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Glia as drivers of abnormal neuronal activity

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Abstract

Reactive astrocytes have been proposed to become incompetent bystanders in epilepsy as a result of cellular changes rendering them unable to perform important housekeeping functions. Indeed, successful surgical treatment of mesiotemporal lobe epilepsy hinges on the removal of the glial scar. New research now extends the role of astrocytes, suggesting that they may drive the disease process by impairing the inhibitory action of neuronal GABA receptors. Here we discuss studies that include hyperexcitability resulting from impaired supply of astrocytic glutamine for neuronal GABA synthesis, and epilepsy resulting from genetically induced astrogliosis or malignant transformation, both of which render the inhibitory neurotransmitter GABA excitatory. In these examples, glial cells alter the expression or function of neuronal proteins involved in excitability. Although epilepsy has traditionally been thought of as a disease caused by changes in neuronal properties exclusively, these new findings challenge us to consider the contribution of glial cells as drivers of epileptogenesis in acquired epilepsies.

Following the pioneering studies of Kuffler and colleagues, the idea that glial cells may act as guardians of the neuronal microenvironment was born. These experiments demonstrated that glia cells in the optic nerve of the salamander that share some characteristics typical of astrocytes in mammals respond to neuronal activity[1]. We will refer to glia as a generalized term that includes all types of glial cells in invertebrates and vertebrates (for a review on glial cell classification and evolution, see ref. 2). This review mainly focuses on astrocytes or astrocyte-like glial cells and we will specify the glial cell type when we describe studies in rodents or humans.

Initial studies focused on the buffering of neuronally released potassium ions (K^+) and their spatial redistribution away from sites of release. Studies in retina, hippocampus and cortex of rodents and humans have since identified the Kir4.1 inward-rectifying K^+ channels encoded by the *KCNJ10* gene as a major pathway for K^+ uptake and release by astrocytes[3]. Their weak voltage dependence allows movement of K^+ either into or out of cells, depending on the electrochemical gradient for K^+. Adjacent astrocytes share cytoplasmic connections via gap junctions primarily encoded by connexins 43 and 30 (ref. 4). Gap junctions allow for cell-to-cell spread of K^+ toward astrocytic endfeet surrounding blood

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vessels. Kir4.1 channels are enriched in these endfeet, mediating the release of excess K^+ into the blood stream. Spatial K^+ buffering has become a widely accepted function of glia that primarily engages astrocytes in the mammalian nervous system[5]. The guardian role of astrocytes has since been expanded to include neurotransmitters, notably GABA and glutamate (Glu). Astrocytic processes highly express the excitatory amino acid transporters (EAATs) 1 and 2, and, in the adult nervous system, EAAT2 (Glt1, encoded by *Slc1a2*) is particularly abundant in the vicinity of synapses enveloped by astrocytic processes[6]. The high density of EAAT2 protein assures rapid clearance of Glu from the extracellular space, thereby terminating the neurotransmitter action at synapses and preventing Glu from reaching neurotoxic concentrations elsewhere[7]. Glial uptake of Glu also serves to recycle this amino acid as a substrate for neuronal synthesis of GABA and Glu. The functions of glial EAATs and Kir4.1, and therefore glial regulation of Glu and K^+, are interdependent, as the Kir4.1 channels establish the negative resting potential that drives the electrogenic uptake of Glu (Fig. 1a).

As the concept of glia as altruistic guardians of the neuronal microenvironment gained traction, it also became evident that any failure of astrocytes to perform their functions may make them co-conspirators that indirectly contribute to injury or diseases (Fig. 1b). This notion is now supported by abundant experimental evidence demonstrating pathologically dysregulated potassium and glutamate accompanying many neurological illnesses[8] (Table 1). Notably, those very illnesses also frequently present with seizures, aberrant synchronous neuronal discharges found as co-morbidity in Alzheimer and Huntington disease, vascular dementia, traumatic brain injury, brain tumors, and even autism. Recurring seizures are the defining feature of epilepsy, a disease affecting 1–2% of the world's population. For the majority of patients, the seizure cause is unknown. However, idiopathic and genetic forms of epilepsy are believed to share the same fundamental alterations in excitation-inhibition balance that causes seizures in symptomatic or acquired epilepsy. In the latter, lesions or structural changes in the brain are often visible. These structural changes may include neuronal cell loss and sprouting of neuronal processes, yet they are always associated with marked changes of astrocytes called reactive gliosis or astrogliosis.

