

Glia and epilepsy: excitability and inflammation

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Epilepsy is characterized by recurrent spontaneous seizures due to hyperexcitability and hypersynchrony of brain neurons. Current theories of pathophysiology stress neuronal dysfunction and damage, and aberrant connections as relevant factors. Most antiepileptic drugs target neuronal mechanisms. However, nearly one-third of patients have seizures that are refractory to available medications; a deeper understanding of mechanisms may be required to conceive more effective therapies. Recent studies point to a significant contribution by non-neuronal cells, the glia – especially astrocytes and microglia – in the pathophysiology of epilepsy. This review critically evaluates the role of glia-induced hyperexcitability and inflammation in epilepsy.

Introduction

Glia outnumber neurons in the cerebral cortex by more than 3:1 by some estimates [1], with oligodendrocytes comprising approximately 75% of cortical glia, followed by astrocytes (~17%) and microglia (~6.5%) [2]. Glia are intimately involved in diverse neuronal functions: guiding migration during development; modulating synaptic function and plasticity; regulating the extracellular microenvironment by buffering neurotransmitter, ion, and water concentrations; insulating axons; regulating local blood flow and the delivery of energy substrates; contributing to the permeability functions of the blood–brain barrier (BBB) [3,4]; and enforcing cellular immunity in the brain to restore function and promote healing [5]. These physiological functions of normal glia help to maintain tissue homeostasis.

Dysregulation of glial functions may cause seizures or promote epileptogenesis [6]. Abnormal glia, including chronically activated astrocytes and microglia, glial scars, and glial tumors, are a prominent feature of epileptic foci in the human brain and in experimental epilepsy models. The major mechanisms by which glia can facilitate the development of seizures and epilepsy include increased excitability and inflammation. Disruption of glial-mediated regulation of ions, water, and neurotransmitters can promote hyperexcitability and hypersynchrony. Uncontrolled glial-mediated immunity can cause sustained inflammatory

changes that facilitate epileptogenesis. This review examines how glial-mediated changes in excitability and inflammation contribute to epilepsy.

Reactive astrocytosis and the epileptic focus

Astrocytes undergo changes in morphology, molecular composition, and proliferation in epileptic foci. This ‘reactive astrogliosis’ process includes a continuous spectrum of changes that vary with the nature and severity of diverse insults [7]. Reactive astrocytes occur in animal models of epilepsy and in brain tissue from patients with mesial temporal sclerosis (MTS), focal cortical dysplasia (FCD), tuberous sclerosis complex (TSC), Rasmussen’s encephalitis, or glioneuronal tumors [8–10]. Interestingly, astrocytes are a specific target of cytotoxic T cells in Rasmussen’s encephalitis, an epilepsy with chronic brain inflammation [7,9]. MTS, the most common pathology associated with temporal lobe epilepsy (TLE), is characterized by astroglial and microglial activation and proliferation [6], with increased complexity and arborization of astroglial processes [11], often approaching glial scar-like formations in late-stage MTS. In epileptic brain, reactive astrocytes exhibit physiological and molecular changes, such as reduced inward rectifying K⁺ current or changes in transporters or enzyme systems that may underlie epileptic hyperexcitability (Figure 1).

Water and K⁺ buffering

Astrocytes regulate water and K⁺ flow between brain cells and the extracellular space (ECS). Neuronal excitability is tightly coupled to ECS K⁺ levels and ECS volume. The ECS is reciprocally related to neuronal and glial cell volumes. Increased ECS and decreased neuronal/glial cell volume reduces excitability. Low-osmolarity solutions contract the ECS and promote epileptic hyperexcitability [12]. Indeed, water intoxication can cause seizures, particularly in infants. Shrinking the ECS may promote seizures by increasing extracellular K⁺ concentrations and possibly by enhancing ephaptic (non-synaptic) neuronal interactions. The diuretics furosemide and bumetanide mediate antiepileptic effects by reducing cell volume by blocking the glial Na–K–2Cl cotransporter [13].

The glial water channel aquaporin-4 (AQP4) is implicated in the pathogenesis of epilepsy [14]. AQP4 mediates the bidirectional flow of water between the ECS and the

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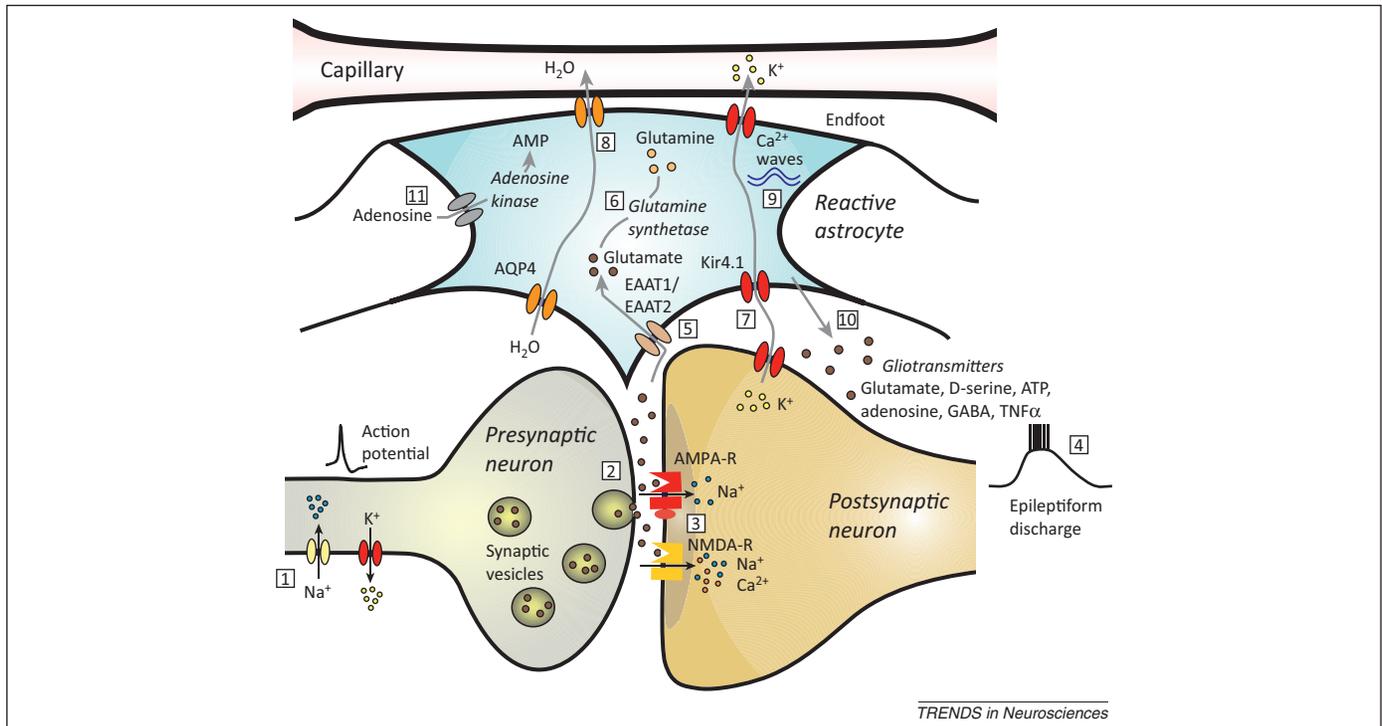


