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Astroglia are the homoeostatic cells of the central nervous system, which participate in all essential functions of the brain. Astrocytes support neuronal networks by handling water and ion fluxes, transmitter clearance, provision of antioxidants, and metabolic precursors and growth factors. The critical dependence of neurons on constant support from the astrocytes confers astrocytes with intrinsic neuroprotective properties. On the other hand, loss of astrocytic support or their pathological transformation compromises neuronal functionality and viability. Manipulating neuroprotective functions of astrocytes is thus an important strategy to enhance neuronal survival and improve outcomes in disease states.

INTRODUCTION

The definition of “an astrocyte” is complicated and continues to be shaped by the ongoing discoveries involving glia in the central nervous system (CNS). The word “astrocyte” was originally applied by Michael von Lenhossek in 1891 to the numerous star-shaped cells observed in histological brain specimens, once it had been realized that “neuroglia” (brain glue) was composed of individual cellular elements (Lenhossek, 1891). Astrocytes are now recognized as a heterogenous population based on their morphology and the expression of different sets of receptors, transporters, ion channels, and other proteins (Matyash and Kettenmann, 2010; Oberheim et al., 2012; Bribian et al., 2015). This raises the intriguing possibility that different subtypes and lineages of astrocytes could be implicated in distinct metabolic/homeostatic functions and suggests that astrocytes can be specialized to serve the functions of various neuronal subpopulations. Moreover, recent studies suggest that astrocytes may exist in different, fairly stable functional states in terms of their interaction with neurons and, under some conditions, may become dangerously neurotoxic (Liddelow et al., 2017; Lin et al., 2017).

If astrocytes are heterogenous, how do we unequivocally distinguish them from other cell types? Mature astrocytes are usually anatomically classified into two general categories: protoplasmic and fibrous (Sofroniew and Vinters, 2010; Oberheim et al., 2012). Protoplasmic astroglial cells are found in gray matter tissue in close association with neurons. They possess a larger quantity of organelles and have relatively thicker, shorter, highly branched processes. Some of these processes closely associate with neuronal synapses, while others extend toward blood vessels. Fibrous astrocytes are present in white matter and have relatively few organelles, fewer but longer processes that extend along axon bundles, providing structural and metabolic support for the axonal tracts. For decades, identification of astrocytes was based on the use of antibodies that recognize astrocyte-specific proteins, but immunohistochemistry is now increasingly supplemented by the use of the promoters of some of these genes to drive expression of various marker proteins in these cells without staining and even in the brain in situ. By far the most widely used immunohistochemical marker is glial fibrillary acidic protein (GFAP), which was the first widely accepted marker (Brenner et al., 1994; Lee et al., 2008) and has stood the test of time well. Up to this day, cells that robustly express GFAP are

**SIGNIFICANCE**

Astroglia participate in all essential functions of the brain. The critical dependence of neurons on constant support from the astrocytes confers astrocytes with intrinsic neuroprotective properties. Manipulating neuroprotective functions of astrocytes is an important strategy to enhance neuronal survival and improve outcomes in disease states.

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commonly identified as astrocytes. The converse, however, does not appear to be correct. Many mature astrocytes do not express detectable levels of GFAP (e.g., in the rodent cortex), and GFAP expression by astrocytes exhibits both regional and local variability that is dramatically regulated by a large number of inter- and intracellular signaling molecules (Morrison et al., 1985; Lee et al., 2006; Oberheim et al., 2012). Therefore, it is important to recognize the limitations of GFAP as an astrocyte marker (Kimelberg, 2004; Sofroniew, 2009). Other molecular markers that have been used for immunohistochemical identification of astrocytes include glutamine synthetase (GS), the Ca^{2+}-binding protein S100B, and the glutamate transporters EAAT1 and EAAT2 (excitatory amino acid transporters, in humans), known as GLAST (glutamate aspartate transporter) and GLT1 (glutamate transporter 1) in rodents (Norenberg, 1979; Goncalves et al., 2008). Several recent studies have conducted large-scale genetic analyses of the astrocyte transcriptomes in rodents and humans and have identified large numbers of molecules enriched in astrocytes (Cahoy et al., 2008; Zhang et al., 2014). Such studies help to identify potential candidates for new and possibly better molecular markers for astrocytes. An interesting addition to the pool of astrocytic markers is SOX9, a transcriptional factor that has been reported as selective for astrocytes in most brain areas (Sun et al., 2017). This protein is characteristically accumulated in the nuclei of the cells, which sometimes is very useful when, for example, one needs to count astrocytes, which is not a trivial task using antibodies against GFAP that predominantly stain processes, creating a mesh-like staining pattern.

An alternative to reliance on immunocytochemical staining of markers is to express a fluorescent molecule, such as GFP, using astrocyte-specific promoters. This technique allows the cells to be identified and studied in the living tissue. Several promoters have been used to achieve this effect, some driving EGFP directly, others driving expression of CRE, which requires a cross with a CRE-dependent reporter mouse. GFAP promoter was the first one used for this purpose (Brenner et al., 1994; Nolte et al., 2001), and mice based on this promoter are still used widely, even though some publications have questioned the specificity of expression in these animals (Fujita et al., 2014). More recently, however, other (perhaps better) drivers of highly specific expression were found for astrocytes. For example, very recent studies employed Aldh1l1-EGFP mice (Liddelow et al., 2017; Lin et al., 2017). We would like to emphasize that expression of the proteins responsible for most cellular activities changes under various conditions, and therefore the activity of all promoters is variable; this can affect the interpretation of the results.

