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Contributions of Astrocytes to Epileptogenesis Following Status Epilepticus: Opportunities for Preventive Therapy?

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Abstract

Status epilepticus (SE) is a life threatening condition that often precedes the development of epilepsy. Traditional treatments for epilepsy have been focused on targeting neuronal mechanisms contributing to hyperexcitability, however, approximately 30% of patients with epilepsy do not respond to existing neurocentric pharmacotherapies. A growing body of evidence has demonstrated that profound changes in the morphology and function of astrocytes accompany SE and persist in epilepsy. Astrocytes are increasingly recognized for their diverse roles in modulating neuronal activity, and understanding the changes in astrocytes following SE could provide important clues about the mechanisms underlying seizure generation and termination. By understanding the contributions of astrocytes to the network changes underlying epileptogenesis and the development of epilepsy, we will gain a greater appreciation of the contributions of astrocytes to dynamic circuit changes, which will enable us to develop more successful therapies to prevent and treat epilepsy. This review summarizes changes in astrocytes following SE in animal models of SE and human temporal lobe epilepsy and addresses the functional consequences of those changes that may provide clues to the process of epileptogenesis.

Keywords

Reactive astrocyte; Gliosis; Status Epilepticus; Review; Epileptogenesis; Inflammation; Epilepsy; Seizure

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Status Epilepticus Can Result in Temporal Lobe Epilepsy

Status epilepticus (SE) is a medical emergency with a poor prognosis. It is defined as continuous seizure activity lasting greater than 5 minutes or two or more sequential seizures without full recovery between seizures (Lowenstein et al., 1999). While the risk of death following SE has declined somewhat in the industrialized world, older patients experiencing prolonged SE are still at high risk of death. Furthermore, patients who survive a prolonged bout of SE often experience cognitive decline and develop epilepsy (Engel, 1996). Patients with temporal lobe epilepsy (TLE) often first present with SE. TLE is a common seizure disorder characterized by complex partial seizures that can secondarily generalize and are often pharmacoresistant to currently available anti-seizure drugs (ASDs). Because SE can result in difficult to treat forms of epilepsy such as TLE, it is the focus of much research.

Administration of chemoconvulsant agents and electrical stimulation have been used in rodents to induce SE that is then followed by the progressive development of epilepsy. Two of the most well studied rodent models of SE are the systemic kainic acid (KA) and pilocarpine (PILO) models. Following injection of either of these two agents, animals begin a bout of SE that can last hours (Curia et al., 2008; Lehmkuhle et al., 2009). While mortality is lower for the KA model, survival can be enhanced in the PILO model by treatment with a variety of anticonvulsant agents after about an hour of SE. After a latent period of several days to weeks following SE, animals develop recurrent seizures that are limbic in origin. Animals treated with a repeated low dose paradigm of KA have been found to develop spontaneous convulsant seizures on average 18 days following SE (Williams et al., 2009), while animals treated with pilocarpine begin to exhibit spontaneous seizures earlier, with some reported seizures occurring within five days following SE (Raol et al., 2006). Likewise, electrical stimulation of the perforant pathway can induce sustained SE that is followed by the development of recurrent seizures. Depending on the stimulus paradigms used to evoke SE in these models, spontaneous seizures can begin within days (Bumanglag and Sloviter, 2008; Norwood et al., 2010). In all of these models, considerable neuronal cell death is observed throughout the brain and astrocytes begin to exhibit signs of reactive gliosis soon after evoking SE (do Nascimento et al., 2012; Glushakova et al., 2012; Lauritzen et al., 2012a).

Animal models of SE-induced epilepsy provide an unparalleled opportunity to unravel the complex changes that occur in the CNS following SE and during the process of epileptogenesis. In particular, they help identify disease-modifying therapies that can prevent the development of epilepsy following SE or stop the progression of seizure activity once epilepsy is established. While much is known about the complex changes that occur in neurons following SE in these animal models, researchers are now investigating the changes that occur in astrocytes following SE. Astrocytes post-SE may develop functional changes that contribute to, or even prevent, network excitability. By understanding these functional changes in astrocytes, it may be possible to identify novel therapeutic targets or strategies for the prevention of epilepsy. In this review, we will focus our attention on recent findings of the effect of SE or related CNS insults on astrocyte function in limbic and cortical regions known to be associated with the initiation of seizure activity and the development of epilepsy.

