New Roles for Old Glue: Astrocyte Function in Synaptic Plasticity and Neurological Disorders

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Previously believed to solely play a supportive role in the central nervous system, astrocytes are now considered active players in normal brain function. Evidence in recent decades extends their contributions beyond the classically held brain glue role; it's now known that astrocytes act as a unique excitable component with functions extending into local network modulation, synaptic plasticity, and memory formation, and postinjury repair. In this review article, we highlight our growing understanding of astrocyte function and physiology, the increasing role of gliotransmitters in neuron-glia communication, and the role of astrocytes in modulating synaptic plasticity and cognitive function. Owing to the duality of both beneficial and deleterious roles attributed to astrocytes, we also discuss the implications of this new knowledge as it applies to neurological disorders including Alzheimer disease, epilepsy, and schizophrenia.

Keywords: Astrocyte; Central nervous system; Synaptic plasticity; Neurological disorders

INTRODUCTION

While neurons are undoubtedly the classic stars of neuroscience research, recent research forces us to expand the spotlight to the star-shaped astrocytic glia. For over a century, astrocytes have been considered both stagehand and scaffold within the central nervous system (CNS). In the recognized supportive role of astrocytes, their known functions ranged from providing structure, literally making up the "glue" that bound the neuronal elements together, to vasomodulation and maintaining the blood-brain barrier. The past 30 years have pushed our understanding into new directions and roles for astrocytes such as modulation of synaptic transmission, long-term potentiation (LTP), and postinjury repair in the nervous system.

In this article, we review recent evidence expanding on the known roles of astrocytes in the CNS. We provide an overview of astrocytes, their incredible heterogeneity, and how they are physiologically stimulated to transmit information and influence synaptic transmission. This is followed with a description of gliotransmitters and their specific roles in mediating or

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modulating long-term potentiation. Finally, we review recent evidence relevant to astrocyte function in CNS pathologies such as Alzheimer disease, depression, epilepsy, and schizophrenia. Where possible, relevant clinical and *in vivo* work has been utilized and where helpful, other reviews have been referenced to provide the reader with understanding on topics beyond the scope of this work.

ASTROCYTES AS DIVERSE NEURAL CIRCUIT ELEMENTS

A pervading thought in neuroscience, and more specifically, glia research, is that neuroglia outnumber neurons 10:1 [1]. Recent evidence, however, shows that this number may be vastly overestimated. Using isotropic fractionation of human brain samples combined with NeuN nuclei labelling, research now establishes the ratio of neuronal to non-neuronal cells is closer to 1:1 [2]. This ratio is in line with other studies [3]. Interestingly, these studies also found that this ratio varies throughout the brain. In cerebral cortex, there is an increase in glia relative to neurons whereas in the cerebellum it is the opposite [2,3].

While the exact reasons for these shifts in glial populations are unknown, it has been suggested that increased neuronal size and coinciding metabolic demand explains the need for increased glial support [2,3]. Indeed, cortical regions show increased glia: neuron ratios across animal species, suggesting that glia may be of evolutionary importance. Similarly, using a combination of glial fibrillary acidic protein (GFAP) and S100 calcium binding protein B (S100B), markers primarily expressed in astrocytes, at least nine different astrocyte populations may be identified that are phenotypically diverse, but region specific to the extent that they may be used to delineate different anatomical regions in the brain [4]. The unique morphology and excitability of astrocytes allows them to taken on several structural roles in the CNS that include maintenance of the blood brain barrier, ion homeostasis, and regulation of neuron-neuron communication [5].

This heterogeneity gives a level of versatility to the astrocyte that allows it to have profound effects on the surrounding neuronal network. A novel study recently published supports the potential evolutionary role of astrocytes in promoting cognitive ability. Using cultured human glial progenitor cells engrafted into neonatal mice, Han et al [6]. demonstrated that these glial progenitors differentiate to become astrocytes and show enhanced function. These glia differentiated into mature astro-

cytes, integrated into the existing host astroglial network, exhibited faster propagation of Ca^{2+} signaling, and promoted LTP. Furthermore, these human glia chimeric mice demonstrated increased cognitive ability as demonstrated by improved performance in the Barnes maze, object-location tasks, alongside contextual and tone fear conditioning tasks [6]. These studies support the notion that astrocytes are heterogeneous elements contributing to cognitive function, either through homeostatic maintenance or other mechanisms.