Electrophysiological approaches played an important part in the study of neurons but less so in the identification and investigation of astrocytes. Because of their leaky K^+ channels, mature astrocytes have very negative membrane potentials and are electrically passive (i.e., do not generate action potentials). Very low membrane resistance also means that recordings made from the somata of astrocytes do not reveal local currents mediated by events in their remote processes. However, the lack of action potentials in astrocytes does not mean they are physiologically “silent.” To the contrary, astrocytes are highly chemically excitable cells. Astrocytes express numerous metabotropic receptors and commonly respond to signaling molecules (neurotransmitters) by dynamic changes in the cytoplasmic concentration of two cations, Ca^{2+} and Na^+. (Agulhon et al., 2008; Gourine et al., 2010; Parpura and Verkhratsky, 2012; Turovsky et al., 2016). Neuronal activity can trigger complex spatiotemporal changes of [Ca^{2+}]_i and [Na^+]_i in astrocytes and generate propagating Ca^{2+} or Na^+ waves, which in turn regulate multiple effector pathways in these glial cells (Bernardinelli et al., 2004; Agulhon et al., 2008, 2012; Gourine et al., 2010; Rose and Verkhratsky, 2016). Astrocytes are also highly mechanosensitive, a well-known feature whose physiological significance is not yet understood (Araque et al., 1998; Paemeleire and Leybaert, 2000; Maneshi et al., 2017).

It is becoming increasingly clear that even though gray matter astrocytes from various parts of the brain may look indistinguishable, they are, in fact, physiologically diverse. For example, we demonstrated that astrocytes located on the ventral surface of the medulla are involved in central respiratory control and highly sensitive to subtle changes in pH, but cortical astrocytes are not (Gourine et al., 2010). More recently, we found that this sensitivity to pH could be mediated by the electrogenic Na^+/HCO_3^- cotransporter NBCe1, which is enriched in this population of astrocytes and is responsible for the acidification-induced influx of Na^+, eventually leading to Ca^{2+} elevation and release of ATP (Turovsky et al., 2016).

Attempts to stratify astrocytes are ongoing. Recently, using astrocytes isolated from the Aldh1l1-EGFP reporter mouse, Lin et al. demonstrated that by using antibodies against CD51, CD63, and CD71, it is possible to subdivide astrocytes into several categories with fluorescence-activated cell sorting based on combinations of expression of these three markers (Lin et al., 2017). In their experiments, five classes of cells were separated using this combinatorial approach. They were also different in terms of their transcriptomes and their ability to support synaptogenesis. Moreover, to some extent these molecular signatures could be even traced in malignant gliomas, potentially indicating a relationship of some astrocyte populations with glioma. It would be very interesting to know whether there are differences in the neuroprotective potential of these proposed subpopulations of astrocytes. Another obvious question concerns the extent to which the genetic profile of the astrocyte is set by the neurons with which it directly interacts, and whether astrocytes can be fine-tuned to “look after” various types of neurons (e.g., cortical pyramidal neurons) versus interneurons versus dopaminergic neurons, and so forth. A very elegant approach to this question has been recently described by Hasel et al., who reported that neuronal activity shapes the genomic profile of co-cultured astrocytes (Hasel et al., 2017). The idea in that study was to use co-cultures of neurones and astrocytes from different
species. This allows in silico discrimination of the changes in gene expression in individual cell populations using next-generation sequencing by mapping individual transcripts to one of the two species. Since the origins of astrocytes and neurons are known, the authors could allocate altered genes to one or the other cell type.

**NEUROPROTECTIVE MECHANISMS IN ASTROCYTES**

Astrocytes Take Up Neurotransmitters

Gamma-aminobutyric acid (GABA) and glutamate are the main fast inhibitory and excitatory neurotransmitters in the mammalian brain that are released mainly from presynaptic boutons/terminals (Danbolt, 2001; Foster and Kemp, 2006; Schousboe and Waagepetersen, 2007). Transmitter concentration determines the extent of receptor activation and signal transmission. It is of critical importance that the resting extracellular concentrations of signaling molecules are kept low (Zhou and Danbolt, 2013). Low extracellular levels can only be maintained by cellular uptake because there is no extracellular metabolism of GABA and glutamate. In case of glutamate, there is an important reason to keep the extracellular levels low. It is generally known that overstimulation by glutamate induces excitotoxicity, a well-recognized component of the pathogenesis of many brain disorders (Platt, 2007; Broer and Palacin, 2011; Lai et al., 2014; Takahashi et al., 2015). Clearing neurotransmitters from the extracellular space is one of the astrocytes’ most critical functions. Astrocytes take up glutamate using two main transporters—EAAT1/GLAST and EAAT2/GLT1 (Rothstein et al., 1996; Danbolt, 2001; Zhou and Danbolt, 2013; Jensen et al., 2015). Rothstein et al. showed that loss of astrocytic GLAST or GLT1 led to elevated extracellular glutamate levels, excitotoxic neurodegeneration, and progressive paralysis (Rothstein et al., 1996). In another study, glutamate uptake activity in GLT1 knockout mice was reduced by about 95%. About half of mice died from spontaneous seizures before they reached 4 weeks of age (Tanaka et al., 1997). Interestingly, lack of GLAST does not lead to spontaneous seizures like those seen in GLT1-deficient mice, but when seizures are initiated, lack of GLAST increases the seizure duration and severity (Watanebe et al., 1999). The difference in mice lacking either GLT1 or GLAST might be due to the brain-specific localization of both transporters. Multiple studies have demonstrated significant regional heterogeneity in the expression of the two glutamate transporters in CNS (Torp et al., 1997; Berger and Hediger, 2000). It is worth mentioning that glioma cells, with the majority believed to originate from astrocytes (Kleihues et al., 1995), lack the expression of EAAT2. And EAAT1 is mislocalized to the nuclear membrane (Ye et al., 1999). This leads to an almost complete absence of glutamate uptake into glioma cells. Even more surprisingly, these cells actually release glutamate, which causes excitotoxic damage to the surrounding neurons. This to some extent explains the formation of seizure activity in the tissue surrounding tumors, a common symptom early in the course of disease (de Groot and Sontheimer, 2011).