Reactive gliosis (astrogliosis)

Astrogliosis refers to the morphological and biochemical changes of astrocytes occurring in association with injury or disease. Reactive astrocytes secrete chemokines, cytokines, growth factors and extracellular matrix (ECM) components that participate in wound healing. Hypertrophy and upregulation of the intermediate filament glial fibrillary acidic protein (GFAP) are some hallmarks of astrogliosis[9]. Under severe injury conditions, a subset of reactive astrocytes even re-enter the cell cycle[9,10]. In general, reactive astrocytes appear to revert to a developmentally immature phenotype comparable in gene and protein expression with astrocyte-like neural stem cells[10,11]. However, astrogliosis is not an all-or-nothing reaction, but is instead a gradual development in which characteristics of reactive astrocytes vary by disease condition and distance from a focal insult such as a brain tumor.

Astrogliosis is prominent in mesiotemporal lobe epilepsy (MTLE), the most common form of epilepsy. MTLE is refractory to pharmacological treatment, yet removal of the affected

brain tissue, including the glial scar containing reactive astrocytes and microglia, is often curative. Microdialysis studies in patients undergoing MTLE surgery show 6–10-fold increased Glu concentrations ([Glu]) near the seizure focus and biochemical tissue analysis suggests a substantially reduced expression of glutamine synthetase (GS, encoded by *Glul*)[12]. GS is an astrocytic enzyme responsible for the degradation of Glu to glutamine following Glu uptake into astrocytes[13]. Similarly, astrocyte-derived primary brain tumors, glioma, which frequently present with seizures, show Glu concentrations in excess of 200 μM, over 200-fold the concentration of normal cerebrospinal fluid[14]. Along with many other similar studies, these examples suggest that reactive astrocytes fail to regulate the neural milieu[15] and become 'incompetent bystanders' that indirectly contribute to an otherwise exclusively neuronal disease process. In stark contrast with this view, recent findings ascribe a more active role to glia in disease, which portray glial cells as the drivers of abnormal neuronal activity (Table 1).

Studies discussed below include 1) hyperexcitability resulting from a selective viral infection of astrocytes[16], which impairs the supply of glutamine for neuronal GABA synthesis, and 2) epilepsy due to genetically induced astrogliosis[17] causing a change in neuronal chloride homeostasis that is 3) replicated in peritumoral epilepsy[18–20]. Here, neuronal GABA receptors become excitatory as a result of a loss of surface expression of KCC2. In all of these examples, pathological changes in astrocytes alter the expression or function of neuronal proteins, which in turn cause abnormal excitability. This conceptual shift from neurons to glia as driver of epileptogenesis is both intriguing and exciting. Seizures and epilepsy have long been thought of as exclusively neuronal diseases caused by intrinsic changes in neuronal properties, but these new findings challenge us to consider the contribution of glial cells as drivers of epileptogenesis in acquired epilepsies and other disorders that present with seizures as co-morbidity.