Figure 1. Schematic model depicting selected interactions between astrocytes and excitatory neurons. Voltage-gated Na^+ and K^+ channels (1) generate action potentials in the presynaptic neuron, leading to the exocytotic synaptic release of neurotransmitter glutamate (2). Glutamate activates AMPA and NMDA receptors (3) in the postsynaptic membrane, causing excitatory synaptic potentials generated by influx of Na^+ and Ca^{2+} . If sufficiently strong, synaptic excitation leads to epileptiform discharges (4). Glutamate is taken up into reactive astrocytes by the EAAT1 (GLAST) and EAAT2 (GLT-1) transporters (5) and is converted to glutamine by glutamine synthetase (6). Glutamine is a substrate for the production of GABA in inhibitory GABAergic neurons (not shown). Loss of glutamine synthetase in reactive astrocytes leads to a decrease in GABA production. K^+ released from neurons by voltage-gated (outwardly rectifying) K^+ channels enters astrocytes via inwardly rectifying K^+ channels (Kir4.1) (7) and is distributed into capillaries. Aquaporin-4 (AQP4) concentrated at astrocytic endfoot processes regulates water balance (8). Ca^{2+} waves (9) stimulate the release of gliotransmitters (10) that can influence neuronal excitability. The inhibitory substance adenosine is taken up into astrocytes by the equilibrative nucleoside transporters ENT1 and ENT2 and concentrative nucleoside transporter CNT2. Excessive adenosine kinase in reactive astrocytes increases the removal of adenosine (11), enhancing hyperexcitability.

blood, thus regulating interstitial fluid osmolarity and ECS volume. Mice lacking AQP4 or components of the dystrophin-associated protein complex that anchors AQP4, including α -syntrophin and dystrophin, have altered seizure susceptibility, and epilepsy can complicate human muscular dystrophy affecting the dystrophin complex [10,14]. In MTS specimens, AQP4 is redistributed from perivascular glia endfeet to the perisynaptic space [15]. This may enhance water entry into the neuropil but impair water egress into the perivascular space, swelling astrocytes, contracting the ECS, and increasing excitability [6]. Thus, glial AQP4 dysfunction can impair water delivery to the ECS, increasing susceptibility to seizure [16].

Glia provide an osmotically neutral spatial buffering system for K^+ using inward rectifying K^+ channels (Kir) that carry K^+ ions into cells accompanied by water entry through AQP4 to maintain osmotic balance. Excessive local concentrations of K^+ predispose to seizures [17]; impaired glial buffering may help cause epilepsy [18]. Conditional knockout of Kir4.1 depolarizes glial membranes, inhibits potassium and glutamate uptake, and potentiates synaptic strength [19]. Reduced Kir4.1 expression (but not other K^+ channels) increases extracellular K^+ in a BBB disruption model of epileptogenesis [19]. In the kainic acid-induced status epilepticus model, AQP4 is markedly reduced, suggesting that impaired water and potassium homeostasis occurs early in epileptogenesis and providing a potential therapeutic target [20]. Moreover,

murine and human polymorphisms or mutations of *KCNJ10*, which encodes the astroglial Kir4.1 K^+ channel, are associated with epilepsy [21]. Because Kir4.1 dysfunction can compromise K^+ spatial buffering [22], both acquired and genetic epilepsies could result from glial pathology. Impaired Kir channel function in the CA1 region in MTS suggests that this pathological mechanism is clinically relevant [23,24]. Impaired gap junction coupling between astrocytes may also disrupt spatial K^+ buffering, but this remains controversial [6,21]. The homeostatic role of astrocytes extends from ions and water balance to neurotransmitter levels and maintaining BBB function.

Regulating neurotransmission

Glutamate uptake by high-affinity membrane transporters is essential for maintaining low ambient levels of glutamate. Uptake is of particular importance when there is intense excitatory synaptic activity, as occurs during epileptic discharges. Uptake mechanisms prevent spill-out of transmitter from the synaptic cleft, thus regulating cross-talk between neighboring synapses and the activation of perisynaptic/extrasynaptic glutamate receptors. Five glutamate transporters are present in the brain. GLAST and GLT-1 (human forms: EAAT1 and EAAT2, respectively) are expressed in glial cells, primarily astrocytes. These transporters, which have an affinity for glutamate of 2–90 μM , are densely concentrated in hippocampal astrocyte membranes [25,26]. As soon as a vesicle releases its

load of glutamate into the synapse, most of the glutamate is removed from the ECS by astrocytic transporters. Astrocytes are optimized for glutamate uptake due to their high (negative) resting potential, which enhances the sodium electrochemical gradient that drives transport, and low cytoplasmic glutamate concentration. Do astrocytic glutamate transporters restrain epileptic activity under normal or pathological conditions? Although antisense knockdown of the neuronal glutamate transporter EAAC1 leads to epilepsy (due to reduced GABA synthesis), knockdown of the astrocyte glutamate transporter GLT-1 does not [27]. However, mice with genetic knockout of GLT-1 display increased levels of synaptic glutamate in response to stimulation and exhibit spontaneous lethal seizures, and seizures in response to ordinarily subconvulsive doses of pentylenetetrazol [28]. Moreover, in rats with cortical dysplasia-like lesions, dihydrokainate, a selective inhibitor of GLT-1, decreased the threshold for inducing epileptiform activity [29]. Interestingly, in a BBB disruption epileptogenesis model, GLAST and GLT-1 (but not EAAC1) were downregulated and there was electrophysiological evidence of reduced glutamate buffering [30].