Astrocytes Become Reactive Following SE and This Persists in Epilepsy

Since their discovery over 100 years ago, glial cells have traditionally been considered only as passive support cells of the nervous system (for reviews on the history of glial research, see Garcia-Marin et al., 2007; Somjen, 1988; Verkhratsky, 2006). However, recent application of cellular imaging, physiology, and genetics to astrocytes has shown that they are active participants at the neuronal synapse; their diverse roles are entwined with the function of neurons. The structural and functional relationship between astrocytes and the neuronal synapse has led to the use of the term “tripartite synapse” to reflect the important contribution of astrocytes in influencing synaptic transmission (Halassa et al., 2007). The processes of astrocytes are in close proximity to neuronal membranes and express a host of receptors, transporters, and ion channels that enable astrocytes to “listen” to the chemical conversation at the synapse. In return, they are able to “respond” and modify the tone of synaptic transmission by regulating the availability of neurotransmitters and ions in the synaptic cleft, such as glutamate and potassium.

As a consequence of SE and subsequent inflammation, astrocytes change their morphology and protein expression in a process termed reactive astrogliosis. A number of molecules and signaling pathways are known to trigger reactive astrogliosis (for a comprehensive review, see (Sofroniew, 2009)) and many of those receptors/pathways are activated as a consequence of SE. Molecules released from dying neurons, such as reactive oxygen species, ATP and glutamate, are all known to induce astrogliosis. In addition, inflammatory cytokines such as TNF- α and IL-1 β are also known triggers and these cytokines can be released by microglia and neurons following SE. Interestingly, cytokines are also released by reactive astrocytes, which can result in a feed-forward system that enhances gliosis (Vezzani et al., 2011). Furthermore, the JAK-STAT pathway is known to be elevated as a consequence of SE and this pathway, when stimulated, can also contribute to the observed changes in astrocytes (Lund et al., 2008; Sofroniew, 2009).

While reactive astrogliosis is one of the most common pathological lesions in epilepsy and other brain insults, it continues to be poorly understood. Dramatic changes in the expression of neurotransmitter receptors, voltage gated ion channels, inflammatory cytokines, and other proteins, have been identified in astrocytes in both animal models and human TLE. However, the time course and the functional significance of such diverse changes that accompany astrogliosis is not clear and it is currently unknown to what extent “reactive astrocytes” either contribute to, or prevent the ultimate development of TLE. Indeed, some components of reactive astrogliosis may be critical compensatory mechanisms following injury, resulting in the dampening of excitability, while other changes, e.g. decreased expression of aquaporin, may in fact contribute to epileptogenesis and/or seizure generation (Lee et al., 2012). Finally, it is possible that some alterations in structure and/or function of astrocytes may even be unrelated to epileptogenesis. We contend that the study of the role of reactive astrogliosis in epileptogenesis is in its infancy and therefore, before disease modifying therapies can be identified for the treatment and/or prevention of SE-induced epilepsy, a mechanistic understanding of structure and function of reactive astrocytes in the disease process must be attained.

Properties of Reactive Gliosis Following SE

Following SE, reactive astrocytes become hypertrophic, increase expression of intermediate filament proteins (for example, GFAP), and develop longer and thicker processes (Binder and Steinhauser, 2006) (Figure 1). Although the functional consequence of changes in domain organization (see below) and hypertrophied processes with increased expression for GFAP are not exactly clear (Fedele et al., 2005; Gouder et al., 2004), some of the changes in expression of other proteins could have direct consequences on the regulatory mechanisms of gene transcription in reactive astrocytes (Ridet et al., 1997). The term “reactive astrocyte” is rather ambiguous because reactive astrogliosis is not an all-or-none response (Sofroniew, 2009). There can be significant diversity within a reactive astrocyte population (Kálmán, 2003; Ridet et al., 1997). Mild to moderate gliosis, found early on in SE models, typically does not induce the proliferation of new astrocytes (Fernaund-Espinosa et al., 1993; Sofroniew, 2009). There are, however, some studies that have addressed the proliferative ability of astrocytes in the CA fields of the hippocampus following KA-induced SE or lesions (Murabe et al., 1982; Niquet et al., 1994). These studies show that while astrocytes can in fact proliferate after insult, the proliferation accounts for a very small number of the new cells in the area, suggesting that in models of SE, reactive astrocytes are comprised mainly of the resident astrocytes present before the insult.

Two of the hallmarks of reactive astrocytes are the increase in expression of intermediate filament proteins (for example, GFAP) in response to injury (Ridet et al., 1997) and hypertrophy of the main cytoskeletal branches. During this process, however, the fine spongiform processes of astrocytes are not immunoreactive for intermediate filaments. GFAP immunolabeling, therefore, does not detect changes in the spatial domains (i.e. cell volume) of reactive astrocytes in the neuropil. Changes in domain organization in reactive astrocytes are important to consider, as they may be indicative of astrocytes that are no longer controlling or modulating the same group of synapses and these changes could indicate pathological astrocyte-astrocyte or astrocyte-neuron relationships.