Virally induced astrogliosis impairs GABA synthesis

Astrocytes are fundamentally important in sustained glutamatergic neurotransmission in the brain. As already alluded to, the synaptic action of Glu, the major excitatory neurotransmitter, is terminated by uptake into perisynaptic astrocytes mainly by the Na^+-dependent EAAT2 (Glt1). Thereafter, Glu is converted to glutamine by astrocytes via GS and released into the extracellular space via the SN1 glutamine transporter. Synaptic terminals reimport glutamine through the system A glutamine transporter SAT1. It then serves as substrate for the *de novo* synthesis of Glu via the mitochondrial phosphate-activated glutaminase (PAG). This so called glial glutamate-glutamine shuttle recycles Glu[21]. Notably, glutamine also serves as a precursor for the synthesis of the inhibitory neurotransmitter GABA[22]. Inhibitory neurons further process Glu via the glutamate decarboxylases GAD65 or GAD67 to GABA.

Although astrocyte-derived glutamine rarely becomes rate limiting for the production of Glu, inhibition of the glutamate-glutamine shuttle at any point along its way causes rapid loss of GABAergic inhibition[22]. Tissue from patients suffering from MTLE is depleted of GS, and the resulting failure to convert Glu to glutamine constitutes a bottleneck for the effective removal of Glu from the extracellular space, which may explain the 6–10-fold

increase in [Glu][12]. However, the reduced expression of GS also starves neurons of glutamine. The reduction of extracellular glutamine affects the availability of GABA and interferes with inhibitory synaptic transmission[15]. In contrast, excitatory synaptic transmission is less affected by a breakdown of the glutamine glutamate cycle and still occurs after inhibition or reduction of GS[16,22,23].

That astrocytes can be blamed for a loss of GABA in inhibitory neurons associated with MTLE has been elegantly demonstrated in a mouse model of reactive astrogliosis. Local injection of an astrocyte-specific adeno-associated virus caused focal astrogliosis and virally infected astrocytes showed a selective loss of GS expression that was comparable to that in MTLE patients. This suggests that astrocyte reactivity induces GS downregulation. A number of factors, such as ECM molecules, pH, and exposure to serum factors after blood-brain barrier breakdown or oxidative stress have been implicated in regulating GS expression[24]. Most of these are, to differing degrees, affected by astrogliosis and might contribute to downregulation of the enzyme.

As a result, inhibitory neurons in the vicinity of virally infected reactive astrocytes are hyperexcitable and show impaired GABAergic transmission, whereas glutamatergic synapses are unaffected. Notably, the deficit in GABA synthesis can be entirely restored by direct glutamine supplementation, bypassing astrocytes as a glutamine source[16,22,23]. This also eliminates the epileptiform neuronal activity. Furthermore, pharmacological inhibition of GS in slices from normal animals results in identical neuronal hyperexcitability that can be corrected by exogenous addition of glutamine[16]. These studies demonstrate that astrocytes constitute an essential metabolic pipeline for the supply of glutamine to inhibitory neurons for GABA synthesis. Although normal astrocytes serve this function well, reactive astrocytes such as those associated with MTLE fail to do so, thereby starving interneurons of their inhibitory neurotransmitter.

Genetically induced astrogliosis impairs GABAergic inhibition

Whereas the study described above induced focal gliosis via targeted viral inoculation of the hippocampus, more widespread gliosis can be achieved in transgenic animals through the knockout of the β1-integrin (*Itgb1*) gene[25]. Integrins are transmembrane receptors composed of an α and a β subunit and mediate cell-cell and cell-matrix signaling[26]. The β1 subunit is the predominant β subunit in brain[27], and its selective ablation in radial glia interferes with the normal development of mature, quiescent astrocytes[25]. Beginning 4 weeks after birth, astrocytes become progressively reactive in the complete absence of gross brain abnormalities, and without cell death or impairment of the blood-brain barrier[25]. By 6 weeks of age, however, the majority of these mice develop spontaneous recurring seizures[17] and virtually all of the transgenic animals develop abnormal interictal spikes. These are short synchronous discharges of groups of neurons observed in the electroencephalograms known to predispose patients to spontaneous seizures.

