Neuron–glia interactions in the pathophysiology of epilepsy

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Abstract | Epilepsy is a neurological disorder afflicting ~65 million people worldwide. It is caused by aberrant synchronized firing of populations of neurons primarily due to imbalance between excitatory and inhibitory neurotransmission. Hence, the historical focus of epilepsy research has been neurocentric. However, the past two decades have enjoyed an explosion of research into the role of glia in supporting and modulating neuronal activity, providing compelling evidence of glial involvement in the pathophysiology of epilepsy. The mechanisms by which glia, particularly astrocytes and microglia, may contribute to epilepsy and consequently could be harnessed therapeutically are discussed in this Review.

Phenobarbital
An anticonvulsant that acts by activating inhibitory postsynaptic neuronal GABA type A ($GABA_A$) receptors. Phenobarbital can activate $GABA_A$ receptors independent of GABA, but it also potentiates the effects of GABA.

Idiopathic
Any condition or disease that occurs spontaneously or with an unknown aetiology.

Since the time of the Greek philosopher Hippocrates, epilepsy, previously called the 'shaking palsy', has been a fascinating, yet often horrifying, syndrome. Once believed to be the result of demonic possession, the invention of electroencephalography in 1929 transformed epilepsy into a well-defined clinical syndrome that became synonymous with the presence of electrographic seizures (that is, abnormal brain waves resulting from hypersynchronous discharge of large populations of neurons in the brain)[1]. As per the current standard of the International League Against Epilepsy, an epilepsy diagnosis requires at least two unprovoked (or reflex) seizures occurring >24 h apart, one unprovoked (or reflex) seizure and a probability of further seizures similar to the general recurrence risk after two unprovoked seizures occurring over the next 10 years, or a diagnosis of an epilepsy syndrome[2]. Seizures can range from subtle, absent staring into space (absence) to profound convulsions of the entire body (tonic–clonic). Seizures can be focal, affecting only a discrete part of the brain, or generalized, encompassing larger brain regions in both hemispheres. Epilepsy affects >1% of the worldwide population, making it the most common neurological illness.

The invention of phenobarbital in 1912 was a turning point in the management of epilepsy, providing at least two-thirds of all affected individuals with seizure freedom and an excellent quality of life. Active research now focuses on providing the same for treatment-resistant patients. Much of this research has focused on how to dampen hypersynchronous discharge and to explain how changes in intrinsic neuronal activity cause such discharge. This research focus led to the widely accepted hypothesis that an imbalance of excitatory and inhibitory currents is largely responsible for epileptic seizures. Possible causes for these imbalances are manifold and include changes in neurotransmitters and mutations in their receptors, ion channels and ion transporters as well as altered network connectivity. Meaningful insight has also come from the study of rare genetic causes of epilepsy, which showed that even subtle changes in a single protein can drive the disease[3]; however, in most instances epilepsy appears to be a polygenic disorder.

On the basis of the presumed underlying causes, epilepsy is classified into two broad categories: genetic, or idiopathic, epilepsy is due to a genetic predisposition of the brain to generate seizures, whereas acquired epilepsy results from a known lesion or acute insult that establishes a series of alterations in cellular, molecular and physiological properties that give rise to seizures. The gradual transformation from a formerly healthy brain into one plagued by spontaneous seizures is called epileptogenesis and can be divided into three phases[4]. First, a brief event or insult, for example, stroke or head trauma, initiates cellular and molecular changes in the affected brain regions. Second, a latent period starts with (tumour or encephalitis) or immediately after (stroke or trauma) the insult and lasts until chronic, unprovoked seizures occur. This can be months or even years later, and behavioural seizures are not apparent during this period. This is the time window during which non-neuronal changes, discussed extensively below, may profoundly contribute to the eventual disease presentation. Third, after the manifestation of recurring seizures, molecular and cellular changes often continue to exacerbate the severity of the disease.

Various biological changes have been described to occur during epileptogenesis and include gliosis[5]; uncontrolled inflammation[6]; an impaired blood–brain barrier (BBB)[7]; neurodegeneration[8]; aberrant neurogenesis[9]; axonal and dendritic plasticity; changes in neural circuits[10]; structural and functional changes in receptors,

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ion channels, transporters and enzymes implicated in excitatory and inhibitory neurotransmission[11]; reorganization of the extracellular matrix (ECM)[12]; and epigenetic reprogramming[13]. These changes may differ qualitatively and quantitatively depending on the precipitating insult or event. Although a majority of epilepsy research has focused on intrinsic neuronal properties and neuronal network function, over the past two decades, evidence for non-neuronal contributions to epilepsy has surfaced with increasing frequency. The evidence that the neuronal environment may be critically altered in epilepsy and even involved in disease aetiology now warrants closer examination, and this is the principal objective of this Review. Here, we highlight evidence that supports the viewpoint that non-neuronal cells may contribute to disease development, disease progression or both. We hope to stir broader conversation to consider astrocytes, microglia, vascular and immune cells and even the ECM produced by neurons and non-neuronal cells as important players in epilepsy and possibly even as potential therapeutic targets in the future.