Anatomically, astroglial are stereotypically identified by their star-shaped morphology. However, as mentioned above, they also exhibit substantial heterogeneity that may explain their expansive roles within the nervous system [7]. While astrocytes may be classified based on morphology, this can often be difficult due to their wide variation in appearance. Thus, the most widely used methods of identifying astrocytes are the molecular marker, GFAP and S100B [8,9]. Both markers have been shown to be sensitive to the major astrocyte types, protoplasmic and fibrous [10]. Protoplasmic astrocytes are commonly found in grey matter and are characterized by their fine, almost cloud-like, processes enveloping neuronal synapses. Fibrous astrocytes, found in white matter, differ in that they exhibit thin and defined processes which are unbranched and whose end-feet meet neuronal nodes of Ranvier. While the aforementioned markers and morphologic phenotypes are useful for broad characterization of astrocytes, other classes of astroglia exist.

ASTROCYTES AND COMMUNICATION

Calcium Waves and Astrocyte Excitability
Astrocytes had long been considered passive members of the CNS without electrical activity. It wasn't until the 1990s when new techniques in Ca^{2+} imaging revealed them as excitable, albeit in ways different from the neuron. The earliest studies demonstrated how cultured hippocampal astrocytes responded to glutamate with increases in intracellular calcium [11], or that mechanical stimulation of a single astrocyte in a primary glial culture could increase intracellular calcium concentration [12]. In both instances, specific increases in intracellular calcium were propagated to cells in the surrounding cultures providing us with some of the earliest evidence of communication between astrocytes. Gap junctions between local astrocytes in addition to extracellular adenosine triphosphate (ATP) link this activity to the surrounding glia and onward in an electrically coupled syncytium [13,14]. Relatively slow Ca^{2+} waves, as com-

pared to the neuronal action potential, are now recognized as hallmark features of astrocytes.

Astrocytes undergo both neuron-dependent and spontaneous excitation [15]. The neuron-dependent excitation of astrocytes is a well-documented way in which synaptic neurotransmitters directly or indirectly through the connected astrocyte syncytium lead to astrocyte excitation. Astrocytes respond to a wide range of neurotransmitters including glutamate, gamma-aminobutyric acid (GABA), acetylcholine, ATP, nitric oxide, and brain-derived neurotrophic factor (BDNF) [15,16]. Further, there is evidence that astrocytes are able to discriminate synapses belonging to different axon pathways. In rat hippocampal slices, Perea and Araque [17] showed that astrocytes not only respond differently to glutamate and acetylcholine, but also to glutamate released from the Schaeffer collaterals compared to glutamate from the alveus terminals. Beyond neuron-dependent excitation, astrocytes are capable of generating spontaneous intracellular calcium oscillations that trigger astrocytic glutamate release causing *N-methyl-D-aspartate* (*NMDA*)-receptor dependent neuronal activation [18]. Similarly, hippocampal astrocytes *in situ* exhibit calcium oscillations that occur independent of neuronal activity or metabotropic glutamate and purinergic receptors and are mediated by inositol-phosphate-3 receptor activation [19].

Ca^{2+} signaling regulation varies by the anatomical region that the astrocyte is found in. A recent study shows that astrocytes along the hippocampal mossy fiber pathway respond to repeated mossy fiber action potentials, are activated by neurotransmitters glutamate and GABA, and cover a large brain region indicating unsuitability in mediating individual synapses [20]. Importantly, observed spontaneous astrocyte Ca^{2+} signaling was independent of this activity and its associated neurotransmitters. In contrast, previous study of hippocampal CA1 and dentate gyrus astrocytes show a high sensitivity towards spontaneous activity by glutamate and single action potentials at a local level [21,22]. Such differences in astrocyte Ca^{2+} response sensitivity, effect size, and overall stimulus suggest differential neuronal population effects. Interestingly, the anatomical placement of these astrocytes along this circuit hints at their function. As part of the trisynaptic circuit, hippocampal input follows the perforant path where entorhinal cortical inputs synapse within the dentate gyrus, which then via mossy fibers is relayed to CA3, which is ultimately relayed to CA1 via schaffer collaterals. Such astrocyte heterogeneity along this circuit suggests differential function based on factors such as synapse number, source of inputs and outputs, in addition to variation in plasticity within these anatomical regions. The extent and significance of this astrocyte variation has yet to be systematically explored.