Biochemical analysis of tissue from these animals provides a clue to the molecular changes that drive epileptic seizures, namely altered expression of two neuronal chloride (Cl^-) transporters, NKCC1 and KCC2. It is well established that these two antagonistically acting

cation/chloride co-transporters (CCCs) establish the transmembrane gradient for Cl^-, which serves as the principle charge carrier for GABA receptors (Fig. 1). Early in development, the Cl^--extruding KCC2 transporter is expressed at low levels and does not antagonize the import of Cl^- via NKCC1, resulting in high intracellular chloride levels. When GABA binds to its receptors, which are GABA-gated Cl^- channels, chloride leaves the cell, causing depolarization and an excitatory response. In just a few weeks after birth, the marked increase in KCC2 expression shifts the balance between NKCC1 and KCC2 action. KCC2 shunts chloride out of the cell, lowering intracellular chloride in mature neurons. These neurons now hyperpolarize in response to GABA exhibiting an inhibitory response[28].

The β1-integrin knockout mice show an atypical loss of neuronal surface expression of KCC2 and increase in NKCC1, which is responsible for the accumulation of intracellular Cl^-. A subset of these animals respond to the NKCC1 inhibitor bumetanide with a reduction in seizure frequency, suggesting that their GABA receptor function reverted to an immature developmental stage. How gliosis changes the neuronal expression and function of NKCC1 and KCC2 remains unknown. However, KCC2 expression and function is regulated through several phosphorylation sites. The increase of extracellular Glu that typifies reactive tissue in which astrocytes are unable to take up Glu (Fig. 1) has been shown to cause KCC2 dephosphorylation and downregulation of both the surface expression and function of KCC2 (ref. 29). Another known regulator of KCC2, BDNF, is released by microglia and causes a transcriptional downregulation of *KCC2* (*Slc12a5*) in spinal neurons, resulting in similarly impaired chloride homeostasis. Ablation of microglia or deletion of the *BDNF* gene in microglia prevented morphine-induced hypersensitivity for pain[30]. In animal models of epilepsy and human epileptic brain tissue, BDNF is upregulated, making it an attractive candidate as the mediator of the above-observed changes in GABAergic function. Taken together, gliosis impairs GABAergic inhibition through changes in neuronal expression of the KCC2 transporter, which is important for setting the inhibitory tone of GABA[28].

The NKCC1 inhibitor bumetanide is now used in clinical studies to treat severe seizures in infants caused by excitatory GABA responses (clinicaltrials.gov, #NCT00830531, https://clinicaltrials.gov/ct2/show/NCT00830531?term=NCT00830531&rank=1), whereas a specific KCC2 activator is not yet available for clinical application. Excitingly, such an activator has been identified for experimental use[31]. It remains to be seen whether this compound is able to alleviate seizures in experimental models of epilepsy that present with KCC2 inactivation.

Malignant glia induce excitatory neuronal GABA responses

Gliomas are glial cell–derived aggressive brain tumors divided into subcategories depending on the morphological characteristics of the cells forming the main mass of the tumor[32]. Astrocytomas are the most common subtype of gliomas, and the cells composing these tumors morphologically resemble astrocytes[33]. In addition, these glioma cells share many characteristics with non-malignant reactive astrocytes[34–37]. Gliomas are a common cause for acquired epilepsy. Indeed, over 80% of glioma patients report seizures during their illness. In many cases these seizures are resistant to anti-epileptic drugs (AEDs) leaving the patient to suffer from tumor-associated epilepsy. One seizure cause, namely the uncontrolled release of

Glu from the growing tumor, is now well established[18,38] and is consistent with vastly enhanced Glu in the vicinity of gliomas in patients[14]. Higher expression levels of the cysteine/glutamate transporter System xc (SXC, encoded by *Slc7a11*), the main transporter responsible for glioma-mediated glutamate release, are predictive of seizure development and shorter survival times in mice inoculated with different patient-derived glioma lines[39]. Treatment of mice bearing SXC-positive gliomas with the SXC inhibitor sulfasalazine reduces the frequency of epileptiform events[18]. Notably, SXC expression also positively correlates with glutamate release in glioma patients and is acutely inhibited after oral sulfasalazine administration in these patients[39].