In TLE, both normal and reduced expression of the astroglial glutamate transporters EAAT1 and EAAT2 were found [119]. Therefore, in some instances impaired glutamate uptake by astrocytes may increase epileptic hyperexcitability. Astrocyte glutamate uptake capacity is enhanced by activating astroglial metabotropic glutamate receptors (mGluRs) [31]. In MTS and FCD, astroglial mGluRs are upregulated [6,8,32], suggesting a compensatory response to prevent seizures. The role of astrocyte membrane transporters in regulating epileptic activity remains suggestive but unproven. Similarly, accumulating evidence suggests that cytoplasmic astrocyte enzymes help maintain excitatory/inhibitory neurotransmitter homeostasis [33–43]. Examples are provided by adenosine kinase (ADK) and glutamine synthetase (GS).

ADK is a predominantly astrocytic enzyme that regulates brain extracellular adenosine levels by phosphorylating adenosine to form 5'-adenosine monophosphate. Astroglial ADK expression is associated with increased levels of ADK. Adenosine is a powerful inhibitory substance released during seizures and implicated in seizure arrest, postictal refractoriness, and suppression of epileptogenesis [33]. Astroglial ADK expression may lower the seizure threshold by reducing extracellular adenosine. This concept is supported by studies showing that; (i) pharmacological inhibition of ADK suppresses seizures; (ii) upregulation of ADK is associated with spontaneous seizures in a model of epileptogenesis; and (iii) resistance to epileptogenesis occurs in transgenic mice with reduced forebrain ADK [34]. Interestingly, ADK is overexpressed in human glial tumor tissue and the peritumoral region infiltrated by glia, suggesting that reduced adenosine could play a role in the development of epilepsy in patients with glial tumors [35]. ADK expression levels are also increased in the seizure foci of TLE patients [36]. Basal adenosine is reduced in epileptic compared with control human hippocampus, consistent with ADK contributing to epileptogenesis [36].

GS, a cytoplasmic enzyme found predominantly in astrocytes, is critical to glutamate homeostasis [37]. GS catalyzes the ATP-dependent condensation of glutamate with ammonia to yield glutamine. The observation that GS levels are significantly reduced in the human hippocampus and amygdala in TLE suggested a role for the enzyme in epileptogenesis [37]. Transient elevations in extracellular glutamate occur during seizures in these and other brain regions, but ambient glutamate levels are also increased interictally, which could predispose to recurrent seizures [39]. GS deficiency may also cause accumulation of glutamate in the cytoplasm of astrocytes, leading to such persistently elevated basal glutamate levels. Reactive astrocytes downregulated GS expression, rapidly depleting synaptic GABA [40]. Thus, glutamine is taken up by GABAergic neurons, where it is converted to glutamate via glutaminase and then to GABA by glutamic acid decarboxylase. Acute inhibition of GS has been found to reduce neuronal and extracellular glutamate in brain, which appears inconsistent with the concept that low GS leads to glutamate release. However, sustained pharmacological inhibition of GS with methionine sulfoximine increases glutamate levels in astrocytes, reduces synthesis of neuronal GABA, and induces seizures [41,42]. A child with GS deficiency due to GS gene mutations suffered severe seizures [43]. Thus, reduced astrocytic GS could play an important role in seizure susceptibility.

Gliotransmission

In the 1990s, the discovery that glutamate released by neuronal synapses activated neighboring astrocytic mGluRs and increased their cytosolic Ca^{2+} indicated that astrocytes sense neural activity [44]. Subsequently, it was proposed that increased intracellular Ca^{2+} induces astrocytes to release glutamate that modulates synaptic activity. Thus, communication between astrocytes and neurons is bidirectional and the astrocyte became the third component of the 'tripartite synapse', along with presynaptic and postsynaptic elements of neurons (Figure 1) [6,10]. An expanding potential range of glial transmitters were proposed, including D-serine, ATP, adenosine, GABA, and tumor necrosis factor alpha (TNF- α) [6,10,45–47]. Evidence of gliotransmission in normal and pathological states is growing [48], although its importance remains controversial [49].

Ca^{2+} waves within the astrocytic syncytium were proposed to propagate signals within connected sets of astrocytes leading to gliotransmitter release [50]. Ca^{2+} -dependent astrocytic release of gliotransmitters such as D-serine modulate NMDA receptor function in nearby synapses [48]. However, others suggest that some 'gliotransmission' is more pharmacological than physiological [51,52]. Although intercellular Ca^{2+} waves occur in cultured astrocytes, scant evidence supports such waves in intact tissue during non-pathological neuronal activity [53]. Under basal conditions, most astrocytic Ca^{2+} elevations are localized to small territories of astrocyte processes. The mechanisms by which gliotransmitters are released from astrocytes may include reversal of glutamate uptake, gap junction (connexin) hemichannels, opening of volume-sensitive ion channels, pore-forming P2X₇ purinoreceptors, and fusion of transmitter-laden vesicles with the

plasma membrane, as occurs in neurons [49]. Active zones with vesicles were found in astrocytes by one group [54], but not by another [51]. The vesicular glutamate transporter VGLUT1 was localized to synapse-like microvesicles within the astrocytic processes by confocal and electron microscopy [49,54,55]; the transcript was detected in astrocytes by one group [54], but not by another [56]. Furthermore, vesicular fusion has been observed only in cultured astrocytes [49], so it is uncertain whether astrocytes release transmitters like neurons. Gliotransmitter release can be triggered by multiple mechanisms, including G protein-coupled receptor-induced increased phospholipase C activity leading to the release of Ca^{2+} from intracellular stores [8] and activation of cyclooxygenase-2–prostaglandin signaling [54]. However, the physiological role of glutamate release from astrocytes remains uncertain [51,52]. A study in transgenic mice engineered to selectively increase or obliterate astrocytic Gq protein-coupled receptor Ca^{2+} signaling concluded that gliotransmission is not necessary for normal brain function [57].