Astrocytes do not destroy glutamate but, in effect, return it to neurons via the well-established glutamate–glutamine cycle (Bak et al., 2006). In brief, glutamate is converted to glutamine by the astrocyte-specific enzyme GS. Glutamine is then transferred to neurons, where it is converted back to glutamate via deamination by phosphate–activated glutaminase, which is enriched in the neuronal compartment. Failure to do so would result in the rapid depletion of the glutamate pool in presynaptic terminals and subsequent disruption of excitatory neurotransmission (Hertz et al., 1999; Tani et al., 2014). Astroglial glutamine is also critical for GABA replenishment in GABAergic neurons. Presynaptic terminals convert glutamine to glutamate, which is then metabolized into GABA by glutamate decarboxylase. This is known as the GABA–glutamine cycle (Walls et al., 2015). Because inhibition in the brain critically depends on GABA, insufficient supply of glutamine to GABAergic neurons may result in seizures (Eid et al., 2013). Although the most common disorder associated with insufficient GABA activity is epilepsy, many other neuropsychiatric diseases involve GABAergic dysfunction as a component (Wong et al., 2003; Gajcy et al., 2010). As GS is an essential part of the glutamine–glutamate/GABA cycle, it is not hard to see why changes in expression and activity of GS may lead to neurological dysfunction (Eid et al., 2004; Steffek et al., 2008; Lee et al., 2010; Rose et al., 2013). Astrocyte-specific elimination of GS in neonatal mice led to a progressive failure to feed, which caused death due to hypoglycemia within 3 days after birth. Death was associated with a precipitous decline in the concentration of glutamine (He et al., 2010).

Astrocytes are also responsible for replenishment of the overall brain pool of glutamate, as they are the only neural cell type expressing pyruvate carboxylase, a key enzyme converting pyruvate into oxaloacetate and a downstream intermediate, 2-oxoglutarate (Shank et al., 1985; Olsen and Sonnewald, 2014). Glutamate is then synthesized from 2-oxoglutarate by transamination of aspartate. This is an essential anaplerotic pathway in the brain, which effectively allows astrocytes to synthesize new glutamate from glucose.

To summarize, astrocytes, on one hand, are responsible for the elimination of glutamate from the extracellular space, creating an environment for effective glutamatergic signaling; on the other hand, they support turnover of glutamate and replenish its stocks.

**Astrocytes Regulate Ion Homeostasis and pH**

Ion and volume homeostasis in the CNS is maintained by a number of mechanisms, residing mainly in glial cells. Apart from the release of neurotransmitters, neuronal activity and action potentials cause substantial local increases of extracellular K⁺ in the restricted extracellular space (Heinemann and Lux, 1977). Without tight regulatory mechanisms, this could dramatically alter the
neuronal membrane potential, leading to hyperexcitability. Such a scenario is prevented by the buffering of extracellular K⁺ by glial cells. Indeed, astrocytes have a strongly negative resting potential and express a large number of K⁺ channels, resulting in high membrane K⁺ permeability (Orkand et al., 1966; Barres, 1991). This, in conjunction with the action of the Na⁺/K⁺ ATPase, enables astrocytes to capture the excess of extracellular K⁺, which can then travel in the astrocytic syncytium through gap junctions down its concentration gradient (the so-called potassium-siphoning mechanism). In theory, this should allow a flux of K⁺ from areas of its high concentration toward areas of lower concentration where it can be extruded either into the extracellular space or into the circulation (Newman, 1987; Chen and Nicholson, 2000; Kofuji and Newman, 2004; Belanger and Magistretti, 2009). Astrocytic Kir4.1 channels seem to be particularly important for K⁺ buffering (Takumi et al., 1995; Higashi et al., 2001; Djukic et al., 2007). Downregulation of Kir4.1 by RNAi led to a significant impairment in K⁺ and glutamate buffering functions of astrocytes, hyperexcitability of neurons, and abnormal synaptic transmission (Kucheryavykh et al., 2007). In the astrocyte-specific conditional Kir4.1 knockout mice, capacity to regulate extracellular K⁺ and glutamate levels is compromised (Djukic et al., 2007; Chever et al., 2010).

M. Nedergaard’s group demonstrated that ammonia triggers neurotoxicity by impairing astrocyte K⁺ buffering (Rangroo Thrane et al., 2013). Aquaporin-4 (AQP4), the predominant water channel expressed by astrocytes in the CNS, has been shown to be colocalized with Kir4.1. The colocalization of these two proteins suggests that AQP4 contributes to the coupled influx of water and K⁺ after neuronal activity (Nagelhus et al., 1999; Potokar et al., 2016). Kir4.1 expression is consistently decreased in a variety of neurodegenerative diseases including amyotrophic lateral sclerosis, Alzheimer disease, Huntington disease, and Alexander disease (Nwaobi et al., 2016). However, whether the reduction is a primary pathophysiological contributor to disease progression or just represents a secondary response to neuroinflammation has yet to be determined.