Together, growing evidence on the excitation of astrocytes establishes them as integral elements in the information-processing pathways of the CNS capable of contributing to excitation of both glial and neuronal elements. It's important to note that Ca^{2+} signaling and its role in astrocyte function is rapidly changing and its effects may be too fine to adequately determine without reassessment [23].

Gliotransmission

Ca^{2+} astrocyte activation is required for the release of astrocyte gliotransmitters [21,24]. Volterra and Meldolesi have previously suggested a precise classification criterion for gliotransmitters. This definition includes synthesis and/or storage in astrocytes, a regulated release stimulated by physiological stimuli, activation of rapid responses in neighboring cells, and finally a role of the gliotransmitters in physiological processes [15]. Even within this growing list of gliotransmitters, molecules released by the astrocyte may not all hold the same breadth of function or effect; some including D-serine may act as strong *mediators* of synaptic plasticity and LTP, while others such as ATP and tumor necrosis factor (TNF)-α may act as *modulators* and regulate plasticity in more subtle ways [25].

By releasing gliotransmitters to communicate over short distances with neighboring neurons, astrocytes actively contribute to functions spanning synapse development/transmission to controlling blood vessel flow. There is now a growing list of molecules secreted by astrocytes in response to stimulation. These include glutamate, ATP, adenosine, D-serine, prostaglandins and TNF-α among many others that are secreted from the astrocyte and act at specific receptors with specific outcomes. Glutamate is perhaps one of the most well-known gliotransmitter released by astrocytes. It acts at the alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and NMDA-receptors, and metabotropic glutamate receptors (mGluRs) and contributes to LTP by increasing excitatory postsynaptic potentials and upregulating AMPA receptors [5]. As discussed later in this review, other secreted gliotransmitters such as D-serine, ATP and TNF-α significantly contribute to LTP [25].

Mechanistically, gliotransmitter release from astrocytes is attributed to both exocytosis-mediated and more recently non-exocytosis pathways. Glutamate release by astrocytes occurs classically through exocytosis in a Ca^{2+}-dependent manner pre-

viously thought specific only to synapses. This may occur through a synaptic-like vesicular (SLMV) compartment that contains crucial elements required for glutamate exocytosis, including the vesicular glutamate transporter, vesicular SNARE protein, and cellubrevin [15,26]. Interestingly, the gliotransmitter D-serine that regulates NMDA-receptor mediated LTP may be coreleased with these vesicles or SLMVs carrying glutamate [27]. As more evidence accumulates, it is possible for gliotransmitters to be released from Ca^{2+}-independent nonexocytotic mechanisms. These include hemichannels such as the Cx43 prevalent in astrocytes, volume-regulated anion channels or VRACs, purinergic channels such as $P2X_7$ or the cysteine-glutamate exchanger [28].

Astrocytes as Neuronal Mediators: LTP

With expanding knowledge on astrocyte signaling and gliotransmission as it pertains to the tripartite synapse, research has expanded on the neuronal functional outcomes of this phenomenon on LTP. LTP is the process by which varied biological processes may be reinforced. This popularly includes memory formation but may also include formation of addiction behavior with drugs. Instead of considering the presynaptic and postsynaptic neurons in isolation, the astrocyte has become increasingly recognized as part of a tripartite model where it acts as an essential 3rd element actively involved in how a signal may be conducted at the neuronal synapse.