Cultured astrocytes are capable of removing 100 μM glutamate from a culture flask in a matter of hours[40] and a similar clearance rate is seen *in vivo* after treating animals with monosodium Glu[41]. Thus, the extent to which glutamate stays elevated in the peritumoral brain is surprising. It is likely that pronounced peritumoral astrogliosis[42] interferes with glutamate uptake as described above[17]. However, definitive experimental evidence will be required to unequivocally draw this conclusion.

Two recent studies, one in mouse[20] and one in acute tissue from glioma patients[19], suggest that concomitant changes in GABAergic inhibition, similar to those described above for genetically induced astrogliosis, appears to be an essential contributor to the disease. More specifically, both studies demonstrated abnormal peritumoral tissue hyperexcitability, and patch-clamp recordings revealed that a majority of peritumoral neurons exhibit excitatory rather than inhibitory GABA responses. These were explained in part by diminished membrane expression of KCC2 with increased expression of NKCC1 in concert with an overall reduction of GABAergic neurons[20]. The imbalance of KCC2 and NKCC1 activity results in increased intracellular Cl^- yielding excitatory GABA responses. An increased intracellular Cl^- concentration can even be observed in mouse hippocampal neurons co-cultured with human glioma cells. These culture studies also suggested that the altered Cl^- gradient results from Glu release causing inactivation of KCC2 via tyrosine phosphorylation[43].

Thus, elevated extracellular Glu released by glioma cells can contribute to tumor-associated epilepsy both directly by enhancing the excitatory drive and indirectly by causing an inactivation of KCC2 (Fig. 1c).

Gliosis, gliotransmission and the composition of the ECM

The common thread emerging from the above studies is the fact that, in each instance, a change in glia, notably astrogliosis or malignancy, is sufficient for the development of epilepsy. How might gliotic astrocytes or glioma cells, malignant glia, signal to neurons? One answer to this question comes from a recent study that explored the role of gliotransmitters, such as Glu and ATP, in pilocarpine-induced status epilepticus[44]. It is noteworthy that gliotransmission, the active vesicular release of neurotransmitters by astrocytes as part of healthy brain function, is controversially discussed in the field. They are several excellent reviews elaborating on studies that found evidence for or against this phenomenon[45–48]. How pathological changes in the diseased brain alter the potential of

astrocytes to release neuroactive substances is even less understood and the release of neuroactive substances through hemichannels, volume-regulated anion channels, P2X7 channels or reversal of glutamate uptake are alternative routes to be considered[48].

However, the following study assessed how inhibition of vesicular release by astrocytes affects the susceptibility of mice to develop seizures. It is well known that pilocarpine-triggered status epilepticus induces marked gliosis[49], and within 2 weeks treated mice develop spontaneous recurring seizures. Notably, seizure onset is delayed and subsequent progressive increase in seizure frequency is attenuated if the release of neuroactive substances from astrocytes is inhibited by expression of a dominant-negative SNARE domain in astrocytes[44] (note that the use of this genetic tool is also under debate[50]). Moreover, chronic inhibition of neuronal NMDARs by infusion of D-AP5 also delayed seizure onset, suggesting that release of gliotransmitters by reactive astrocytes acts via neuronal NMDARs to induce neuronal hyperexcitability.

A study from the Nedergaard laboratory also supports the idea that calcium (Ca^{2+})-mediated release of glutamate from astrocytes has a key role in seizure activity[51]. Here, the authors found that photolytic uncaging of Ca^{2+} in astrocytes initiates the release of glutamate. This induced local depolarization shifts, which can cause groups of neurons to fire synchronously, which is the basis for epileptiform discharges. When seizure activity was induced by local application of the potassium channel inhibitor 4-AP *in vivo*, increases in astrocytic Ca^{2+} preceded seizure-like neuronal burstings. When antiepileptic drugs were administered systemically to the animals, both epileptic discharges and the 4-AP–induced elevated astrocytic Ca^{2+} were reduced. Similar to the finding of Clasadonte *et al.*[44], glutamate release causes hyperexcitability through activation of NMDAR[51].