Another fundamental supportive function of astrocytes is their contribution to pH regulation (Belanger and Magistretti, 2009). Many neuronal functions including energy metabolism, membrane conductance, neuronal excitability, synaptic transmission, and gap junction communication are strongly affected by relatively small shifts in pH (Deitmer and Rose, 1996; Obara et al., 2008). An important feature of glial cells, endowing them with a robust pH buffering capacity, is high expression of carbonic anhydrase, which converts CO₂ into H⁺ and HCO₃⁻—effectively allowing them to act as a CO₂ sink (Cammer and Tansey, 1988; Agnati et al., 1995). Regulation of extracellular pH by astrocytes is carried out by transport of H⁺ with Na⁺/H⁺ exchanger, by operation of monocarboxylate transporters (MCTs) that cotransport one H⁺ with one lactate molecule and by Na⁺/HCO₃⁻ exchanger (Deitmer and Rose, 2010) or cotransporter (Song et al., 2013). Additionally, GLTs, which take up glutamate, also cotransport protons (1:1) from the extracellular milieu (Verkhratsky et al., 2015). In this context, it is interesting that the astrocytes on the ventral surface of the medulla overlying the central chemoreceptor area are intrinsically sensitive to CO₂ and pH. It has been recently demonstrated that Na⁺/HCO₃⁻ exchanger, which is particularly highly expressed in these cells, could act as an acidification sensor, leading to an influx of Na⁺ with consequent reversal of Na⁺/Ca²⁺ exchanger, followed by Ca²⁺ entry and downstream signaling events (Turovsky et al., 2016).

Finally, astrocytes may play a role in the regulation of Ca²⁺ concentrations. A decrease in extracellular Ca²⁺ concentration due to high neuronal activity evokes [Ca²⁺]ᵢ increases in astrocytes, with some of this cytosolic astrocytic Ca²⁺ being excreted into the extracellular space via Na⁺/Ca²⁺ exchanger (Zanotti and Charles, 1997). It was later shown that a drop in extracellular Ca²⁺ due to synaptic activity could evoke release of ATP from astrocytes, probably because of opening of the connexin 43 hemichannels. This process in some areas could lead to increased activity of GABAergic neurons (Torres et al., 2012).

**Astrocytes Supply Neurons with Energy Substrates**

Astrocytes make important contributions to CNS metabolism. While the brain is a very energy-hungry organ, it contains few energy reserves and is therefore highly dependent on the uninterrupted supply of glucose from the circulation. The morphological characteristics of astrocytes are ideally tailored to sense neuronal activity at the synapse and respond with the appropriate metabolic supply via their end-feet, some of which also directly contact intracerebral blood vessels (Belanger et al., 2011); this idea dates back to the famous predictions made by Ramón y Cajal (1913). 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” proposed over two decades ago by Pellerin and Magistretti (1994). The essence of this model is that neuronal activity triggers glucose metabolism in astrocytes. Glucose then undergoes glycolysis, and the produced pyruvate is converted to lactate, which is eventually released from astrocytes and taken up by neurons to be used for oxidative phosphorylation (Pellerin and Magistretti, 1994; Fillenz, 2005). This idea is still hotly debated; for further discussion, please see our recent reviews (Mosienko et al., 2015; Teschemacher et al., 2015). The greatest controversy involves why neurons would even need lactate if there is glucose present. There is no immediately obvious answer to this question (Dienel, 2017). At least one important recent study performed using state-of-the-art in vivo imaging in mouse cortex provided evidence that there is a gradient of lactate from astrocytes to neurons, which makes such transfer physically possible (Machler et al., 2016).
Another commonly recognized feature of astrocytes is their capacity to store glucose in the form of glycogen. Indeed, in the CNS glycogen is predominantly found in astrocytes, although it is also present in minute amounts in neurons (Phelps, 1972; Koizumi, 1974; Pfeiffer-Guglielmi et al., 2003; Dienel, 2012). The glycolytic units resulting from glycogen breakdown are fed into the glycolytic pathway of astrocytes; some of it becomes lactate, which may be released into the extracellular space (Dienel and Cruz, 2015). Storage of energy in the form of glycogen may be important for brain resilience in situations where glucose becomes scarce, although this depot has a fairly small capacity. 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 caused by subsequent hypoxic-ischemic injury (Brucklacher et al., 2002; Herzog et al., 2008; Canada et al., 2011; Evans et al., 2013).

It is interesting that more than 25 years ago it was postulated that the main regulator of glycogenolysis in astrocytes is central noradrenaline (Stone and Ariano, 1989). From the published transcriptions (http://bioinf.nl:8080/GOAD2/databaseSelectServlet), it appears that the predominant receptor for noradrenaline on astrocytes is the Gs-coupled ß1 adrenoceptor subtype (Adrb1) (Zhang et al., 2014); this is fully consistent with unpublished observations from our laboratory. In contrast, ß2 receptors are essentially all localized to microglia, while ß3 receptors are not expressed in the brain at any significant level. Surprisingly, a recent study localized ß2 receptors to hippocampal astrocytes but not to microglia, which calls for a further clarification of this fairly important question (Gao et al., 2016). ß2 adrenoceptors seem to be also able to drive glycogenolysis in a pertussis toxin-sensitive manner, implicating involvement of a Gi/o signaling pathway (Hutchinson et al., 2011). Curiously, adrenoceptors appear in transcriptions at marginally low levels (Zhang et al., 2014), but nevertheless, selective ß1 agonists have a clear effect on astrocytic Ca2+ in cortical slices, and their activation leads to release of neurotransmitters (Pankratov and Lalo, 2015). Hence, it is likely that one of the key consequences of the activation of the locus coeruleus (the key source of the front brain noradrenergic line), which is a landmark of arousal and behaviorally engaged state of the brain, is activation of glycogenolysis.