Regardless of whether gliotransmission is involved in normal brain function, it might occur in pathological states. Gliotransmission may be central to epileptic synchronization [32,58], but this remains controversial [59]. In the intact neocortex *in vivo*, blockade of GABA-mediated neurotransmission, which increased neuronal discharges but did not evoke seizures, increased Ca^{2+} spike frequency within astrocytes and coordinated Ca^{2+} signaling in neighboring astrocytes. This supports enhanced neuron–glia communication in the intact brain during hyperexcitability [53]. A particularly radical notion is that the paroxysmal depolarization shift, the fundamental electrophysiological event in epileptic brain and the intracellular analog of the interictal spike, is due to glutamate release not from neurons, as believed for decades, but from astrocytes [58]. This release may depend on Ca^{2+} oscillations in astrocytes, which could be attenuated by antiepileptic drugs (AEDs) [58]. Simultaneous patch-clamp recording and Ca^{2+} imaging in entorhinal cortex slices and in the whole guinea pig brain isolated *in vitro* provided a different view [60]. Focal seizure-like discharges were accompanied by Ca^{2+} elevations in astrocytes during seizure-like activity, but not during brief interictal events. Astrocytic activation was mediated by neuronal release of glutamate and ATP. Selective inhibition of astrocyte Ca^{2+} signaling blocked ictal discharges in neurons, whereas stimulation of Ca^{2+} signaling enhanced these discharges. Thus, there is bidirectional neuron–astrocyte communication during seizures. These studies suggest that astrocytes may be required for seizure initiation but not for interictal activity [60]. Astrocytic Ca^{2+} oscillations in *in vivo* seizure models may also mediate seizure-induced excitotoxicity [61].

Other gliotransmitters and mechanisms of glial modulation of neurotransmitters could promote seizure activity. D-Serine is a prime gliotransmitter candidate relevant to epilepsy: it is the principal endogenous ligand for the glycine site of NMDA receptors and NMDA receptors cannot function without an agonist bound to this site [62]. Because NMDA receptor activation can trigger epileptiform activity and epileptogenesis, D-serine could regulate these functions. Inhibiting Ca^{2+} signaling in astrocytes reduces

GABAergic inhibition of neighboring neurons [42]. Similarly, activation of interneuronal purinergic receptors by astrocytic release of ATP facilitates inhibition in hippocampus [63]. This evidence suggests that astrocyte modulation of GABAergic inhibition could influence the generation and spread of epileptic activity. In addition to gliotransmission, astrocytes can influence neuronal homeostasis and excitability by affecting BBB integrity and by activating inflammatory mechanisms.

Vasculature and the BBB

Astrocytes are intimately related to the microvasculature, because their endfeet wrap around the endothelial cells. Astrocyte endfeet ensheathing blood vessels contribute to BBB function by releasing chemical signals that help to form and maintain tight junctions between endothelial cells. They also regulate the movement of water and molecules between the blood and brain parenchyma.

The brain microvasculature undergoes several structural, molecular, and functional changes in epilepsy. Vessel proliferation in TLE positively correlates with seizure frequency [64] and is associated with alterations in BBB permeability [64,65]. Vascular endothelial growth factor (VEGF) is released from astrocytes in *in vivo* seizure models and in brain slices exposed to kainate; VEGF contributes to BBB damage and induces microvasculature proliferation (angiogenesis) by activating VEGF receptor 2 on microvessels [66].

Proinflammatory chemokines and cytokines released by astrocytes can interact with their cognate receptors overexpressed by brain microvessels in epilepsy, thus affecting BBB permeability at multiple levels (e.g., by disrupting tight junction proteins [66], increasing transendothelial vesicular transport, or guiding leukocyte or viral particles through the BBB into the brain parenchyma). Leukocyte transmigration by interacting with adhesion molecules on endothelial cells may alter BBB permeability to serum proteins and circulating molecules [67,68]. Astrocyte-derived interleukin-1 beta (IL-1 β) can compromise BBB integrity during seizures also in the absence of circulating leukocytes [69]. Brain extravasation of serum albumin due to BBB damage increases excitability [70,71] and promotes epileptogenesis [70]. One key mechanism is the albumin-mediated activation of transforming growth factor beta (TGF- β) receptor II signaling in astrocytes, resulting in transcriptional downregulation of Kir4.1 and GLT-1 [30,72]. This signaling also promotes synthesis of inflammatory molecules in astrocytes, helping to perpetuate the inflammatory milieu [72].

Release of inflammatory mediators or glutamate by astrocytes may increase multidrug transport proteins on endothelial cells [73]. These proteins are overexpressed in resected tissue specimens from drug-resistant epilepsy patients [73]. In particular, p-glycoprotein (encoded by the multidrug resistance-1 gene) is overexpressed at the luminal side of endothelial cells, in astrocytic endfeet, in dysplastic neurons in developmental glioneuronal lesions, causing uncontrolled epilepsy, and in TLE [74]. Because p-glycoprotein transports various AEDs from the brain to the blood, its overexpression may limit access of AEDs to the brain, thus reducing their therapeutic efficacy [73].

This set of evidence highlights important pathophysiological interfaces between glial-mediated inflammation, microvasculature, and excitability.

Glia-mediated immunity and inflammation

The transformation of resting to activated (reactive) astrocytes and microglia in response to insults and stressors is fundamental to maintain brain homeostasis and limit injury (Figure 2). Both cell types are activated by pathogens or local non-infectious injuries, leading to the release of proinflammatory mediators. Anti-inflammatory molecules and growth factors then help to orchestrate and resolve the inflammatory tissue response.

Glia-mediated inflammation induced by various brain insults can promote seizures and epileptogenesis, especially when normal feedback mechanisms fail to limit and extinguish inflammation. Formerly considered an epiphenomenon, recent evidence strongly suggests that glia-mediated inflammation plays a role in the pathogenesis of seizures and epilepsy (Box 1). In different animal models of epilepsy, but not other non-epilepsy causes of gliosis, activated astrocytes extend their processes outside their usual non-overlapping domains [75]. Together with dendritic sprouting and new synapse formation, loss of astrocytic domain organization may contribute to the structural bases of recurrent excitation in epilepsy [75].