Notably, NMDAR activity is known to regulate KCC2 function in hippocampal neurons, which in turn acquire excitatory GABA responses[52]. Similarly, Glu, the neurotransmitter acting on NMDARs, has been shown to be the driver of tumor associated epilepsy[18,42]. Hence Glu may be one important glia-neuronal signal that causes intrinsic neuronal changes associated with reactive and malignant astrocytes.

Another well-understood change that characterizes both reactive gliosis and glial malignancy is the active remodeling of the extracellular matrix (ECM). The brain contains a variety of ECM molecules, many of them produced by astrocytes. Of particular relevance for epilepsy are the chondroitin sulfate proteoglycans (CSPGs) that form perineuronal nets (PNNs) around GABAergic interneurons. CSPGs suppress axonal and dendritic outgrowth and have been proposed to suppress regeneration after CNS injury[53] and decrease of plasticity when the brain matures[54]. Notably, formation of PNNs coincides with the end of the critical period for ocular dominance in the visual cortex. If PNNs are digested with the enzyme ChondroitinaseABC, neuroplasticity can be restored in the adult visual cortex, suggesting that PNNs restrict axonal and dendritic sprouting once major circuits have formed. Interneurons become hyperexcitable when PNNs are digested[55]. This suggests that the composition of the ECM influences neuronal excitability. How this may occur is not entirely clear, but one recent study[56] provides food for thought. The authors propose that the composition of the ECM determines the transmembrane Cl^- gradient and therefore whether

GABA is inhibitory or excitatory. Using mice with genetically encoded Cl^- sensors, the authors found that digestion of the ECM increases intracellular neuronal [Cl^-] from ~6–20 mM, which changes the transmembrane Cl^- gradient so that GABA becomes excitatory[56]. The authors argue that the CSPG ECM surrounding neurons is rich in impermeable, stationary sulfate anions that reduce the concentration of free, membrane permeant Cl^- (refs. 56,57). Following digestion, the impermeant anions are replaced by permeant Cl^-, shifting the equilibrium conditions for the cation chloride transport toward higher intracellular Cl^-, which results in increased excitability. This study elicited a heated debate[57–59], as it questions the relevance of the chloride transporters NKCC1 and KCC2 for maintenance of intracellular chloride concentrations.

Digestion and remodeling of the ECM, including CSPGs, is important in the progression and invasion of primary brain tumors, which are rich in matrix-degrading enzymes. Similarly, reactive astrocytes secrete CSPGs that are known to suppress neurite outgrowth. Whether this ‘scar matrix’ is sufficient to change intracellular [Cl^-] in neurons remains to be seen. However, similar to gliomas, reactive glia also destabilize the ECM by the release of proteases[60,61] and this has been well documented in stroke patients and animal models of the disease[62,63]. Stroke is the major risk factor for seizures in people over the age of 65. In this case, the reduction of polyanionic molecules in the ECM might shift the neuronal Cl^- concentrations toward high and result in depolarizing membrane potentials following GABA binding.

Changes in the ECM might not just affect Cl^- currents. The ECM component vitronectin regulates hyperpolarization-activated cation currents and inactivating potassium currents in mouse hippocampal neurons and might therefore modulate neuronal excitability[64,65]. Although little evidence points to the presence of vitronectin in the healthy or injured brain, this ECM molecule is secreted by glioma cells[66–68] and might contribute to the alteration of neuronal cation currents in this condition specifically.