It is also worth noting that the conventional view on the mechanisms of lactate release from astrocytes—that it necessarily requires MCTs—is no longer valid. Channel-mediated release of lactate was reported, although the nature of that channel has not yet been identified (Sotelo-Hitschfeld et al., 2015). Recently, we found that lactate can be released via connexin hemichannels. Hemichannel blockers strongly attenuate lactate release triggered by transient hypoxia and by stimulation of Schaffer collaterals in the hippocampus in brain slices. As mentioned above, one trigger, which effectively opens hemichannels, is lowering of extracellular Ca2+ (Kargarlannis et al., 2016). Hence, it is possible that dips in extracellular Ca2+ during episodes of neuronal activity open hemichannels and cause release of not only ATP but also lactate (Torres et al., 2012). For further discussion of the roles of lactate in astrocyte-to-neuronal communication, see Barros (2013), Mosienko et al. (2015), and Teschemacher et al. (2015).

**Astrocytes Contribute to Control of Cerebral Blood Flow**

To maintain normal brain function, it is critical that cerebral blood flow (CBF) is matched to the neuronal metabolic needs. Apart from purely metabolic considerations, this is critical because brain activity leads to release of copious amounts of heat, which must be drained from the activated areas to prevent overheating. Astrocytes are the essential elements of the mechanism that controls CBF (Howarth, 2014). However, it is still not clear how astrocytes control the vasculature, although the unique and close relationship of astrocytes with cerebral blood vessels has been recognized since Ramón y Cajal’s time. A popular hypothesis of astrocytic control of CBF in response to neural activity has been that neuronally released glutamate acts on astrocytic metabotropic glutamate receptors (mGlurS) to raise astrocytic [Ca2+]i, initiating downstream production of arachidonic acid (AA) followed by formation and release of vasoactive substances (Zonta et al., 2003; Mulligan and MacVicar, 2004; Takano et al., 2006; Attwell et al., 2010; Choi et al., 2012). However, some studies have put this hypothesis into question. For example, it has been shown that in mature mice, mGlur5, which is usually expected to mediate astrocytic Ca2+ responses to glutamate, is down-regulated, suggesting that this coupling mechanism might be more relevant for the very young brain (Sun et al., 2013). Other evidence suggests that neuron–glia signaling may be mediated by neuronally released ATP acting on glial P2Y receptors rather than via activation of mGlurS by glutamate (Calcagnì et al., 2011; Habbas et al., 2011; Nizar et al., 2013; Sun et al., 2013). Group I mGlurS and the ATP receptor P2Y1 are both G protein–coupled receptors associated with Gq signaling that induces Ca2+ release from internal stores by generating IP3. However, in mice where the astrocyte–specific subtype 2 of IP3 receptor was knocked out, neurovascular coupling seemed to be preserved (Nizar et al., 2013). A recent study by Mishra et al. might explain why release of Ca2+ from stores does not affect neurovascular coupling (Mishra et al., 2016). They provided evidence that astrocyte [Ca2+]i is raised not by release from intracellular stores but by entry through ATP-gated channels. Alternative hypotheses of astrocyte control of vessel diameter also include the efflux of K+ through Ca2+-activated K+ channels in astrocyte end-feet (Filosa et al., 2004). Somehow in this vein is a recent study by Longden et al. that described a mechanism present in brain capillaries and operating via an inward-rectifier Kir2.1 channel (Longden et al., 2017). The idea in that study is that this channel can be activated by a local elevation in the extracellular K+, resulting in further hyperpolarization of the
endothelial cell. This creates a propagating wave of hyperpolarization that travels to the upstream arterioles, causing vasodilation and an increase in perfusion of activated areas of the brain. This is an attractive idea because it could help to tie together a number of previously made suggestions, but it may need further support from other groups working in this area.

The role of astrocyte \([\text{Ca}^{2+}]_i\) transients in the control of CBF in vivo during functional hyperemia remains controversial. Although some studies argued that astrocytic \(\text{Ca}^{2+}\) transients are not fast and frequent enough to account for neurovascular coupling (Nizar et al., 2013), the bulk of evidence shows convincingly that, at least at the level of end-feet, responses occur within reasonable time frames (Takano et al., 2006; Lind et al., 2013; Otsu et al., 2015). Hence, we still do not know for certain whether \(\text{Ca}^{2+}\) transients in astrocytes really are critically important for neuron-to-blood vessel coupling (Gurden et al., 2006; Petzold et al., 2008; Schummers et al., 2008). Irrespective of the role of \(\text{Ca}^{2+}\), we have demonstrated that rapid degradation of extracellular ATP and ADP by viral vector–mediated expression of the enzyme transmembrane prostatic acid phosphatase drastically attenuates BOLD fMRI responses in rat neocortex to stimulation of somatic afferents (Wells et al., 2015). Even though in that study the source of ATP was not identified, the bulk of published studies point to astrocytes as the main sources of extracellular ATP, which appears to be an essential contributor to neurovascular coupling. Mishra and colleagues’ work suggested, though, that neuronal activity also results in postsynaptic release of ATP by neurons, which acts on ATP receptors on astrocytes (Mishra et al., 2016). This process should have not been affected in our experiments cited above (Wells et al., 2015), consistent with the idea of astrocytes as the key source of ATP in the case of neurovascular coupling.