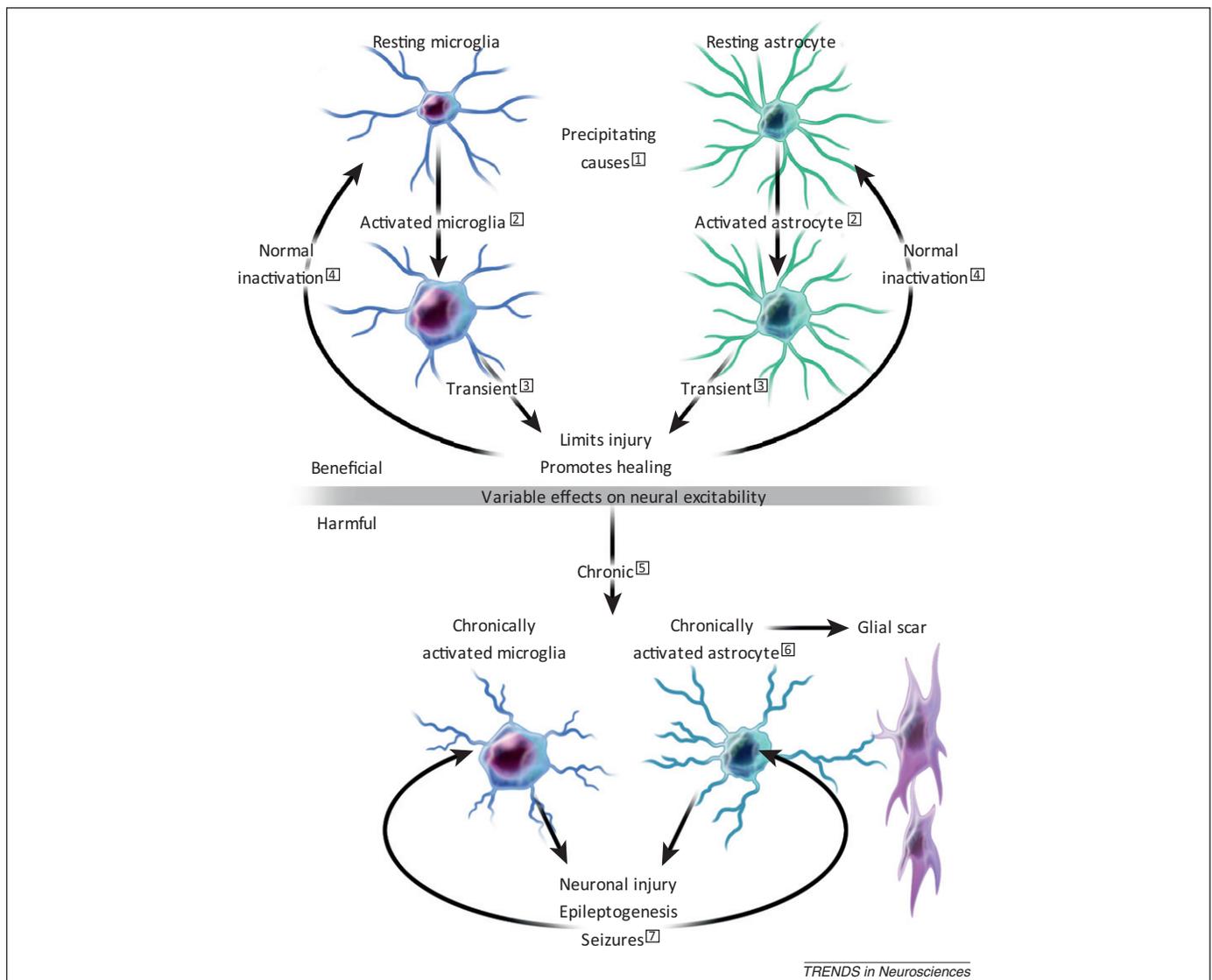


Figure 2. Intersecting roles of astrocytes and microglia in inflammation and excitability. (1) Hypoxia, trauma, infection, stroke, autoimmunity, and seizures can be among the precipitating causes of astrocytic and microglial activation. (2) Cytokines, Toll-like receptor (TLR) ligands, glutamate, ATP, NO, NH_4^+ , and β -amyloid are among the soluble molecules released by activated glia. (3) Activated astrocytes exhibit homeostatic functions such as increased glutamate uptake (a function also displayed by microglia), glutathione release to decreased oxidative stress, adenosine release to control neuronal excitability, regulation of fluid/ion homeostasis, and release of anti-inflammatory mediators to control innate immunity activation (also shared by M2-type microglia). (4) Normal inactivation of activated microglia is partly mediated by astrocytes, which: (i) inhibit microglial phagocytosis; (ii) lower microglial production of tumor necrosis factor alpha (TNF- α), NO, and reactive oxygen species; and (iii) release anti-inflammatory molecules such as the IL-1 receptor antagonist (IL-1ra). (5) Chronic uncontrolled astrocytic and microglial activation is associated with excessive release of proinflammatory molecules, blood-brain barrier (BBB) damage with serum albumin and IgG brain extravasation, ionic imbalance, and decreased glial glutamate reuptake and GABA synthesis in neurons. This set of phenomena has numerous detrimental effects, including neuronal injury and seizure induction. (6) Chronically activated astrocytes can form a glial scar. The effects of such a scar are both beneficial and pathologic and include decreased axonal regeneration, neuronal protection from oxidative stress associated with glutathione production, and restricted spread of inflammatory cells and infectious agents [7,36,103]. (7) Epileptogenesis, seizures, and neuronal injury can all arise from chronic pathological glial activation and cause proinflammatory changes that maintain chronic, pathological activation of astrocytes and microglia.

Box 1. Epilepsy therapy: glial targets?

AEDs prevent seizures, but have not been shown to modify the underlying epilepsy. Studies on the mechanisms of AED action focus on neurons, ion channels and transporters, and excitatory and inhibitory neurotransmission [106]; they rarely examine whether these drugs have effects on glia or immune function. However, anti-inflammatory effects of AEDs could be relevant to their clinical activity. For example, carbamazepine and levetiracetam can reduce inflammatory mediators in glial cell cultures [107,108] and valproate may impair microglial activation [109].

