the presence of astrocytes, neurons show increased resistance to toxic doses of nitric oxide,[69,70] hydrogen peroxide,[71-73] superoxide anion combined with nitric oxide,[69,74] or iron.[69,74]

This neuroprotective capacity of astrocytes may derive from the fact that they possess significantly higher levels of a variety of antioxidant molecules (including glutathione, ascorbate, and vitamin E) and display greater activities for ROS-detoxifying enzymes (including glutathione S-transferase, glutathione peroxidase, and catalase).[68,72,75-78] In addition, it appears that astrocytes may also play an active role in preventing the generation of free radicals by redox active metals, as they participate in metal sequestration in the brain.[79] This is achieved in part through their high expression levels of metallothioneins and ceruloplasmin, which are involved in metal binding and iron trafficking, respectively.[80-82]

Glutathione (GSH) is the most important antioxidant molecule found in the brain.[83] This thiol compound can act as an electron donor, and thus fulfills its antioxidant role either by directly reacting with ROS or by acting as a substrate for glutathione S-transferase or glutathione peroxidase. Both neurons and astrocytes can synthesize the GSH tripeptide (L-glutamyl-L-cysteinylglycine) by the sequential action of glutamate cysteine ligase and glutathione synthetase. However, neurons are highly dependent on astrocytes for their own GSH synthesis, as illustrated by the fact that GSH levels are higher in neurons when they are cultured in the presence of astrocytes.[84] Astrocytes release GSH in the extracellular space, where it is cleaved by the astrocytic ectoenzyme γ-glutamyl transpeptidase (γGT) to produce CysGly, which can then be taken up by neurons directly or after undergoing further cleavage by extracellular neuronal aminopeptidase N to form glycine and cysteine.[83] This shuttling of GSH between astrocytes and neurons is essential in providing precursors for neuronal GSH synthesis (*Figure 2*, green box). This is especially true for cysteine, the rate-limiting substrate for GSH synthesis, since neurons, unlike astrocytes, cannot use the cysteine-oxidation product cystine as a precursor.[83] The importance of this cooperative process for neuronal defense against oxidative stress is evidenced by the reduced ability of GSH-depleted astrocytes to protect neurons against oxidative injury.[85,86] Conversely, increasing the capacity to synthesize GSH specifically in astrocytes by increasing their capacity to uptake cystine significantly enhances the neuroprotective effect of astrocytes against oxidative stress.[87]

The recycling of ascorbate is another example of cooperation between astrocytes and neurons for antioxidant defense. Ascorbate can directly scavenge ROS, and is also an important cofactor for the recycling of oxidized vitamin E and GSH.[68] Astrocytes are responsible for the uptake of the oxidation product of ascorbate, dehydroascorbic acid, from the extracellular space and its recycling back to ascorbic acid. The latter can then either be used intracellularly in astrocytes, or released into the extracellular space to be utilized by neurons for their own antioxidant defense.[68]

Astrocytes in the diseased brain: a fine balance

Considering the extensive functional cooperativity that exists between neurons and astrocytes, one can expect that alterations of astrocytic pathways in response to pathological stimuli will result in (or at least contribute to) neuronal dysfunction. Interestingly, several neurological diseases share common pathogenic processes, such as oxidative stress, excitotoxicity, metabolic failure, or inflammation—many of which are known to be counteracted by the function of astrocytes in the normal brain (see previous sections). This may reflect a common underlying phenomenon by which disease progression is associated with chronic and/or escalating harmful stimuli that eventually exhaust the neuroprotective mechanisms of astrocytes. Even worse, deleterious pathways may then be turned on in astrocytes, directly contributing to the pathogenic process. A role of astrocytes has been described in a number of brain pathologies, and a complete review is beyond the scope of this article (see refs 88-90). Instead, we focus on three pathological processes that well illustrate the dual role of astrocytes in neuroprotection and neurotoxicity, namely neuroinflammation, Alzheimer's disease, and hepatic encephalopathy.