Two independent groups investigated whether domain organization is preserved in reactive astrocytes. Wilhelmsson et al. (2006) dye filled astrocytes in the hippocampus following disruption of the perforant path from the entorhinal cortex. In this model, astrocytes increased the expression of GFAP and were reactive (presumably due to the axonal degeneration of the perforant path) but did not change their domain volume and did not interdigitate with neighboring astrocytes; thus, reactive astrocytes in this model maintained their spatial domains. In contrast, Oberheim et al. (2008) diolistically labeled astrocytes in the hippocampus of three mouse models of epilepsy: the ferrous chloride model of post traumatic epilepsy, KA-induced SE model, and the SWXL-4 mouse, a genetic model of epilepsy. Hypertrophied astrocytes (observed with GFAP) in these models of epilepsy had increased domain volumes and almost a complete loss of domain organization (observed with diolistic labeling). These changes were observed 1 week post injection of ferrous chloride, and 6 months after, during a time of chronic seizure activity. Interestingly, in ferrous chloride injected animals whose seizures were reduced with the anti-epileptic drug valproate, astrocytes were less hypertrophied, had domain volumes similar to controls, and did not lose their domain organization by interdigitating extensively with neighboring

astrocytes. Therefore, changes in domain organization paralleled the severity of seizure activity.

The results from these studies on the changes in spatial domains of reactive astrocytes suggest that a loss of domain organization may be specific to astrocytes in epileptic tissue. In support of this notion, Oberheim et al. (2008) noted that reactive astrocytes in a mouse model of Alzheimer's disease did not lose their domain organization, even though the astrocytes in this model were reactive (i.e. hypertrophied processes and an increase in GFAP expression). The research highlights two salient points. The first point is that reactive astrocytes, as determined by increased immunoreactivity to GFAP and hypertrophied processes, may not be homogeneous. Astrocytes may respond differently according to the type of CNS insult, even though they may appear identical by immunohistochemistry (i.e. reactive, as revealed by GFAP). Indeed, recent genomic analysis of reactive astrocytes revealed a different pattern of gene expression depending on the type of insult (Zamanian et al., 2012). The CNS insults compared in this study were ischemia and LPS injection. Therefore, while it is not clear what patterns of gene expression would be observed following SE, it is possible that an entirely different gene expression pattern would be observed. Second, reactive astrocytes that have lost their domain organization in epileptic tissue may confer unique functional properties that are specific to this neurological disorder. Based on the differences in structural changes, therefore, reactive astrocytes in various models of neuronal injury may have different functional properties. This is particularly important to note because reactive astrocytes are often considered a homogenous population, although the data increasingly indicates that the term reactive gliosis encompasses a very wide range of features with important functional consequences to studying pathologies. An additional point to consider is that the work described above was performed in rodents. However, there exists a dramatic heterogeneity of human astrocytes. It is not currently known how the individual types of human astrocytes differentially respond to either SE or prolonged TLE (Oberheim et al., 2009).

Potassium Buffering Is Not Altered Shortly after SE

Astrocytes play a critical role in maintaining neuronal homeostasis by buffering extracellular potassium (K^+) through the highly permeable, inwardly rectifying potassium channel $K_{IR}4.1$ (Higashi et al., 2001; Poopalasundaram et al., 2000). Neurons extrude K^+ with each action potential repolarization and during periods of high activity, K^+ concentration in the extracellular space can be rapidly elevated (Dichter et al., 1972; Nicholson and Sykova, 1998). Thus, the tight regulation of $[K^+]_o$ via astrocytic uptake is critical for maintaining neuronal homeostasis. Given the importance of K_{IR} channels in spatial buffering and the role of potassium in neuronal function, it is no surprise that its role has been heavily investigated in epilepsy. For instance, conditional knock-out of the $K_{IR} 4.1$ channel in mice results in behavioral ataxia, seizures, and early lethality is observed resulting from deficient K^+ uptake in astrocytes (Djukic et al., 2007). Likewise, in the sclerotic hippocampus of patients with TLE, the K_{IR} channel blocker Ba^{2+} was shown to be ineffective in increasing extracellular potassium levels measured by potassium selective microelectrodes in response to stimulus as was expected, suggesting dysfunction in K_{IR} channels (Kivi et al., 2000). This finding was also observed in some brain slices obtained from epileptic rats that had received

pilocarpine to induce SE (Gabriel et al., 1998). These results were corroborated using patch-clamp electrophysiology in acute hippocampal slices obtained from surgical specimens of patients with TLE, which showed reduced inward rectifying potassium conductances in reactive astrocytes from this tissue (Hinterkeuser et al., 2000). Together these findings support the altered ability of astrocytes to effectively buffer potassium in epileptic tissue. However, until recently, changes in K_{IR} and potassium uptake have not been investigated in reactive astrocytes during the latent period in an animal model of SE-induced MTLE. Studies from our laboratory (Takahashi et al. 2010) have demonstrated that potassium conductances were not altered in astrocytes during the latent period one-two weeks following SE in the KA-treated rat. This finding suggests that soon after SE and prior to the development of epilepsy, astrocyte potassium buffering capability remains intact. Further investigation is necessary to determine how and if K_{IR} function contributes to epileptogenesis. Indeed, therapeutic strategies which result in the continued expression and function of specific K_{IR} channels in astrocytes might prove to be useful in delaying the onset of epilepsy following SE.