LTP mechanisms have been extensively studied in the past two decades. The most well studied mechanisms are those involving NMDA-dependent glutamate signaling and a cAMP-dependent presynaptic form of plasticity that we will elaborate on here. For LTP induction, both the presynaptic and postsynaptic neurons must be depolarized, following which there much be high frequency stimulation of the NMDA-type glutamate receptors that results in a large amount of calcium influx into the post-synaptic neuron [29]. This influx leads to activation of calmodulin kinase II, phosphorylation of AMPA receptors, and their exocytosis to the postsynaptic membrane [29,30]. The requirement of AMPA receptors for NMDA-dependent LTP induction was first understood from experiments involving activation of postsynaptically silent synapses in the hippocampus [31,32]. It is now a well evidenced in the literature as the mechanism of LTP induction [29].

The corollary to LTP is long-term depression (LTD) that as the name suggests is a reduction in activity-dependent reduction in synaptic strength. It has gained increasing attention in

recent years with its functional importance studied in monocular deprivation studies that lead to blindness in the unused eye. Of the several mechanisms that have been posited, NMDA-receptor dependent LTD is a leading one. Experimentally, LTD may be induced through low-frequency stimulation (1–3 Hz for 5–15 minutes) of the presynaptic neurons [29]. Repetitive stimulation such as this results in a prolonged increase in calcium in the postsynaptic neuron due to repeated stimulation of the NMDA receptors [29]. The calcium influx selectively activates phosphatases that cause endocytosis of, and reduce the AMPA receptors at the synaptic cleft.

Glutamate is the primary excitatory neurotransmitter in the brain and also the primary neurotransmitter for LTP and LTD induction, acting through the ionotropic NMDA and AMPA-type glutamate receptors. Astrocytes play significant roles in regulating LTP induction. They are the primary recyclers of glutamate and sequester up to 90% of the neurotransmitter at the synaptic cleft [33-35]. In fact, early experiments that quantified glutamate-uptake by astrocytes (astrocyte transporter currents) played a pivotal role in our understanding of LTP induction, namely that LTP induction is not related to further glutamate release, but rather an intracellular response involving AMPARs as described above [36,37]. Astrocytes also release glutamate through a calcium-regulated mechanism that allow for astrocyte-neuron communication [26,38,39]. Triggers include neuron-dependent excitation of astrocytes through neurotransmitters including synaptic glutamate itself that can act through astrocytic metabotropic glutamate receptors [15]. In the hippocampus, glutamate release from astrocytes also synchronizes neuronal activity and control synaptic strength [40].

ASTROCYTES AND NEUROLOGICAL DISORDERS

As we have increasingly understood a more complex and widespread role of astrocytes in the CNS, studies have also demonstrated the involvement of astrocytes in neurological disorders. Astrocytes can be both causative, and protective, in pathological states of neurodegeneration. Below we discuss recent evidence in our understanding of how the astrocyte contributes to observed neurological disorder pathology.

Alzheimer Disease

Alzheimer disease (AD) is characterized at the cellular level by widespread neurofibrillary tangles composed of hyperphosphorylated tau protein and neuritic plaques composed of the

amyloid beta proteins. While the exact etiology of AD is unknown, the prevailing hypothesis that aggregation of fibrillogenic amyloid beta over time leads to cellular, and thus cognitive, dysfunction is well supported by genetic and molecular study [41]. Additionally, AD related degeneration coincides with astrocyte reactive gliosis [42].

Mouse models of AD show evidence of altered intracellular calcium signaling within the astrocytes. For instance, in the APP/PS1 mouse model of AD producing excess amyloid, time-lapse calcium imaging shows significant increase in spontaneous astrocyte activity related to the age-specific development of plaques (3.5 months vs. 6 months) [43]. Both the frequency and amplitude of these transient calcium currents were higher in the APP/PS1 mice, coordinated over long distances and independent of local neuronal activity. Furthermore, application of amyloid-beta to mixed culture of hippocampal astrocytes of neurons causes selective induction of calcium currents in astrocytes compared to neurons [44]. While there is calcium-dependent glutathione depletion in both neurons and astrocytes, the 50% observed death in neurons could be secondary to the neuronal-dependence on astrocytes for antioxidant support.