Clinical anti-inflammatory or immunosuppressive treatments can control seizures that are resistant to conventional AEDs in some epileptic syndromes [110,111]. For example, intravenous immunoglobulin (IVIG) can suppress seizures in some epilepsies [112], an effect that may be partially mediated by reducing proinflammatory cytokines and suppressing astrocyte activation [113,114]. IVIG increases circulating levels of IL-1ra, blocking IL-1 β signaling [113], which has anticonvulsant properties in animal models [86,104,105]. Adrenocorticotropic hormone (ACTH) is a first-line treatment for infantile spasms; anti-inflammatory effects mediated via increased adrenal corticosteroid production may play a role [115]. Clinical trials are in progress with VX-765 (e.g., [116]), a selective inhibitor of caspase 1, the enzyme that cleaves the precursor form of IL-1 β to the active peptide. VX-765 has anticonvulsant [89] and antiepileptogenic [90] activity in *in vivo* models. Notably, the drug conferred seizure protection in mice with epilepsy resistant to conventional AEDs [89]. Seizure protection was associated with normal brain IL-1 β production, unlike the increased production in astrocytes in epileptic animals [89].

Glia may also provide a biomarker of epileptogenesis that can be assessed noninvasively using magnetic resonance imaging or positron emission tomography [117]. These methods may help identify patients who can benefit from specific anti-inflammatory treatments.

Activated astrocytes release cytokines that induce transcriptional and post-transcriptional signaling in the astrocyte itself (autocrine actions) and in nearby cells (paracrine actions). For example, astrocytes release IL-1 β (Figure 3) and high-mobility group box 1 (HMGB1) protein. These cytokines activate nuclear factor kappa B (NF- κ B), an important regulator of proinflammatory gene expression. NF- κ B transcriptional signaling is upregulated in MTS and TSC tissue [76,77]. IL-1 β and HMGB1 signaling occurs through activation of the proinflammatory IL-1 receptor/Toll-like receptor (IL1R/TLR) system. This system is activated in epilepsy models and in MTS, TSC, and FCD tissue (Figure 3) [78–80]. In mice, activation of IL1R/TLR signaling promotes seizure onset and recurrence, whereas its pharmacological blockade or genetic inactivation drastically reduces seizure activity [79,81]. Activation of IL1R/TLR signaling in neurons reduces the seizure threshold by inducing sphingomyelinase-mediated ceramide production. This cascade of events activates Src kinase-mediated phosphorylation of the GluN2B subunit of the NMDA receptor. Consequently, NMDA-mediated neuronal Ca²⁺ influx is enhanced, promoting excitability and excitotoxicity [79,81].

Other astrocytic changes resulting from brain injury or seizures may alter immune activity. For instance, miRNA-146a (miR-146a) modulates innate and adaptive immunity by activation of IL-1R/TLR signaling. miR-146a increases in astrocytes following experimental seizures and in MTS [36,82], but its role in experimental or human epilepsy remains unexplored. Microglia are brain-resident macrophage-like cells that contribute, together with

astrocytes, to innate immune mechanisms. Activated microglia promote astrocytic activation and vice versa [83].

Microglia play a central role in brain immunity as phagocytes and mediators of humoral and cellular immune mechanisms. The functional outcome of microglial activation is context dependent, chiefly determined by the type, extent, and duration of tissue stressors and the cell types expressing receptors for the molecules released by microglial cells [84]. Prolonged or excessive microglial activation can cause cellular dysfunction and death. Activated microglia can assume various proinflammatory or anti-inflammatory phenotypes, but the mechanisms and cell interactions regulating these phenotypes are largely unknown [5].

Microglia are integral to inflammatory processes in experimental models and human epilepsy. In epilepsy models, microglial and astrocytic activation can result from seizures alone, without cell loss [85–87]. The mechanism by which microglia sense neuronal hyperexcitability is uncertain. Microglial activation can persist without concomitant synthesis of inflammatory cytokines in these activated cells. For example, IL-1 β is detectable in microglia following a seizure but its expression fades after several hours. Still, the microglia remain morphologically activated as if in a ‘primed’ state [88]. Microglia, as well as astrocytes, may remain morphologically activated in experimental epileptic tissue also following inhibition of cytokine synthesis [88–90].

Activated microglia produce proinflammatory mediators within 30 min of seizure onset [91], well before morphological cell activation is detectable [92]. In animal studies, the intensity of expression of these mediators correlates with seizure frequency [88]. Microglia are activated in human epilepsy, including MTS, FCD, TSC, and Ramussen’s encephalitis. Notably, the extent of microglial activation correlates with the seizure frequency and disease duration in these drug-resistant epilepsies [93,94].

Activated microglia can decrease the seizure threshold in animal models by releasing proinflammatory molecules with neuromodulatory properties (Table 1) [78,95,96]. This may occur through effects on astrocytes. For example, chemokine-activated microglia cooperate with astrocytes in releasing TNF- α [47], and other cytokines, which in turn promotes astrocytic glutamate release thereby contributing to cell loss and seizures [96]. Proinflammatory mediators released by astrocytes can feed back onto microglia (Figure 2).

Lipopolysaccharide (LPS), a Gram-negative bacterial wall component, activates microglia via TLR4. LPS can induce immediate focal epileptiform discharges in rat neocortex mediated by IL-1 β release [97]. Endogenous ligands of TLR4, including HMGB1 and IL-1 β , can be generated by microglia or astrocytes following brain injury, mimicking the effect of LPS. Consequently, microglia may help generate seizures by releasing, and responding to, endogenous inflammatory mediators such as HMGB1 and IL-1 β [78,81,96]. Other modulators of microglial function include TGF- β produced by astrocytes [98] and cluster of differentiation 200 (CD200) and the atypical chemokine fractalkine (CX₃CL1) released from astrocytes and neurons [84]. These molecules are induced in seizure models or following