Gap Junctions Between Astrocytes Increase Following SE

Astrocytes are by far the most extensively gap junction coupled cells in the central nervous system and are critical in maintaining homeostatic balance of ions and molecules (for reviews, see Kielian, 2008; Nagy and Rash, 2000; Theis et al., 2005). A complete gap junction is formed by the direct contact of two opposing hemichannels, or connexons, on two different cells. Each connexon consists of a hexameric structure consisting of six connexin (Cx) proteins with a hydrophilic pore at its center. This pore allows transfer of ions, second messengers, metabolites, and other small molecules (Nakase and Naus, 2004), allowing not only electrical coupling between cells but also chemical and metabolic coupling. Astrocytes express predominately Cx43 and Cx30 and form a metabolically coupled syncytium in CNS tissue (Rash et al., 2001).

Our laboratory recently showed that the number of coupled astrocytes in brain slices was significantly increased in KA-treated rats early in the process of epileptogenesis, and consistent with that observation, CX43 expression was greatly elevated (Takahashi et al., 2010). Interestingly, in brain slices obtained from normal rats, epileptiform burst-like activity has been shown to enhance coupling between astrocytes in a rapid activity dependent manner (Rouach et al., 2008). The extent of coupling in astrocytes is thought to underlie spatial buffering and the ability to redistribute potassium and glutamate (Theis et al., 2005). Gap junction coupling has also been implicated in spreading depression, a pathophysiological phenomenon in the CNS characterized by a combined reaction of neurons and glia in the form of a propagating wave of neuronal depolarization followed by neuronal inactivation. Interestingly, a conditional knock out of the main gap junction protein in astrocytes, connexin (Cx)43, results in an animal with decreased intracellular coupling among astrocytes and enhanced velocity in the wave of spreading depression (Theis et al., 2003), suggesting that the redistribution of ions and intracellular molecules in astrocytes is critical for minimizing spreading depression and hyperexcitability. Therefore, in animals experiencing SE, an increase in gap junctionally-coupled astrocytes could be viewed as compensatory, with an enhanced capacity for buffering both glutamate and potassium.

The data concerning the expression of Cx43 and Cx30 in other models of chronic epilepsy and in human resected tissue are conflicting. In the kindled rat and tetanus toxin-induced model of epilepsy, no change in mRNA of Cx43 was observed four weeks following injury (Elisevich et al., 1997a). Likewise, no change in mRNA or protein expression of Cx43 or Cx30 was detected in kindled rats and in the kainate-treated rat four weeks following injury (Sohl et al., 2000). However, in human resected tissue from patients with intractable epilepsy, Cx43 mRNA and protein are generally shown to be increased (Collignon et al., 2006; Fonseca et al., 2002; Naus et al., 1991) except for (Elisevich et al., 1997b). There is evidence of increased gap junction coupling by measuring calcium signaling induced by glutamate exposure using fluorescence recovery after photo bleaching (FRAP) assays in surgically resected tissue (Lee et al., 1995). These findings suggest that gap junction coupling, along with increased Cx43 and Cx30 expression, might be a common feature in human epilepsy, something not always recapitulated in animal models of chronic epilepsy. Some of the discrepancies between studies could be due to the dynamic nature of the connexin protein. Connexins have a rapid turnover rate of ~1 hour and the antigens that have been used to label Cx43 in the past were difficult to detect and were sensitive to fixation (Hossain et al., 1994; Laird et al., 1991).

Glutamate Transporter Function is Enhanced Following SE

Unlike some neurotransmitters that can be enzymatically degraded after being released into the synaptic cleft, glutamate must be actively removed via Na⁺-dependent glutamate transporters. Fast efficient clearance of glutamate is essential for high signal-to-noise ratios in neurotransmission and for the prevention of excitotoxicity (for review see Danbolt, 2001; Marcaggi and Attwell, 2004; Tzingounis and Wadiche, 2007). The main glutamate transporters, GLAST and GLT-1 are predominately expressed in astrocytes (Lehre et al., 1995), and are responsible for >90% of the total glutamate uptake (Tanaka et al., 1997). The alterations that occur to this system, as well as the closely linked processes governing glutamate and glutamine cycling, during epilepsy are complex and the literature is often conflicting in regards to changes in glutamate transporter expression and function following SE.