**Astrocytes and the Blood-Brain Barrier**

Integrity of the blood-brain barrier (BBB) is fundamental to the well-being of neurons. The BBB is formed by the endothelial cells connected by tight junctions, surrounded by the basal lamina and sealed by the astrocyte end-feet (Chow and Gu, 2015). Astrocytes are important in the development and maintenance of BBB characteristics in endothelial cells through the release of growth factors like vascular endothelial growth factor (VEGF), glia-derived neurotrophic factor, basic fibroblast growth factor, and angiopoietin 1 (Dehouch et al., 1990; Rubin et al., 1991; Hayashi et al., 1997; Haseloff et al., 2005; Alvarez et al., 2013). These factors are important in the formation of tight junctions, expression of relevant enzymes, and polarization of the transporters (Wong et al., 2013).

Disruption of BBB is a common feature of various neurological disorders, such as cerebral ischemia, trauma, glioblastoma, stroke, multiple sclerosis, epilepsy, Alzheimer disease, and Parkinson disease, all of which are accompanied by changes in the permeability of the BBB and phenotypical changes of both endothelial cells and astrocytes (Daneman, 2012; Alvarez et al., 2013; Cabezas et al., 2014). Reactive gliosis, which typically follows damage and accompanies BBB disruption, has an additional detrimental effect on the barrier function and neuronal survival (Daneman, 2012; Obermeier et al., 2013; Cabezas et al., 2014).

**Astrocytes Defend Against Oxidative Stress**

The mammalian CNS is particularly prone to damaging effects of reactive oxygen species (ROS) because of its high rate of oxidative metabolism and large fatty acid content in myelin and other membranes (Belanger et al., 2011). The unsaturated carbon–carbon bonds, needed to ensure sufficient fluidity of the fatty acid side chains of the phospholipids, are most susceptible to oxidative damage by ROS (Dringen, 2000). ROS cause breakdown of a large number of lipids and proteins. Because the reduction of molecular oxygen by the respiratory chain is never complete, ROS are always produced and need to be neutralized. For further information, see the reviews by Kimelberg and Nedergaard (2010), Gandhi and Abramov (2012), Kim et al. (2015), and Gebicki (2016).

Astrocytes possess a much more effective defense system against ROS than neurons (Wilson, 1997). Therefore, cooperation of astrocytes with neurons is essential for neuronal resilience against ROS (Belanger and Magistretti, 2009). This idea is supported by a large number of studies demonstrating that when cultured in the presence of astrocytes, neurons are much more resistant to toxic concentrations of nitric oxide, hydrogen peroxide, or superoxide anions combined with nitric oxide or iron (Langeveld et al., 1995; Lucius and Sievers, 1996; Tanaka et al., 1999; Chen et al., 2001; Fujita et al., 2009).

Greater activity of ROS–detoxifying enzymes (including glutathione [GSH] S-transferase, GSH peroxidase, and catalase) and significantly higher levels of antioxidant molecules (such as GSH, ascorbate, and vitamin E) in astrocytes contribute to their ability to improve the survival of neurons. In addition, astrocytes may also prevent generation of free radicals by redox active metals, as they participate in metal sequestration in the brain (Makar et al., 1994; Huang and Philbert, 1995; Dringen et al., 1999).

GSH is the most potent intrinsic antioxidant molecule in the brain. As a tripeptide comprising the amino acids glutamate, cysteine, and glycine, GSH is generated by the successive actions catalyzed by \(\gamma\)-glutamyl cysteine ligase and GSH synthetase. Since intracellular glutamate and glycine concentrations are relatively high, cysteine appears to be the rate-limiting substrate for GSH synthesis (Dringen, 2000). Extracellular cysteine is readily autooxidized to cystine. Cystine uptake is mediated by cystine/glutamate exchange transporter (xCT), which is expressed primarily on astrocytes (Qiang et al., 2004; Seib et al., 2011). Astrocytes then reduce it back to cysteine, which is used to synthesize GSH, and consequently release it into the extracellular space. GSH acts against...
ROS either by directly reacting with ROS or as a substrate for GHS S-transferase or GSH peroxidase (Dringen, 2000). Although neurons also can synthesize GSH, they depend on astrocytes for the supply of cysteine because they are not very efficient in using extracellular cystine as a cysteine precursor.

Ascorbic acid, the reduced form of vitamin C, is another key antioxidant in the CNS. Ascorbic acid is synthesized in the liver of most mammals. However, higher primates, including humans, lack the functional enzyme for the final step of synthesis, rendering them dependent on exogenous sources of ascorbic acid (Nishikimi et al., 1992; Lachapelle and Drouin, 2011). This dietary dependence led to its categorization as a vitamin. Ascorbic acid is concentrated in the brain (Spector and Lorenzo, 1973). It is believed that ascorbic acid may be released from glial reservoirs into the extracellular space, from where it is taken up by neurons. In neurons, ascorbic acid scavenges ROS generated during periods of high activity leading to formation of the oxidized form dehydroascorbic acid, which is released from neurons and taken up by astrocytes (Wilson, 1997; Covarrubias-Pinto et al., 2015). Astrocytes reduce dehydroascorbic acid back to ascorbic acid and either use it themselves or release it into the extracellular space. This recycling of ascorbate is another good example of cooperation between astrocytes and neurons in antioxidant defense (Covarrubias-Pinto et al., 2015). Ascorbic acid has also been implicated in regulation of the astrocyte–neuron lactate shuttle (Castro et al., 2009). During glutamatergic synaptic activity, neurons produce glutamate, which stimulates ascorbic acid release from astrocytes. Ascorbic acid then enters into neurons and, within the cell, can inhibit glucose consumption and stimulate lactate transport. Imbalance of ascorbic acid homeostasis has been demonstrated in several neurodegenerative disorders (Shaw, 2005; Acuna et al., 2013; Warner et al., 2015). Ascorbic acid deficiency exacerbated oxidative stress–induced neuronal death during neurodegeneration (Kim et al., 2015). Based on these considerations, ascorbic acid has been tested as a neuroprotective agent in humans for treatment of various neurodegenerative diseases including Parkinson disease, amyotrophic lateral sclerosis, and Huntington disease (Fitzgerald et al., 2006; Tyzack et al., 2014) and represent a cellular target for treatments aimed at promoting posttraumatic plasticity and recovery. However, it is important to acknowledge recent data demonstrating that astrocytes, when pathologically activated by microglial cytokines, can in fact have a detrimental effect on synapse formation and/or maintenance (Longden et al., 2017).