Gliosis

The presence of a prominent gliotic scar has become a hallmark of chronic focal epilepsy[5]; indeed, gliosis is probably omnipresent in all forms of epilepsy. Gliosis refers to a spectrum of physicochemical and physiological changes in glial cells, particularly astrocytes and microglia, that occur universally in response to diverse forms of CNS insults and diseases[5]. The extent and nature of these changes vary with the type and severity of the injury. Defining features of gliosis include hypertrophy of the cell bodies and processes; upregulated expression of several proteins, including intermediate filament proteins (glial fibrillary acidic protein (GFAP) and vimentin) in astrocytes and ionized calcium-binding adaptor molecule 1 (IBA1) and CD68 in microglia; cellular proliferation; and the formation of a tenacious scar rich in ECM molecules such as chondroitin sulfate proteoglycans (CSPGs)[14]. Gliosis may impart either beneficial or detrimental effects on nearby cells in a highly context-dependent manner[5]. Reactive glia release cytokines, chemokines, growth factors and other molecules to mitigate or prevent the brain damage due to insult by controlling inflammation, removing debris and inducing synaptic plasticity and tissue remodelling. However, unrestrained reactive gliosis may cause excessive inflammation, neuronal death and tissue damage. Recent findings suggest that reactive astrocytes can assume distinct neuroprotective or neurotoxic phenotypes explaining the diametric role of reactive glia in CNS pathophysiology[15].

The question of whether gliosis is a cause or consequence of seizures is debatable. In the case of trauma-associated epilepsy, gliosis precedes the development of epilepsy and hence could be causative. The best evidence for gliosis being causative of epilepsy comes from studies in which gliosis was induced by the conditional astrocyte-specific deletion of the β1 integrin gene *Itgb1*. These animals showed widespread gliosis without other pathologies[16] and developed spontaneous, recurrent seizures, suggesting that astrogliosis alone was sufficient to cause epilepsy. An unrelated study similarly found that virally induced astrogliosis in the hippocampus was sufficient to cause neuronal hyperexcitability[17]. Furthermore, astrocyte-specific deletion of the *Tsc1* gene, which causes tuberous sclerosis complex, induces abnormal neuronal organization and astrogliosis, and this disease also presents with epileptic seizures[18]. Lastly, seizures are one of the most common symptoms of Alexander disease, caused by heterozygous mutations in the astrocyte-specific *GFAP* gene. Astrocytes in affected individuals express insufficient glutamate transporters, causing excitotoxic neuronal loss and seizures, also supporting a causal link between astrocytic dysfunctions and epileptic seizures[19,20]. Note that reactive astrogliosis appears to be present in most animal models of acquired epilepsies as well as in tissues from people with epilepsy and, as the above studies suggest, is typically associated with epilepsy. However, there are examples in the literature in which the interdependence between seizures and astrogliosis appears absent or questionable. For instance, one study that examined tissue resected from people with focal cortical dysplasia found an absence of gliosis in the nonlesional epileptogenic cortex[21]. In addition, in pilocarpine-treated epileptic mice, seizure frequency did not correlate with reactive gliosis but instead with a loss of GABAergic interneurons in the dentate gyrus[22]. Taken together, these findings show that gliosis has been unequivocally acknowledged as an integral part of epilepsy histopathology and as a likely contributor to epileptogenesis in many, but not all, forms of epilepsy. How gliosis contributes to seizures is an area of active investigation, and the possible mechanisms are discussed extensively below and summarized in FIG. 1 and TABLE 1.