In animal models, as would be expected, knock down of GLT-1 expression in the mouse leads to neuronal loss and seizure activity that is caused by the build up of extracellular glutamate (Tanaka et al., 1997). Similarly, eight weeks following stereotactically injected KA into the amygdala of rats, spontaneous motor seizure activity is observed and hippocampal tissue homogenates show a reduced expression of GLT-1 and GLAST (Ueda et al., 2001). In human tissue from patients with TLE, immunohistochemistry consistently demonstrates reductions in GLT-1 and GLAST expression (Mathern et al., 1999; Proper et al., 2002; Sarac et al., 2009). However, similar studies in human tissue using western blots on hippocampal homogenates do not show a change in GLT-1 or GLAST expression (Bjornsen et al., 2007; Tessler et al., 1999). However, the changes in transporter protein expression described above do not provide a direct assessment of the changes in transporter dependent glutamate uptake in epilepsy. A more quantitative measurement of transporter function, and hence glutamate clearance, in epileptic tissue is to directly record synaptically evoked glutamate transporter dependent currents in reactive hippocampal astrocytes.

In a recent study, we recorded from astrocytes in hippocampal brain slices obtained from control animals and those that had been subjected to KA-induced SE. We demonstrated that there is no effect of SE on the amplitude of glutamate transport currents, nor any concomitant change in the expression of GLT-1 expressed in astrocytes within 1-2 weeks after SE. However, we did determine that there is a significant decrease in the decay kinetics of glutamate transport currents recorded in reactive astrocytes, suggesting that following SE, astrocytes have an enhanced capacity to more quickly take up extracellular glutamate (Takahashi et al., 2010). Previous work by other investigators demonstrated that the time course of glutamate transport currents is not affected by changes in stimulus strength, the probability of release of glutamate, or the diffusion kinetics of glutamate. Instead, the rate of decay of the current is likely due to a variety of mechanisms, including the number and type of glutamate transporters present at the synapse and the extracellular volume (Diamond, 2005; Diamond and Jahr, 2000). Therefore, there are several hypotheses that could explain the faster kinetics of the glutamate transport currents in reactive astrocytes. For example, it has recently been demonstrated that the GLT-1 transporter is rapidly trafficked to activated synapses. (Benediktsson et al., 2012). Therefore, it is possible that following the intense neural activity that occurs during SE, existing transporters may be trafficked to activated synapses. It is also known that several astrocyte signaling pathways can regulate glutamate transporter trafficking (Robinson, 2002) and while we did not detect a change in GLT-1 expression in our studies, it is possible that trafficking of existing transporters could contribute to the changes we observed. Alternatively, decreased extracellular volume contributes to the observed decrease in glutamate transporter current decay kinetics observed during development (Thomas et al., 2011) and thus may also play a role in epilepsy, where the extracellular space is reduced and known to contribute to hyperexcitability (Hochman, 2012). Finally, the rapid buffering of glutamate observed following SE may also be facilitated by the increase in gap junctions between astrocytes (Takahashi et al., 2010). Indeed, recent work in animals with Cx43 and Cx30 deleted from astrocytes, and consequently no gap junctions between astrocytes, demonstrated that the decay time constant of the glutamate transport current was significantly slowed, consistent with the notion that extensive networks are essential for proper buffering of glutamate (Pannasch et al., 2011). Thus, the maintenance of extensive networks of coupled astrocytes could very well be viewed as an essential mechanism for dampening excitability in epileptic networks.

Glutamate Receptor Expression

Astrocyte function depends in part on cell surface receptor expression. Astrocytes in juvenile animals and in cell culture have been shown to express nearly every transmitter receptor as their neuronal counterparts, including metabotropic and ionotropic glutamate receptors; for review see (D'Antoni et al., 2008; Kimelberg, 1995; Lalo et al., 2010). In brain slices obtained from juvenile animals, the functional expression of α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) (Jabs et al., 1994; Schroder et al., 2002; Seifert et al., 2004; Seifert et al., 1997) and N-methyl-D-aspartate receptors (NMDA) (Serrano et al., 2008; Steinhauser et al., 1994) have been described. Expression for group I mGluRs is increased in reactive astrocytes 1 week after SE (Aronica et al., 2000), in human MTLE (Notenboom et al., 2006), and in reactive astrocytes following neuronal injury (Ferraguti et

al., 2001; Ulas et al., 2000), however, the electrically passive, GFAP-expressing, protoplasmic astrocyte of the adult hippocampus does not express ionotropic glutamate receptors under basal conditions. Interestingly, kainate receptors (KARs) and NMDA receptors are expressed in reactive astrocytes following ischemia in adult animals (Gottlieb and Matute, 1997; Krebs et al., 2003). Furthermore, two recent reports have demonstrated that the protoplasmic GFAP+ astrocyte of the cortex acquires electrophysiological responses to kainate application following ischemic insult or within an Alzheimer's disease model, again suggesting possible KAR expression in response to injury (Peters et al., 2009; Wang et al., 2008). Interestingly, preliminary immunohistochemical studies from our laboratory suggests that following SE, astrocytes begin to express KAR subunits (Vargas et al., 2006).