Neuroinflammation

The brain can mount an immune response as a result of various insults such as infection, injury, cellular debris, or abnormal protein aggregates. In most cases, it constitutes a beneficial process aiming to protect the brain from potentially deleterious threats. In some situations, however, the insult may persist and/or the inflammatory process may get out of control. Chronic neuroinflammation sets in as a result, and may negatively affect neuronal function and viability, thus contributing to disease progression. Neuroinflammation has indeed been implicated in several neuropathologies including Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, and stroke.[91]

While microglial cells are generally considered the main resident immune cells of the brain, it is important to note that astrocytes are immunocompetent cells as well, and that they act as important regulators of brain inflammation. Like microglia, astrocytes can become activated—a process known as astrogliosis, which is characterized by altered gene expression, hypertrophy, and proliferation.[92] Activated astrocytes can release a wide array of immune mediators such as cytokines, chemokines, and growth factors, that may exert either neuroprotective or neurotoxic effects.[93] Additionally, activated astrocytes can release potentially deleterious ROS and form a glial scar which may impede axon regeneration and neurite outgrowth.[94] This has led to considerable debate as to whether activation of astrocytes is beneficial or detrimental to neighbouring neurons. The most likely answer is that it is neither exclusively one nor the other, and that the overall consequences of an immune activation of astrocytes is the result of a complex interplay between pro- and anti-inflammatory—as well as neurotoxic and neurotrophic—processes.

Cytokines, for instance, are major effectors in this fine balance as they exert a dual role, potentially sustaining or suppressing neuroinflammation (hence their traditional labeling as pro- or anti-inflammatory). In this regard, dissecting out the exact neuroprotective and neurotoxic contributions of astrocytes in neuroinflammatory processes has proven to be extremely challenging because they are capable of releasing such an extensive repertoire of cytokines in response to various stimuli (some examples include interleukin (IL)-1β, TNFα, IL-6, IL-10, IL-15, INFβ, and TGFβ).[93] Adding another level of complexity, astrocytes express several cytokine receptors and can therefore also be a target of cytokine signaling through autocrine or paracrine mechanisms.[11]

While cytokines are categorized as proinflammatory or anti-inflammatory, understanding their exact individual effect is far more complex, as many of them interact with each other (either antagonistically or synergistically) and may additionally have pleiotropic effects.[11,95] As a result, cytokines can potentially mediate both neuroprotective and neurotoxic processes at once. For example, ample evidence indicates that IL-1β may exacerbate neuronal injury both in vivo and in vitro.[96-99] In contrast, IL-1β has also been implicated in neuroprotective processes such as remyelination,[100] blood-brain barrier repair,[101] ischemic tolerance,[102] and neurotrophic factor production.[103-106] Importantly, astrocytes can themselves respond to IL-1β by releasing a number of potentially neuroprotective trophic factors such as nerve growth factor (NGF), ciliary neurotrophic factor (CNTF), glial cell-line derived neurotrophic factor GDNF, and fibroblast growth factor (FGF)-2.[11,107-109]

Taken together, studies such as those mentioned above provide important information about the multiple

effects of individual cytokines. However, they also have major limitations, in that they can only take into account a few pro- and anti-inflammatory pathways at a time. As such, they may only reflect a small fraction of an infinitely more intricate process in which astrocytes take part. For this reason, the use of genetically manipulated animal models specifically preventing the proliferation of reactive astrocytes or the activation of their core inflammatory pathways, has provided important new insight into their overall role in response to brain injury. For instance, it has been demonstrated that the selective attenuation of astrocytic proinflammatory processes, through genetic inactivation of the transcription factor NF-κB specifically in this cell type, affords substantial neuroprotection following spinal cord injury.[110] By contrast, using a transgenic mouse model in which dividing reactive astrocytes were selectively ablated, Sofroniew and colleagues have demonstrated that following various types of brain injury, reactive astrocytes play an essential role in temporally and spatially restricting neuroinflammation, as well as in promoting blood-brain barrier repair, limiting brain edema, and preserving neuronal viability.[94,111-113]