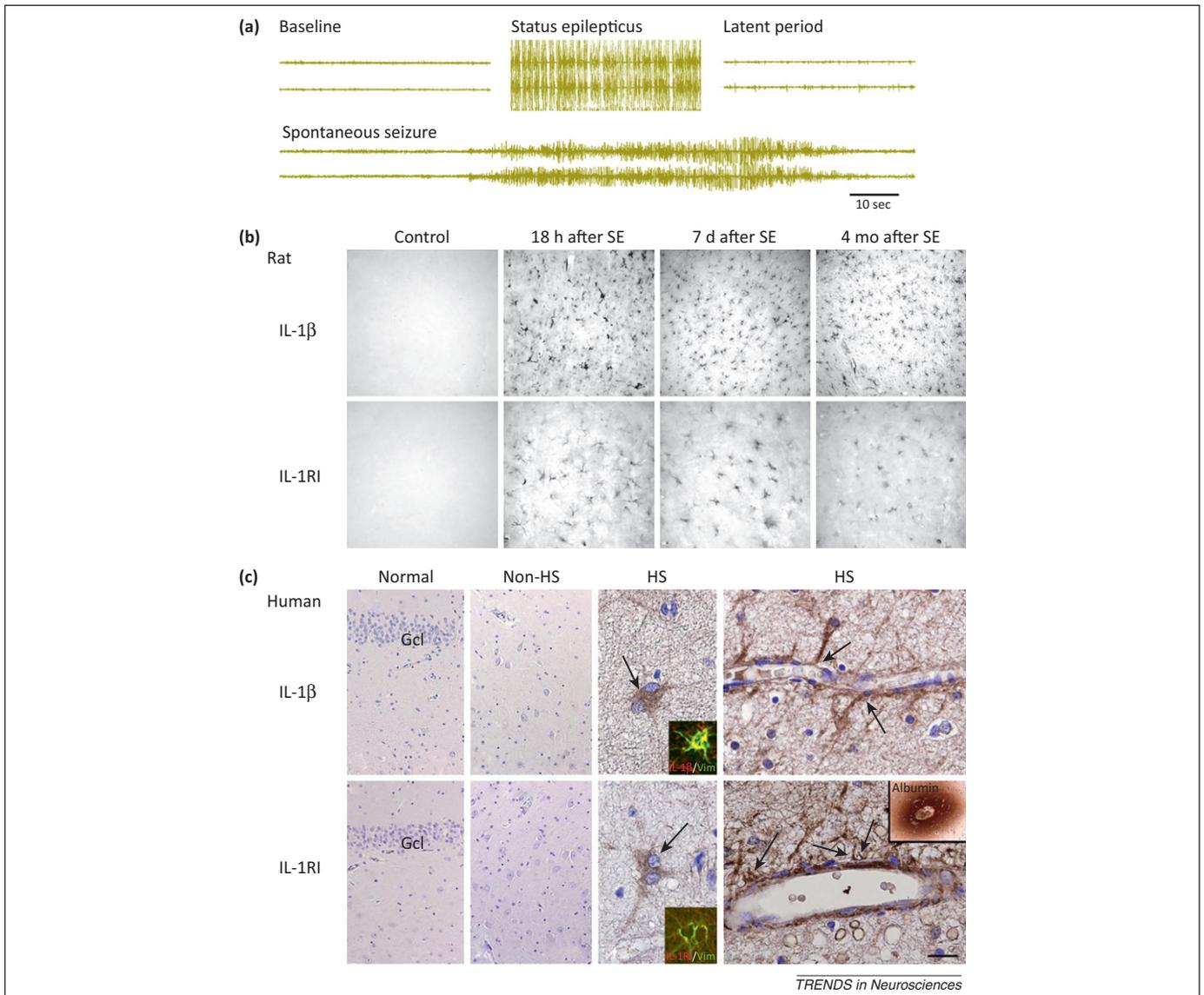


Figure 3. Inflammatory changes in hippocampal tissue of a rodent model of epilepsy and in surgical specimens from patients with mesial temporal lobe epilepsy (TLE). (a) Electroencephalographic recordings from the right (upper traces) and left (lower traces) frontoparietal cortex of pilocarpine-treated rats. Treatment induces status epilepticus (SE), which is followed by a latent period (with sporadic spiking) and development of chronic spontaneous seizures. The pattern is similar in the hippocampus (not shown). (b) Immunoreactivity to the cytokine interleukin-1 beta (IL-1 β) (upper traces) and IL-1 receptor type I (IL-1RI) (lower traces) is markedly increased in cells with glial morphology in the frontoparietal cortex of treated rats at completion of SE (18 h), during the latent phase (7 days), and in chronic epileptic rats (4 months) compared with controls. (c) IL-1 β (upper traces) and IL-1RI staining (lower traces) in hippocampal tissue from an autopsy control subject and in a surgically resected specimen from a patient with TLE and associated hippocampal sclerosis (HS). A surgical hippocampal specimen from a patient with epilepsy not involving the hippocampus proper is also depicted (Non-HS). Staining is absent from the control and Non-HS tissue. In the HS sample, reactive astrocytes (arrow) stain intensely positive for IL-1 β ; inset shows colocalization (yellow) of IL-1 β (red) with vimentin (Vim; green) in a reactive astrocyte. The large panel shows a blood vessel with strong IL-1 β immunoreactivity in perivascular astrocytic endfeet (arrows). There is also strong IL-1RI immunoreactivity in the sclerotic hippocampus, including astrocytic endfeet (arrows); inset shows serum albumin staining around a blood vessel demonstrating blood–brain barrier (BBB) damage. These studies reveal chronic activation of inflammatory pathways during epileptogenesis (rats) and in the chronic epileptic state (rats and humans), supporting a possible pathogenic role in epilepsy. Abbreviation: gcl, granule cell layer. Scale bar: 90 μ m (control), 40 μ m (Non-HS), 100 μ m (HS). Reproduced, with permission, from [83].

high-frequency neuronal activity and can affect synaptic transmission and plasticity and cell survival [99–101]. Astrocytes can also influence microglia through the release of ATP, which acts on microglia via purinergic receptors [102].

Like all immune effector cells, astrocytes may help limit the immune response by controlling microglial activation. Better defining this mechanism could provide therapeutic targets for epilepsy and other brain disorders (Box 1). In *in vitro* experimental settings, astrocytes can reduce the production of proinflammatory and neurotoxic TNF- α , nitric oxide and reactive oxygen species from microglia

and inhibit microglial phagocytosis [103]. In *in vivo* seizure models, astrocytes are key sources of anti-inflammatory molecules such as the IL-1 receptor antagonist (IL-1ra), an endogenous competitive IL-1 receptor blocker that controls IL-1 β -mediated inflammation. IL-1ra has powerful anticonvulsant effects in experimental seizure models [86,104,105] and mice overexpressing IL-1ra in astrocytes are intrinsically resistant to seizures [86]. In MTS and experimental epilepsies, astrocyte expression of IL-1ra is significantly lower than that of IL-1 β , indicating that, unlike peripheral organs, the anti-inflammatory response is poorly induced in the epileptic brain [91,94].