The modulating role of KARs and their unique ability to couple to G-proteins makes this family of glutamate receptor an important component of CNS function. It is currently unknown what functional role KARs expressed in astrocytes following KA-induced SE play in the disease process of epileptogenesis. However, it is enticing to hypothesize that increased calcium signaling and subsequent gliotransmission mediated in part by the pathological expression of ionotropic KARs and increased expression of mGluRs in reactive astrocytes could cause the synchronization of neurons thus contributing to hyperexcitability.

Blood-Brain Barrier Disruption Following SE

Astrocytes form close associations with the endothelial cells of capillaries and help maintain proper function of the blood brain barrier (BBB). Disruption of the BBB is often observed in epileptic brain regions, but has generally been thought to result from seizure activity, rather than contribute to the generation of seizures. A body of clinical and experimental evidence now suggests that opening of the BBB triggers a chain of events that may contribute to the processes underlying epileptogenesis. Van Vliet and coworkers have demonstrated that pronounced levels of BBB leakage occur during the acute and latent phases following SE in humans and rats (van Vliet et al., 2007). In addition, following BBB disruption, uptake of the serum protein albumin by astrocytes through TGF- β receptors has been associated with reactive gliosis (Ivens, Kaufer et al. 2007). Studies in cultured astrocytes from rat brains show that albumin induces calcium signaling (Nadal et al., 1995) and that TGF- β receptor activation causes a down regulation of K_{IR} channels (Perillan et al., 2002), suggesting that albumin can directly modulate astrocyte functions and enhance excitability. Together these data suggest that disruptions of the blood brain barrier may directly contribute to processes underlying epileptogenesis through astrocyte-mediated mechanisms (Ivens et al., 2007).

It is also important to note that changes in BBB permeability following SE and other insults may also have significant consequences for delivery of pharmacotherapies in drug-resistant epilepsies (Loscher and Potschka, 2005). For instance, increased expression of multidrug-resistance-associated proteins (MDPs) in endothelial cells of blood vessels in the brain following seizures has been implicated as a possible mechanism in drug-resistant epilepsies. In chronic epilepsy, increased levels of MDPs are thought to facilitate the efflux of antiepileptic drugs such as carbamazepine and valproate, thereby preventing the accumulation of therapeutic levels of antiepileptic drugs in brain tissue (Loscher, 2007). One study demonstrated that increased expression of MDPs in reactive astrocytes and blood

vessels in the hippocampus occurs immediately following electrically evoked status epilepticus (SE) in the rat, indicating that changes in MDP expression may occur earlier than initially thought (van Vliet et al., 2005). Furthermore, increased expression of MDPs during the acute and chronic phase was also accompanied by decreased levels of an administered ASD, phenytoin (PHT). These results suggest that the development of pharmacoresistant epilepsy via the increased expression of MDPs may be initiated after a single bout of SE and should be taken into account when considering potential therapeutic interventions aimed at disrupting the process of epileptogenesis.

Additionally, reductions in MCT1, a key BBB carrier molecule for monocarboxylates and amino acids, have been observed on the endothelial cell membrane of microvessels in the hippocampus of human patients with TLE (Lauritzen et al., 2011) as well as two rodent models of SE (Lauritzen et al., 2012b). Reduced levels of MCT1 in the epileptogenic hippocampus may impair the uptake of monocarboxylates resulting in decreased concentrations of blood-derived fuels, such as ketone bodies, which could contribute to enhanced seizure susceptibility. Intriguingly, the transport of drugs such as valproic acid across the blood brain barrier is also facilitated by MCT1. Thus, concentrations of anti-seizure drugs in the MCT1-deficient epileptogenic hippocampus may be severely reduced, representing a possible explanation for pharmacoresistance in TLE (Lauritzen et al., 2012b).

Brain inflammation occurs as a consequence of seizures and is intimately linked to changes in BBB permeability. Reactive astrocytes have been identified as key players in the mechanisms involved in CNS inflammation (Aronica et al., 2012). Indeed, mRNA of inflammatory mediators are induced within 30 minutes of SE onset (De Simoni et al., 2000) and upregulation of astrocyte genes related to immune and inflammatory responses persists for up to 1 week following SE (Gorter et al., 2006). It is now recognized that a myriad of immune responses, many of which are mediated by astrocytes, can occur as a consequence of seizure activity and may contribute to the development of epilepsy. A more complete discussion of these topics are included in this special issue (see Carson and Fabene's reviews).