Consistent with a role of astrocytes in containing neuroinflammation, it is interesting to note that astrocytes appear to participate in the suppression of microglial activation through negative feedback loops. Activated microglial cells release high levels of proinflammatory cytokines and toxic ROS which may negatively impact neuronal survival.[114] Several in vitro studies have demonstrated that astrocyte-conditioned medium or the presence of astrocytes attenuates microglial activation in response to various proinflammatory stimuli.[115-117] The exact nature of the astrocyte-derived factors involved has not been fully elucidated, but transforming growth factor (TFG)β is thought to contribute to this process.[115] This may in part explain the neuroprotective effect of TGFβ in experimental models of excitotoxicity or ischemia.[118-120]

To summarize, if inflammatory activation of astrocytes unquestionably has consequences for neuronal function and viability, it must be emphasized that the overall effect is dependent on the fine balance between a number of factors including the type, duration, and severity of the insult, the complex interplay between the various cytokines released by astrocytes and surrounding cells, and the receptors for cytokines and growth factors expressed by these neighboring cells.

Alzheimer's disease

Alzheimer's disease (AD), the most prevalent neurodegenerative disorder, is characterized by the progressive decline of cognitive functions including memory and mental processing, and by disturbances in behavior and personality.[121] Typical histopathological features of the AD brain are amyloid-β (Aβ) plaques which may contain dystrophic neurites, intracellular neurofibrillary tangles, vascular amyloidosis, neuronal and synaptic loss, and reactive gliosis. Though the exact pathophysiological mechanisms leading to synaptic loss and the resulting cognitive decline have not been fully elucidated, a central role of Aβ peptides in concert with neuroinflammation is generally accepted.[122] Alois Alzheimer himself in 1910 suggested that glial cells may participate in the pathogenesis of dementia[123]; however, their exact role is still a matter of debate, as available evidence can argue both for neuroprotective or neurotoxic effects.

Reactive astrocytes, like microglia, are observed in close association with Aβ plaques in the brains of AD patients,[124,125] and both cell types have been shown to be capable of internalizing and degrading Aβ peptides.[126-129] This is thought to be a neuroprotective mechanism by contributing to the clearance of Aβ from the extracellular space, thus avoiding the accumulation of toxic extracellular Aβ. Several observations support an active role of astrocytes in Aβ clearance. For example, astrocytes surrounding plaques in autopsy material from the brain of AD patients contain intracellular Aβ deposits.[128,130] In addition, when exogenous astrocytes were transplanted into the brain of Aβ plaque-bearing transgenic mice, they migrated towards Aβ deposits and internalized Aβ-positive material.[129] Similarly in ex vivo studies, binding, internalization, and degradation of Aβ could be observed when cultured astrocytes were seeded on top of plaque-bearing sections prepared either from the brains of AD patients or transgenic mice models of AD.[127,129] The physiological importance of Aβ clearance by glial cells in vivo is evidenced by the increased Aβ accumulation and premature death observed in a transgenic mouse model of AD when microglial activation was impaired.[131] Interestingly, glial cell activation and astrocytic accumulation of Aβ can be observed even preceding plaque formation,[128,132] suggesting that astrocyte cells attempt to scavenge Aβ early in the progression of the disease, which likely reflects an effort to limit its extracellular deposition.

Although their contribution to the clearance of Aβ deposits is thought to be protective, there is also evidence to suggest that microglia and astrocytes contribute to the progression of AD. One obvious explanation is that the physiological functions of astrocytes may be directly affected by Aβ. For instance, in a elegant study using fluorescence imaging microscopy in live mice bearing AD-like pathology, intracellular Ca^{2+} signaling was reported to be abnormally increased in astrocytes, sometimes propagating as intracellular calcium waves.[133] These Ca^{2+} transients were only observed after the mice developed senile plaques and were uncoupled from neuronal activity, suggesting that Aβ interacts directly with the astrocytic network.[133]