Besides such pathological changes observed in AD brains that are associated with reactive astrogliosis, one other avenue of active research has been in understanding the observed cognitive impairments, namely, the slow-onset memory loss. GABA signaling has provided a potential explanation for the cognitive deficits. In APP/PS1 mice, the application of bicuculine, a GABA_A antagonist, to hippocampal slices of both adult APP/PS1 and old nontransgenic mice increased the observed LTP, while application of picrotoxin, another GABA_A antagonist could rescue the memory deficits in APP/PS1 mice [45]. Further, in both the cerebellum and hippocampus, tonic inhibition is directly correlated with astrocytic GABA which is released through the Bestrophin-1 channel [46,47]. Indeed, it was recently shown in the APP/PS1 model that reactive astrocytes may use monoamine oxidase-B to produce and release GABA from the Best1 channels [48]. The increased GABA was directly related to impaired presynaptic probability, spike probability, synaptic plasticity, and learning and memory. Both putrescine and amyloid-beta caused Maob-mediated GABA production and release from Best1 channels, while the resulting deficits could be fully rescued by using Maob inhibitors such as selegeline or targeting the Best1 channels. The study is the clearest evidence thus far of astrocytes playing a key role in mediating memory loss in the hippocampus.

Major Depression

Depression is a common disorder that is characterized by anhedonia, loss of interest, psychomotor retardation, and suicidality. It is a significant cause of disability worldwide with 16.2% lifetime and 6.6% 12-month prevalence in the United States population [49]. Several studies in humans have recorded pathological glial loss in the brains of major depressive disorder (MDD) patients. These include glial reduction in the subgenual prefrontal cortex, anterior cingulate gyrus, and prefrontal cortex [50-52]. In the hippocampus, several magnetic resonance imaging studies reveal a decreased volume [53]. Interestingly, while another study found depth of hippocampal slices from post-mortem brains of MDD patients, at the cellular level there was a 30% increase in glial density across hippocampal pyramidal subfields and the granular cell layer. Compared to other regions in the brain with glial loss, in the hippocampus however there was a unique reduction in neuropil that consists of glial cells, their processes, dendrites and axonal processes that may explain the volume loss [54]. These studies show that astrocytes are pathological hallmarks of MDD.

Several studies have attempted to delineate how astrocytes may mediate MDD behaviors. Previous research establishes that L-alpha-aminoadipic acid (L-AA) selectively ablates astrocytes. Targeting the prefrontal cortex, studies show that L-AA astrocyte ablation leads to anhedonia in sucrose preference test, anxiety in novelty suppressed feeding test, and overall increased helplessness in forced swimming and two-way active avoidance tests *in vivo* [52,55]. Additionally, the role of astrocytes in mediating depressive-live behavior may be directly related to its release of the gliotransmitter ATP. Mice that are susceptible to chronic social defeat, a model that mimics depression, may have lower levels of ATP in the prefrontal cortex and the hippocampus. In their study, Cao et al. [56] showed that administration of ATP produces rapid antidepressant effects while blocking ATP release in mice lacking IP3 receptor type 2 or by inhibiting vesicular gliotransmission produces depressive-like behavior. Furthermore, using a transgenic Gq GPCR astrocyte mouse model to selectively activate astrocytes through Ca^{2+} signaling and ATP release, Cao et al. [56] were able to rescue some depressive behaviors in mouse models of depression. Supporting the role of astrocyte purinergic signaling, at least one study looking at the ventral PFC in patients with suicide showed altered expression of genes involving ATP biosynthesis in addition to GABA transmission [57].

Epilepsy

Different mechanisms have been put forth to describe the role of astrocytes in epilepsy. Human studies of patients with temporal lobe epilepsy have shown evidence of reactive astrogliosis. One manner in which astrocytes may mediate epilepsy includes dysfunction of the glutamate-glutamine cycle. The increase in GFAP and vimentin that occur with reactive astrogliosis has been associated with a concurrent decrease in glutamine synthetase, an enzyme that converts glutamate to glutamine which is a precursor to the neurotransmitter GABA. It has been hypothesized that this results in a lack of inhibitory synaptic transmission in the surrounding neurons. In their study, Ortinski et al. [58] showed how adeno-associated virus induction of reactive astrogliosis in mouse CA1 pyramidal neurons reduced the inhibitory input on the surrounding neurons. Reduced inhibitory postsynaptic current was observed that could be increased with exogenous administration of glutamine.