Increased Expression of Adenosine Kinase Following SE

In addition to the list of the changes in astrocytes following SE, alterations in the astrocyte-mediated adenosine/adenosine kinase system have shown intriguing potential as a target in the prevention and treatment of epilepsy. For over 40 years, adenosine has been recognized as an endogenous anticonvulsant. Adenosine levels are elevated in patients following seizures, suggesting that adenosine is released during a seizure and may contribute to seizure termination (Boison, 2005). Astrocytes regulate adenosine levels via the production of the enzyme adenosine kinase (ADK) and during epilepsy, reactive astrocytes produce increased levels of ADK. Studies from transgenic animals have shown that ADK overexpression leads to the development of spontaneous seizures and results in mortality following KA-induced SE, presumably due to a reduction in adenosine (Li et al., 2008). Furthermore, SE may cause an imbalance of adenosine/ADK that leads to epileptogenesis. Gouder et al. (2004) demonstrated that ADK is increased in reactive astrocytes around 1 week post SE, prior to the development of spontaneous seizures. Intriguingly, reduction of ADK in the brain can

prevent the development of spontaneous seizures following KA-induced SE and prevents cell death, indicating that ADK reduction confers a neuroprotective effect following status (Li et al., 2008). Recently Theofilas et al. demonstrated that an antisense viral approach to modulate astrocytic ADK expression was able to trigger or prevent seizures in mice, providing the first line of evidence that gene therapy may be successfully applied to this system in order to control epilepsy (Theofilas et al., 2011).

Changes in Astrocyte Calcium Signaling After SE

While astrocytes do not exhibit prominent electrical excitability as do neurons, they are able to dynamically regulate calcium using internal stores (Charles et al., 1991; Cornell-Bell et al., 1990; Finkbeiner, 1992). Calcium transients in astrocytes occur in response to the activation of g-protein coupled receptors (e.g. mGluRs) and are thought to modulate the release of a number of gliotransmitters that could influence synaptic function (Aguado et al., 2002; Araque et al., 1998; Fellin et al., 2004; Halassa and Haydon, 2010; Parpura and Haydon, 2000; Pasti et al., 1997; Shigetomi et al., 2008). Consistent with effective synaptic modulation, astrocytic calcium transients have been shown to correlate with structured neuronal network activity (Aguado et al., 2002; Kuga et al., 2011; Winship et al., 2007). These observations naturally lead to the question of potential changes in astrocytic calcium dynamics during and following SE and how any changes might affect neural network function. Astrocyte calcium dynamics and SE have been studied in only a couple of papers to date (Ding et al., 2007) (Smeal et al., In press). Ding et al. used two-photon microscopy to study cortical astrocytic calcium dynamics *in vivo* using a pilocarpine model of SE (Ding et al., 2007). They found that astrocytic calcium activity increased during and after SE. Interfering with the increase in calcium activity in astrocytes that followed SE was neuroprotective, suggesting that glutamate release from astrocytes was contributing to excitotoxic cell death following SE (Ding et al., 2007).

Recent work from our laboratory using two photon microscopy and a fast scanning technique showed that calcium waves mediated by the astrocytic syncytium in CA3 region of the hippocampus can be very fast and widespread *in vitro* (Smeal et al., In press). This fast wave activity was much reduced in brain slices prepared from juvenile rats that experienced SE induced with kainic acid. Therefore, based on these two studies, calcium dynamics in reactive astrocytes following SE appear to be altered. The functional consequences of these changes in the context of epileptogenesis however, remains to be determined.

One of the primary difficulties in studying astrocyte calcium dynamics related to SE is the inability to bulk load calcium indicating dyes in brain slices obtained from the older animals that are used in the models of SE. The labeling of cells *in vitro* using the acetoxymethyl (AM) ester calcium indicator dyes becomes much more difficult as the animals age past a few weeks (Peterlin et al., 2000; Reeves et al., 2011). Transfection of protein calcium indicators using viruses is a potential solution, but has the drawback of causing astrocytes to become reactive independent of the insult that causes the SE (Ortinski et al., 2010). In order to bypass these loading problems, we have recently used the *in utero* electroporation technique (Chen and LoTurco, 2012; Gee et al., 2011; LoTurco et al., 2009) to introduce

genetic calcium indicators into animals that are subsequently used in the standard SE animal models. Using plasmids that contain modifications to introduce the genetic calcium indicator (GCaMP-3) into the genome and subsequently target it to the cell membrane (Shigetomi et al., 2011) has resulted in well-labeled astrocytes in a number of brain regions including the hippocampus (Figure 2). Calcium transients can be detected even in the fine processes (Figure 2). Future experiments using this technique will study both the potential changes of calcium activity within the fine structure of individual astrocytes and in the calcium dynamics of large networks of astrocytes following SE and during the process of epileptogenesis.