The involvement of glial cells in the pathogenesis of AD is supported by several in vitro studies demonstrating that their interaction with Aβ impairs neuronal viability or worsens the neurotoxic effect of Aβ.[134-138] Upon their activation by Aβ, astrocytes and microglia can release a number of inflammatory mediators which may be toxic for surrounding neurons. Examples include proinflammatory cytokines such as IL-1β and IL-6, and reactive oxygen and nitrogen species (RN/ROS) such as NO and $O_2^{\cdot-}$.[132,139-143] Proinflammatory cytokines have been shown to exacerbate the microglial response to Aβ and to enhance its neurotoxic effects.[144-146] Moreover, it appears that proinflammatory cytokines can also increase the expression of the amyloid precursor protein and its processing through amyloidogenic pathways.[147-149] Aβ accumulation may therefore establish a vicious circle whereby neuronal stress and glial activation initiates an inflammatory response, which in turn promotes the synthesis and accumulation of more Aβ, thus perpetuating glial cell activation. This may in part explain why age is the most important risk factor for developing AD since increased neuroinflammation is associated with normal aging.[150] This enhancement of the basal inflammatory state, together with the gradual accumulation of Aβ which is also seen in the normal aging brain, may provide the trigger necessary for this vicious circle to set in. Because of their central role in neuroinflammation (see previous section), glial cells may provide a valuable therapeutic target for the treatment of AD. This is supported by studies testing newly identified anti-inflammatory molecules which selectively suppress pro-inflammatory cytokines production in glia, resulting in a significant attenuation of synaptic dysfunction and neurodegeneration and in behavioral improvements in experimental models of AD.[151,152]

Besides proinflammatory cytokines, RN/ROS produced by activated astrocytes and microglia may contribute to disease progression by inducing oxidative stress, a hallmark of AD.[142,153] Astrocytes have been proposed to take part in this process. For example, Aβ causes intracellular Ca^{2+} transients and stimulates the production of ROS by NADPH oxidase in astrocytes but not in neurons.[154-156] In mixed cultures, these effects were accompanied by decreases in GSH levels in both astrocytes and neurons, resulting in neuronal cell death.[154-156]

Conversely, in the presence of microglia, astrocytes may provide significant protection through the negative regulation of microglial reactivity following exposure to Aβ.[137,157] However, this must be interpreted with caution since, as previously discussed, increased microglial phagocytosis associated with their activated state may be neuroprotective. In line with this, microglial phagocytosis was shown to be markedly suppressed in the presence of astrocytes, which resulted in increased persistence of senile plaques when presented to microglia in vitro.[158]

In summary, the apparently conflicting roles of astrocytes in the progression of AD may be explained by the coexistence of potentially protective and deleterious pathways in activated astrocytes. As the disease progresses, the overwhelming combined effect of Aβ accumulation, neuroinflammation, and oxidative stress may tip the scales away from the neuroprotective functions of astrocytes and towards the activation of deleterious pathways.

Hepatic encephalopathy

Hepatic encephalopathy (HE), a neuropsychiatric syndrome occurring as a result of chronic or acute liver failure, is one of the first identified neurological disorders involving astroglial dysfunction as its primary cause. In its acute form, the symptoms of HE can progress rapidly from altered mental status to stupor and coma, and may cause death within days. The most important cause of mortality in acute liver failure is brain herniation, which occurs as a result of cytotoxic swelling of astrocytes, leading to intracranial hypertension.[159] Although HE is a multifactorial disorder, ammonia is thought to play a central role in its pathogenesis.[159] Ammonia rapidly accumulates in the blood as a result of acute liver failure and can readily cross the blood-brain barrier. Because the brain does not possess an effective urea cycle, it relies

almost exclusively on glutamine synthesis for the detoxification of ammonia.[159] As mentioned before, this is accomplished by the enzyme glutamine synthetase (GS) which is exclusively localized in astrocytes.[29] Ammonia detoxification is an essential homeostatic function of astrocytes, as excess hyperammonemia has profound effects on various brain functions.[159] However, the astrocytic accumulation of osmotically active glutamine as a result of ammonia detoxification is thought to contribute at least in part to the swelling of astrocytes in hyperammonemic conditions. This is supported by the demonstration that inhibition of GS with methionine sulfoxide prevents brain edema in experimental hyperammonemia.[160] Alternatively, glutamine may also induce astrocytic swelling via other mechanisms, including oxidative and nitrosative stress.[161] Interestingly, glutamine efflux from asctrocytes through the system N transporter appears to be negatively regulated by elevated extracellular glutamine in hyperammonemic conditions.[162] Such a mechanism may contribute to trap glutamine in astrocytes and promote swelling.