Astrocytes may also result in epileptiform activity through their effects on adenosine levels. This comprises the adenosine kinase (ADK) hypothesis of epileptogenesis. Adenosine plays neuroprotective roles in seizure regulation with its levels typically elevated following a seizure. It may mediate seizure arrest and postseizure refractoriness. Adenosine is converted to adenosine monophosphate via the enzyme ADK which is predominantly found in the astrocytes. Reactive astrogliosis causes an increased amount of available ADK that can potentially act on adenosine and reduce its levels leading to seizure aggravation [59]. Finally, increasing evidence is demonstrating that calcium oscillations in, and glutamate release from astrocytes could also lead to epileptiform discharges. Cultured astrocytes from human epileptic foci show increased spontaneous calcium-oscillation frequency [60,61]. Inducing cortical epileptiform activity may increase calcium-oscillations in astrocytes that can be suppressed though the use of anticonvulsants. Further, astrocytes may also cause excitations in nearby neurons that resemble interictal paroxysmal depolarizations. Other studies have posited that glutamate release from astrocytes instead of being necessary to cause epileptiform discharges could play more of a modulatory role [62,63]. Other studies have shown calcium-dependent glutamate release from astrocytes may also contribute to neuronal death seen in status epilepticus [64].

Schizophrenia

Astrocyte role in the pathogenesis of schizophrenia comes from recent studies involving the gliotransmitter D-serine and NMDA neurotransmission that bear on the glutamate hypothesis of schizophrenia. According to this hypothesis, a relative deficiency of glutamate and consequent hypofunction at the NMDA receptor may contribute to schizophrenia [65]. Early evidence for the hypothesis came from studies involving NMDA receptor antagonists, such as phencyclidine that induced schizophrenia-like positive and negative symptoms in subjects [66]. Studies have shown that activation of the NMDA receptor requires binding of both glutamate and a coagonist D-serine that is a predominant endogenous ligand that binds at the 'glycine' site of the receptor [67,68]. Using D-amino oxidase to selectively degrade D-serine is enough to significantly reduce NMDA receptor-mediated neurotransmission [67].

More importantly, D-serine is also a gliotransmitter that is localized to the astrocytes that contain the enzyme serine racemase to convert L-serine to D-serine [69]. Accumulating evidence has demonstrated decreased levels of D-serine in schizophrenia patients, or polymorphisms of the serine racemase gene [70]. In fact, D-serine has proven an efficacious therapeutic agent in the treatment of schizophrenia [70-72]. Recent evidence has put forth a more compelling case for astrocyte function. DISC1 is a gene that was found to be altered in a Scottish family and associated with schizophrenia, depression and bipolar disorder [73]. DISC1 functions in neurodevelopment and regulation of hippocampal adult neurogenesis [73-75]. One recent study has shown how mutant DISC1 may physiologically impair the function of D-serine by binding to serine racemase and causing its degradation and thus resulting in a diminished availability of D-serine at the NMDA receptor [76]. Furthermore, astrocytic DISC1 expression influences adult hippocampal neurogenesis and hippocampal-dependent behaviors through abnormal production of D-serine [77]. Taken together, these studies suggest that astrocyte derived D-serine mediated by DISC1, in addition to NMDA receptor antagonism and reduced glutamate activity may be involved in schizophrenia pathogenesis.

CONCLUSIONS

Astrocytes play an essential role in the CNS, with roles extending beyond a supportive role as previously thought and into functions such as local network modulation, synaptic plasticity, memory formation, and postinjury repair. Increasing evidence is shedding new light on astrocyte function in major neurological disorders. Future studies will benefit from a focus on astro-

cyte role in disease etiology as well as exploring possible ways to harness our understanding towards therapeutic benefit.

AUTHOR CONTRIBUTION STATEMENT

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