Glia as drivers of abnormal neuronal activity

The above studies converge on just a few common disease mechanisms and indeed point to ‘reactive gliosis’ as a single unifying theme central to the disease. Regardless of the underlying insult, the transition from normal to reactive glia is sufficient to cause changes in the intrinsic properties of neurons associated with them (decreased GABA, increased intracellular Cl^-). These neuronal changes both cause a loss of GABAergic inhibition and resemble a reversion to a more immature developmental stage where GABA acts as an excitatory rather than inhibitory neurotransmitter. In epilepsies that present with marked focal gliosis, such as MTLE, seizures may originate from the brain adjacent to the gliotic lesion if the lesion itself has become devoid of neurons. Similarly, the tumor mass itself is free of neurons, but nevertheless affects the neighboring neuronal network function such that seizures result.

We suggest that many if not all acquired epilepsies may share this underlying common mechanism and all hinge on the presence of reactive gliosis. This viewpoint is certainly supported by the presence of gliosis in essentially all idiopathic and acquired forms of

epilepsy. If true, this would support a shift in the therapeutic target from neuron to glia, particularly making the glial scar the target of future therapy. This principle idea is consistent with the century-old observations that surgical treatment of mesiotemporal lobe epilepsy is only curative if the glial scar is removed.

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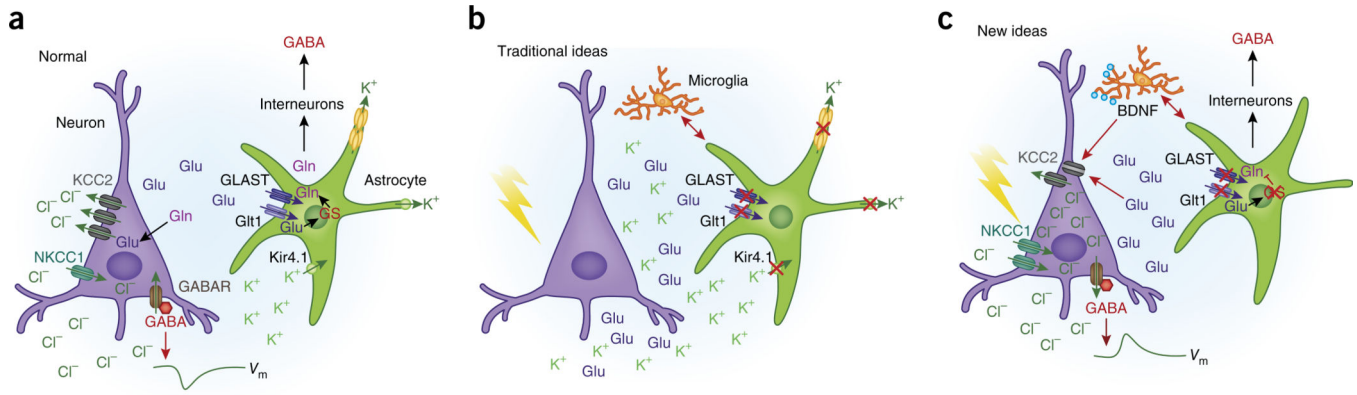

Figure 1.

Astrocytes are active modulators of neuronal activity. (**a**) In the healthy brain, astrocytes are important for brain homeostasis, including the clearance of potassium (K^+) from the extracellular space and termination of neuronal transmission. They express the glutamate transporters EAAT1 and EAAT2, which clear the extracellular space of excess Glu. Glu is transformed into glutamine (Gln) by GS and Gln cycles back into neurons, where it serves as precursor for Glu and GABA synthesis. Mature neurons in the healthy nervous system express low levels of the cation-cotransporter NKCC1 and high levels of KCC2, which shunts Cl^- out of the cells. This assures low intracellular Cl^- concentrations and a Cl^- electrochemical gradient that mediates Cl^- inflow after opening of the GABA receptor (GABAR). The GABAR is a GABA-gated chloride channel that usually causes hyperpolarization of the membrane after binding of the inhibitory neurotransmitter GABA. (**b**) Reactive astrocytes fail as guardians of the neuronal microenvironment in epilepsy. Pathologically elevated extracellular potassium and Glu concentrations result from a failure of astrocytic homeostatic functions. These can be a consequence of the downregulation of transporters and ion channels, loss of these proteins from the membrane, or morphological or physiological changes in reactive astrocytes. (**c**) New studies point to a direct role of reactive astrocytes in modulating neuronal function. Reduced activity of GS not only limits Glu uptake, but also affects the production of GABA, resulting in a loss of inhibition and enhanced excitatory drive.