Table 1. Mechanisms of glia-mediated neuronal hyperexcitability

Cellular component	Change in epilepsy	Functional effect	Mechanism of hyperexcitability	Source of data	Refs
Non-inflammatory mediated					
<i>Ion channels (astrocytes)</i>					
Kir4.1	↓ Expression	↓ Spatial K ⁺ buffering	↑ Extracellular K ⁺	MTS; human and transgenic murine models	[20,21,30]
<i>Communicating junctions/pores (astrocytes)</i>					
Gap junctions	↓ Gap junction	↓ Spatial K ⁺ buffering	↑ Extracellular K ⁺	Rodent hippocampal slice	[6,21]
AQP4	AQP4 dysfunction	↓ H ₂ O delivery to extracellular space	Shrinkage of extracellular space	MTS, <i>in vivo</i> rodent	[14]
<i>Transporters (astrocytes)</i>					
EAAT1/EAAT2	↓ Expression of transporters	↓ Glutamate astrocyte uptake	↑ Extracellular glutamate	MTS; transgenic murine models, Rat <i>in vivo</i>	[28,30]
<i>Chemical transmission (astrocytes and neurons)</i>					
mGluRs	↑ Expression	↑ Glutamate uptake	Compensatory reduction in hyperexcitability	MTS, FCD; rodent hippocampal slice	[6,8,32]
Neuronal GABA transmission	↓ GABA transmission	↓ Inhibitory tone	↓ Opposition to excitation	Slice models	[42]
Gliotransmitters	↑ Release of glutamate, D-serine, and ATP from glia	↑ Extracellular excitatory gliotransmitters	↑ Activation of glutamate and purinergic receptors	Slice models, transgenic murine models	[32,51,54]
<i>Cytosolic enzymes (astrocytes)</i>					
Adenosine kinase	↑ Expression	↑ Adenosine phosphorylation	↓ Basal adenosine	MTS; rodent hippocampal slice, <i>in vivo</i> murine models	[33,34]
Glutamine synthetase	↓ Expression	↓ Conversion of glutamate to glutamine	↑ Basal glutamate; ↓ GABA synthesis	Human hippocampus and amygdala, slice models	[37,40]
Inflammatory mediated					
Glia-derived proinflammatory molecules	↑ Release	Neuromodulatory functions	↓ Seizure threshold	Rodent brain slice, <i>in vivo</i> rodent, transgenic murine models	[78,81, 95,98]
IL-1R/TLR signaling in glia and in neurons	↑ Activity	↑ NF-κB-dependent transcription of proinflammatory genes ↑ Phosphorylation of GluN2B and ↑ neuronal Ca ²⁺ influx	↓ Seizure threshold; ↑ excitotoxicity	MTS, FCD, TSC; <i>in vivo</i> rodents, transgenic murine models	[79–81]
Astrocyte glutamate transporters	↓ Activity	↓ Glutamate astrocyte uptake	↑ Extracellular glutamate	Human astrocytic cell cultures	[118]
Microglia-derived proinflammatory molecules	↑ Release	↑ Gliotransmitter release from astrocytes	↑ Neuronal stimulation	MTS, FCD, TSC; <i>in vivo</i> rodents; glial cell cultures	[84,103]
BBB dysfunction	↑ Permeability	Albumin-mediated ↑ TGF-β receptor type II signaling leading to ↑ transcription of inflammatory genes, ↓ Kir4.1, ↓ astrocyte glutamate transporter	↑ Inflammation; ↓ K ⁺ buffering; ↑ synaptic glutamate; ↑ neuronal stimulation	MTS; rodent slice, <i>in vivo</i> rodent	[66,69,70]
Multidrug transport proteins in endothelial cells and in perivascular astrocytes	↑ Expression	↓ AED levels in brain tissue	↓ Seizure control	MTS, FCD, TSC; <i>in vivo</i> rodent, transgenic murine models	[73,74]

Concluding remarks

Although neurons are the final cellular elements expressing seizure discharges, evidence grows for glia-mediated excitation and inflammation in modulating or triggering seizures (Table 1). Moreover, glia could support the initiation, development, and establishment of epileptogenesis

when their homeostatic functions are disrupted. The roles of glia in excitation and inflammation, traditionally considered independent pathways, may best be understood as overlapping and reciprocal. Excitation can promote inflammation. Inflammation can promote excitation. The neuronal mechanisms in epilepsy are likely to be more fully

Box 2. Outstanding questions

- Which alterations in astrocytes and microglia represent primary pathogenic factors and which are secondary to the epilepsy and unrelated to pathogenesis?
- What are the roles of different glial functions in epileptogenesis, seizure initiation, seizure spread, and seizure termination?
- Do gliotransmitters released by astrocytes play a role in normal physiology? Do they play a role in epilepsy?
- Can drugs targeting glia be useful in epilepsy therapy, either to prevent the occurrence of seizures or to modify the underlying disease?
- Does the astrocytic syncytium play a role in the initiation or spread of seizures? Could the Ca^{2+} waves that spread via the astrocytic syncytium through gap junctions facilitate seizure spread? Could agents that impair gap junction (connexin) function be useful anticonvulsant drugs?
- Does microglial and astrocytic activation and the concomitant cytokine release represent a common primary or reinforcing mechanism in human epilepsy? If so, would targeted anti-inflammatory therapies be useful in epilepsy therapy and is there a critical window in which they must be administered?
- What mechanisms turn off microglia and astrocytes in normal brain inflammatory responses? Can such mechanisms be used therapeutically for epilepsy?

understood by accounting for the excitatory and inflammatory effects of glia, taking into account the newly described direct neuromodulatory actions of inflammatory mediators (e.g., cytokines, chemokines and prostaglandins). A major challenge is untangling the concatenated cascades of proinflammatory and anti-inflammatory pathways (Box 2). A deeper appreciation of these divergent functions will suggest ways to reduce the contribution of glia to seizures and epileptogenesis, while at the same time enhancing their homeostatic role. In summary, understanding the roles of glia may provide insights into key unanswered questions in epilepsy, including how epileptogenesis occurs and why some patients are resistant to medications. As the fundamental mechanisms come into better focus, strategic targets for therapeutic interventions will emerge where neurons, glia, excitation, and inflammation converge.

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