It is thought that astrocytes are also involved in the elimination of synapses in the CNS, the process that underlies the fine tuning and plasticity of neuronal inputs (Barres, 2008). This may be achieved by secretion of certain factors or proteolytic enzymes, which demolish the extracellular matrix and reduce the stability of the synaptic contact (Stevens et al., 2007; Verkhratsky et al., 2016). Subsequently, astroglial processes may enter the former synaptic cleft and literally substitute the synapse. It has been suggested that astrocytes play a role in synaptic pruning via Draper/Megf10 and Merk/integrin alpha(v)beta5 phagocytic pathways (Barres, 2008) and release of signals that induce expression of the C1q protein, which activates the classical complement pathway (Stevens et al., 2007). Contribution of glia to formation and elimination of synapses is one of the hot topics in today’s neuroscience.

**Astrocytes Assist Synapse Formation and Maintenance**

Astrocytes play an essential role in the development of the nervous system by regulating formation, maturation, maintenance, and stability of synapses (Guillamon-Vivancos et al., 2015). Growing axons are directed toward their targets by astrocyte-derived guide molecules, such as tenasin C and proteoglycans (Powell and Geller, 1999). In the absence of glia, cultured retinal ganglion cells developed little synaptic activity, which was 100 times higher when they were co-cultured with astrocytes, and the increase was due to the formation of additional synapses (Pfrieger and Barres, 1997). This increase in the number of synapses was mediated by the astrocyte-secreted factor, thrombospondin, an important matrix molecule induced by purinergic signaling (Christopherson et al., 2005; Risher and Eroglu, 2012). Thrombospondins are a family of five homologous proteins. Astrocytes express at least four types of thrombospondins during development and following damage to brain tissue. Thrombospondins facilitate formation of ultrastructurally normal excitatory synapses at both presynaptic and postsynaptic levels (Barres, 2008; Guillamon-Vivancos et al., 2015). In addition to thrombospondins, cholesterol has also been shown to be an important astrocytic factor for synapse maturation. CNS neurons produce enough cholesterol to survive and grow, but the formation of numerous mature synapses demands additional amounts, which are provided by astrocytes (Mauch et al., 2001; Ferris et al., 2017; Van Deijk et al., 2017). Astrocytes also participate in formation of the inhibitory synapses (Elmariah et al., 2005). Therefore, it is plausible that astrocytes might help restore synapses after injury (Emirandetti et al., 2006; Tyzack et al., 2014) and represent a cellular target for treatments aimed at promoting posttraumatic plasticity and recovery. However, it is important to acknowledge recent data demonstrating that astrocytes, when pathologically activated by microglial cytokines, can in fact have a detrimental effect on synapse formation and/or maintenance (Longden et al., 2017).

**Astrocytes Produce and Release a Variety of Growth Factors and Other Trophic Molecules**

Although it is generally accepted that co-cultured astrocytes have ‘‘trophic’’ action on neurons, and that they synthesize and release a range of molecules that can be classified as trophic factors (e.g., brain-derived neurotrophic factor, VEGF), it is not clear whether any such factors are exclusive to astrocytes. In this regard, transforming growth factors (TGF)-β1 and TGF-β2 are probably among the best-documented candidates (Dhandapani et al., 2003), and they may be responsible for the neuroprotective effect of estrogens (Dhandapani and Brann,
2003; see review by Gomes et al., 2005). Meteorin is another example of a “trophic” factor involved in astrocyte-to-neuron signaling. It was first identified as a retinoic acid–responding gene suggested to be involved in glial differentiation and regulation of axonal extension (Nishino et al., 2004). Meteorin is long peptide (291 amino acids in the mouse) and is highly expressed by Bergman glia in the cerebellum but also by astrocytes and some discrete neuronal populations (Jorgensen et al., 2009). In addition to the effects on glia and neurons, it is implicated in the maturation of the BBB by stimulating endothelial cells (Park et al., 2008). Recently, Lee et al. found that Meteorin is upregulated in reactive astrocytes in a photothrombotic ischemia mouse model and functions as a negative feedback effector in reactive gliosis (Lee et al., 2015). However, the cellular receptor(s) for Meteorin is still unknown.

**Astrocytes Improve Viability and Maturation of Stem Cell–Derived Neurons**

Human-induced pluripotent stem cell (hiPSC)-derived neurons currently attract a lot of attention as invaluable tools for modeling disease and as potential means of therapy (An et al., 2012; Israel et al., 2012; Chen et al., 2014; Zhang et al., 2017). HiPSCs provide an unlimited source of genetically personalized cells of various phenotypes, including neurons with a diminished risk of immunorejection (Grskovic et al., 2011; Ichida and Kiskinis, 2015). However, one of the critical concerns that limits application of hiPSC-derived neurons is their long-term functional stability. When cultured in the absence of astrocytes, such neurons lack the adequate environment and usually can only survive short-term. As described earlier, it has long been recognized that astrocytes are essential partners of neurons and produce numerous diffusible and nondiffusible neuron-supporting signals and trophic factors (Barres, 2008).