In contrast with its acute form, chronic hepatic encephalopathy, which is associated with more modest increases in brain ammonia, does not result in overt cerebral edema,[163] suggesting the existence of compensatory mechanisms taking place in astrocytes in order to prevent excessive swelling. This is thought to be accomplished by the release of osmolytes such as taurine and myo-inositol by astrocytes in response to glutamine accumulation. However, it appears that when osmolyte pools are depleted as a result of excessive hyperammonemia, for example during acute liver failure, this protective mechanism is exhausted and astrocytes swell as a result. This, together with an impaired capacity of astrocytes to fulfill their role in ammonia detoxification, seriously compromises brain function in acute liver failure.

Conclusion

Astrocytes are known to be the most important neural cell type for the maintenance of brain homeostasis. It is safe to assume that, as technology advances in the years to come, we will continue to uncover the multiple facets of astroglia. It has already become quite clear however that it is unrealistic to approach brain function and dysfunction from a uniquely neuronal standpoint. Because of their involvement in such a wide range of homeostatic functions, any brain insult is likely to have an impact on astrocytes. Their capacity to adapt to these changes weighs heavily in the fine balance between neuroprotection and neurotoxicity as illustrated by the three neuropathological conditions discussed above. In this context, understanding astrocytic function is key to providing a better grasp of brain function in general and how it may go awry. This may lead to the identification of better suited therapeutic targets, as they should take into account the multiple interactions and interdependencies between neural cell types. ❑

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El papel de la astroglía en la neuroprotección

Los astrocitos constituyen el principal tipo celular neural responsable del mantenimiento de la homeostasis cerebral. Ellos forman áreas anatómicas altamente organizadas que están interconectadas en extensas redes. Estas características, junto con la expresión de una gran variedad de receptores, transportadores y canales iónicos, los favorece de manera ideal para detectar y modular dinámicamente la actividad neuronal. Los astrocitos cooperan con las neuronas a varios niveles, incluyendo el tránsito y reciclaje de neurotransmisores, la homeostasis iónica, la neuroenergética y la defensa contra el estrés oxidativo. Las neuronas dependen en forma crítica de su soporte constante, lo que le confiere a los astrocitos propiedades neuroprotectoras intrínsecas, las cuales también se discuten aquí. A la inversa, los estímulos patogénicos pueden alterar la función astrocítica, comprometiendo así la funcionalidad y la viabilidad neuronal. Se utilizan como ejemplos la neuroinflamación, la Enfermedad de Alzheimer y la encefalopatía hepática para discutir cómo los mecanismos de defensa de los astrocitos pueden estar sobrepasados en las condiciones patológicas, lo que contribuye a la progresión hacia la enfermedad.

Rôle de l'astroglie dans la neuroprotection

Les astrocytes sont le principal type de cellules neuronales responsables de l'entretien de l'homéostasie cérébrale. Ils s'interconnectent en réseaux étendus, formant des régions anatomiques très organisées. Cette organisation qui s'accompagne de toute une série de récepteurs, transporteurs et canaux ioniques, les met en position idéale pour pressentir et moduler de façon dynamique l'activité neuronale. Les astrocytes coopèrent avec les neurones à différents niveaux, dont le recyclage et la circulation des neurotransmetteurs, l'homéostasie ionique, la neuroénergétique et la défense contre le stress oxydant. Les neurones sont très dépendants du soutien constant des astrocytes, ce qui donne à ces derniers des propriétés neuroprotectrices que nous analysons dans cet article. Á l'opposé, lorsque des stimuli pathogènes troublent la fonction astrocytaire, la fonctionnalité et la viabilité des neurones sont compromises. En prenant pour exemples la neuro-inflammation, la maladie d'Alzheimer et l'encéphalopathie hépatique, nous montrerons comment les mécanismes de défense astrocytaires peuvent être débordés en situation pathologique, participant ainsi à la progression de la maladie.

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