Disease Modifying Therapies Targeting Astrocytes

The vast majority of anti-seizure (ASD) drugs target ion channels and receptors that directly modulate neuronal function (Bialer and White, 2010; White et al., 2007). While this approach can confer seizure control to a majority of patients with epilepsy, it is estimated that between 25-35% of patients do not have their seizures adequately controlled with existing ASDs and the side effects of these drugs also negatively impact the quality of life of patients with epilepsy. Thus, novel ASDs that target inflammation and as a consequence, reactive astrocytes, may provide viable approaches to seizure control. Indeed, there is currently a clinical trial investigating the ability of VX-765, a caspase-1 inhibitor that prevents the production of IL-1 β , to reduce seizures in patients with refractory partial seizures. This is the first clinical trial designed to target inflammatory processes in patients with epilepsy and a positive outcome will set the stage for novel therapeutic approaches for the treatment of existing seizure disorders.

There is currently no strategy to prevent the development of epilepsy in patients at risk following a CNS insult such as SE. However, initial work with VX-765 in rodents suggested that this compound may possess disease modifying properties that could alter the development of epilepsy (Ravizza et al., 2008). Furthermore, the increase in experiments that study the function of astrocytes following SE has opened the door for the development of novel compounds or approaches for targeting astrocyte function in an effort to interfere with the process of epileptogenesis. In addition to the VX-765 studies already discussed, the manipulation of the adenosine kinase system as described in this issue by Boison and colleagues may prove to be an important new therapeutic direction. Likewise, as more information is gleaned about the role of reactive astrocytes in the progression of epilepsy, the more opportunities we will have for interfering with the process of epileptogenesis.

Conclusions

SE results in rapid structural and functional changes to not only neurons, but astrocytes as well. Numerous studies performed in resected epileptic tissue and in chronic models of epilepsy have noted many changes in astrocyte function that might contribute to seizure activity. However, less is known about early changes in function in astrocytes following SE and to what extent astrocytes prevent or contribute to the development of epilepsy following SE. Ideally, understanding the wide array of astrocyte functions will give us clues as to how to exploit those processes that dampen excitability, such as glutamate uptake, gap junctions,

and potassium buffering, and how to minimize those processes that contribute to network excitability, such as decreased expression of aquaporins and secretion of cytokines and gliotransmitters. The development of new tools for wide scale imaging of networks of neurons and astrocytes with genetically encoded calcium indicators will undoubtedly contribute to our quest for disease modification and prevention of acquired epilepsy.

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- Review of astrocyte structure and function after status epilepticus (SE)
- Pro-convulsant and anti-convulsant changes in astrocytes are described
- Genetically encoded calcium indicators will detect network changes in SE
- Astrocytes may prove to be an important therapeutic target following SE

Figure 1.

Reactive astrocytes are present in the hippocampus one week following KA-induced SE. (A) Immunoreactivity for GFAP is observed throughout the hippocampus and cortex in horizontal brain sections from both control and KA-treated rats. dg = dentate gyrus, ecx = medial entorhinal cortex. (B) Close up of CA1 and stratum radiatum from the area indicated by the white box in (A). Sections from KA-treated rats (right) show characteristics typical of reactive astrocytes including a hypertrophied morphology and an increase in the immunoreactivity for GFAP. Scale bar, 50 μ m. (C) Western blot of crude hippocampal membrane fractions from control (Ct) and kainate-treated (Kt) animals probed with an antibody directed against GFAP. Actin served as a lane loading control. (D) Densitometry

values of immunoreactivity for GFAP from kainate-treated samples ($n = 6$) were found to be significantly increased compared to control ($n = 8$) samples (** $p < 0.0001$, Student's t test). Figure reprinted from (Takahashi et al., 2010) with permission from Elsevier.

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Figure 2.

Ca^{2+} transients recorded at high resolution in the soma and throughout the processes in an astrocyte expressing Lck-GCaMP3 are observed in a series of images extracted from a movie using a standard x-y raster. Changes in fluorescence as a consequence of increased Ca^{2+} are highlighted in pseudo-color and superimposed on the mean image of the movie. At time 0.0 second, there is activity throughout the astrocyte. Time 66.9 sec to 74.3 sec shows a slow wave moving from the main processes to the soma. The final two panels demonstrate isolated Ca^{2+} transients in the astrocytic processes. Scale bar = 100 μM .