Table 1

Factors contributing to seizure development

Traditional			
Kir4.1	Potassium channel	Expressed mainly on astrocytes Open at rest, responsible for resting membrane potential and clearance of extracellular potassium	Functionally impaired in epileptic patient tissue and animal models Downregulated in patient tissue Single nucleotide polymorphisms (SNPs) and mutations associated with different forms of epilepsy Conditional knockout mice prone to stress-induced seizures[69]
EAAT2 (Glt1)	Glutamate transporter	Clearance of extracellular glutamate Expressed in astrocytes	Differentially regulated in patient tissue Glt1 KO mice develop spontaneous seizures[18,70]
EAAT1 (GLAST)	Glutamate transporter	Clearance of extracellular glutamate Expressed in astrocytes	Differentially regulated in patient tissue GLAST KO mice have a lower threshold to develop chemically induced seizures[18]
Aquaporin-4 (AQP4)	Water channel	Mediates water flow across the membrane Expressed in astrocytes Colocalize and act in concert with Kir4.1	Dysregulated in human epilepic tissue (up or down or altered subcellular distribution) SNPs in AQP4 associated with TLE AQP4 KO mice have prolonged seizure duration, impaired K^+ clearance and gap junctional coupling[69]
Connexin 30, 43	Gap junction/hemichannel proteins	Couple astrocytes and allow molecules and ions to directly pass between cells through a regulated gate Implicated in potassium buffering	Dysregulated in human epilepsy and rodent models (up- and downregulation) Astrocyte-specific deletion in mice causes spontaneous epileptiform activity and a decreased threshold for evoked seizures[70]
New concepts			
KCC2	CCC	Exports Cl^- out of the cell Expressed in neurons	Downregulated in tissue of epilepsy patients[71] and in mouse models of epilepsy[17,20,72] CCC misregulation causes impaired Cl^- homeostasis and depolarizing GABA responses[20,71,72]
NKCC1	CCC	Imports Cl^- into the cell Expressed in neurons, glia, endothelial cells	Upregulated in human epileptic tissue[19] and rodent models of epilepsy[17] NKCC1 inhibitor bumetanide acts as an anticonvulsant in animal models[73] Clinical trial (NCT00830531) tests the efficacy of bumetanide in the treatment of neonatal seizures
GS	Enzyme catalyzing the condensation of glutamate with ammonia to glutamine	Expressed in astrocytes Essential enzyme of the glutamate-glutamine shuttle	Downregulated in patient tissue[13] and after virally induced astrogliosis in mice[16] Loss of GS reduces GABAergic inhibition[16]
ECM	Might determine Cl^- equilibrium potential of neurons[56]	Intracellular source mainly nucleic acids Extracellular source ECM	ECM remodeling in epilepsy[60]
GAT	GABA uptake	Expressed in neurons or astrocytes Terminates GABAergic neuronal transmission Astrocytes adjust GAT levels in response to	GATs are explored as drug targets to modulate extracellular GABA levels for anticonvulsant action Tiagabine is the only approved GAT inhibitor for clinical use[75]

GABA release[74]

Differential expression of the CCCs NKKC1 and KCC2, induced directly or indirectly by reactive glia, result in a reversed ratio of KCC2 to NKKC1 and, consequently, high neuronal Cl^-. The inhibitory neurotransmitter GABA then actually becomes excitatory, as opening of the GABA receptor results in outflow of Cl^- and a depolarization of the neuronal membrane.