Extensive experimental evidence demonstrates that astrocytes play a critical role in promoting both morphological and functional maturation of human neurons derived from hiPSCs (Roy et al., 2006; Pasca et al., 2015). For example, the presence of astrocytes significantly enhanced neuronal dendritic complexity, the expression of ionic channels and neurotransmitter receptors, and the frequency and amplitude of synaptic events in hiPSC-induced neurons (Tang et al., 2013). In another study, co-culture of rat astrocytes with hiPSC-derived neurons facilitated long-term survival of hiPSC-derived neurons for more than 3 months and spontaneous firing activity. Moreover, the presence of astrocytes protected the neurons from damage, and they had elongated neurites, contrasting with the damaged neurites in the absence of astrocytes (Odagawa et al., 2014). Other examples include embryonic stem cell–derived motor neurons in mice, which were stabilized by culturing them on an astrocytic “feeder” layer (Bryson et al., 2014). These and many other observations show that astrocytes are crucial partners to the induced neurons and justify the need for a better understanding of this close relationship.

**ASTROCYTES CAN SOMETIMES BE “BAD GUYS”**

As described above, astrocytes normally play a neuroprotective role, but, upon pathological stimuli, they can release a range of cytotoxic mediators, including ROS, reactive nitrogen species, proinflammatory cytokines, and chemokines (Benveniste, 1992; Rama Rao and Kielian, 2015). These mediators are detrimental to neurons and ultimately result in neuronal dysfunction and promote neuronal cell death. Astrocytes respond to all forms of injury and disease in the CNS through a process referred to as reactive astrogliosis. Studies over the past 20 years provide compelling evidence that reactive astrogliosis is not simply an all-or-nothing phenomenon but, rather, is a finely graded continuum of molecular, cellular, and functional changes that range from subtle alterations in gene expression to glial scar formation. These changes can exert both beneficial (Anderson et al., 2016) and
detrimental effects (Liddelow et al., 2017) in a context-dependent manner regulated by many potential signaling events. Reactive transformation of astrocytes is beyond the scope of this review, but there have been some excellent reviews published on this topic in recent years (Burda and Sofroniew, 2014; Pekny and Pekna, 2014; Pekny et al., 2014; Liu and Chopp, 2015; Filous and Silver, 2016; Pekny et al., 2016). Here we only want to emphasize one recent high-profile study, which focused attention on the mechanisms by which activated (reactive) astrocytes may compromise neuronal networks (Liddelow et al., 2017). Liddelow et al. used a model whereby astrocytes are activated by the inflammatory stimulus lipopolysaccharide (LPS). LPS injections induced in astrocytes upregulation of many genes of the classical complement cascade, and the authors ascribed a neurotoxic phenotype, the so-called A1 state, to this transformation. In contrast, ischemic insults lead to upregulation of many of the factors with trophic activity, and this state, termed A2, was postulated to be neuroprotective. The neurotoxic A1 phenotype was not the result of a direct action of LPS on astrocytes but required a step mediated by microglia, which is sensitive to LPS. Transformation into the A1 phenotype seemed remarkably stable, rendering astrocytes unable to properly support neuronal functions and synaptogenesis. Currently, the identity of the “benevolent” A2 phenotype is much less clear, as is how, in relation to these two proposed states, various populations of resting astrocytes can be classified. The study also suggested that genomic profiling reveals a much more complex picture than just two clearly defined states. Multiple papers where astrocytes were used in vitro to support neurones, including stem cell-derived neurones, show that on the whole, astrocytes are extremely important for the well-being of neurones, even though cultured astrocytes are always “reactive” to some extent. We do not know whether their supportive effects could be in all cases matched to the “beneficial A2” genetic profile, leaving the possibility that the specific means by which astrocytes support neurones might be very different in each particular case.

TARGETING ASTROCYTES: PERSPECTIVES FOR THEIR THERAPEUTIC POTENTIAL

Considering that astrocytes may act as a double-edged sword, exerting both detrimental and neuroprotective effects, it is conceivable that enhancing their beneficial roles while minimizing their deleterious effects holds considerable therapeutic potential for treatment of neurological diseases. We recently reviewed and highlighted some of the potentially targetable processes in astrocytes, which might be of interest for future drug development (Liu et al., 2017).

CONCLUDING REMARKS

In this brief review, we show that astrocytes actively support and protect neurones against toxic and traumatic insults (summarized in Fig. 1). Harnessing the natural capacity of astrocytes to protect neurones could be seen as a promising therapeutic strategy aimed at eventually improving neuronal function or resilience (Liu et al., 2017). Recent revelations of the role of astroglia in the flux of macromolecules or ions in the brain (termed “glymphatic system”) have opened a new range of mechanisms by which astrocytes may contribute to the well-being of the brain by clearance of potentially pathogenic molecules during sleep or, in fact, by a contribution to the sleep–wakefulness control (Xie et al., 2013; Ding et al., 2016). We believe that many of the mechanisms that astrocytes employ to assist neuronal networks represent potential opportunities for therapies aimed at processes localized in astrocytes, rather than neurones.

CONFLICTS OF INTEREST

The authors declare no known conflicts of interest.

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ROLE OF AUTHORS

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