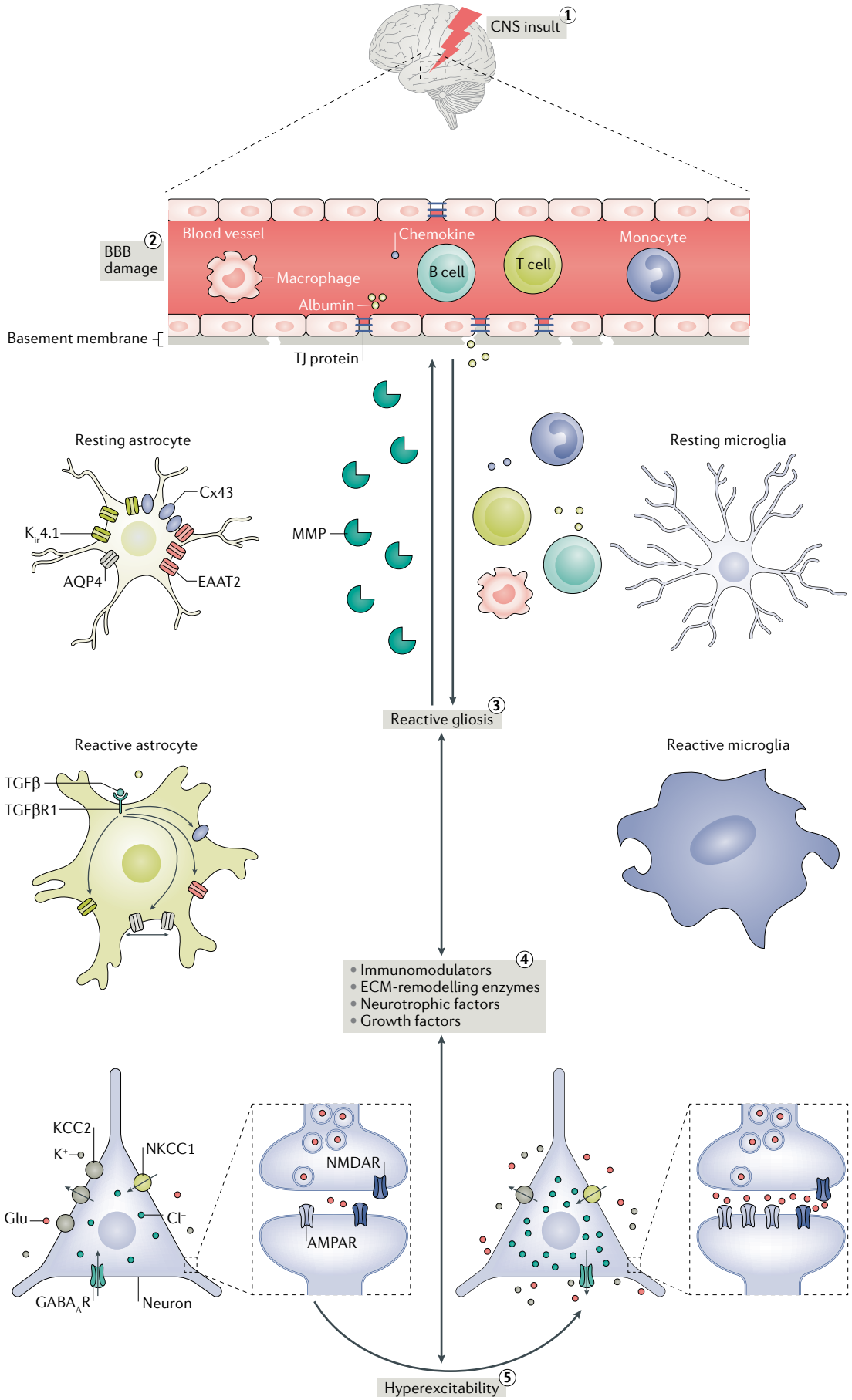
Ion and water homeostasis

Neuronal activity hinges on Na^+ and K^+ ions moving across the cell membrane into or out of the extracellular space (ECS). This space is very small in volume and therefore even modest ion fluxes can change ion concentrations considerably. Indeed, a single action potential raises extracellular K^+ concentration ($[K^+]_o$) by almost 1 mM, and during seizures repeated neuronal firing increases $[K^+]_o$ from a resting level of ~3 mM to ~12 mM (REFS[23,24]). In normal brain, $[K^+]_o$ is quickly readjusted to ~3 mM by the neuronal Na^+/K^+-ATPase, and if necessary excessive extracellular K^+ can also be cleared via astrocytic inwardly rectifying potassium (K_{ir}) channels. Once inside the astrocyte, K^+ is spatially buffered via redistribution through gap junctions (GJs) into the syncytial network of GJ-coupled astrocytes[25].

A failure to adequately buffer K^+ has been extensively discussed as one way in which gliotic astrocytes may contribute to epilepsy, particularly because simply increasing $[K^+]_o$ is sufficient to induce robust epileptiform activity in hippocampal slices from animals or humans[26,27]. Astrocytic K^+ buffering is mainly attributed to $K_{ir}4.1$, the most abundant K_{ir} channel in the CNS that is predominantly expressed in astrocytes[28]. Loss of $K_{ir}4.1$ expression and function has been reported in multiple animal models of acquired epilepsy and in tissues from people with epilepsy (reviewed in REF.[29]).

Gliotic scar
A dense fibrous mass of reactive glia formed in response to CNS injury. It is a part of the tissue-remodelling process that occurs following injury and provides both beneficial and detrimental effects in a context-dependent manner.

Sclerosis
A pathological stiffening of tissue at the injury site due to overgrowth of fibrous connective tissue that replaces original tissue.


CNS insult
1
BBB damage
2
Blood vessel
Chemokine
Monocyte
Macrophage
B cell
T cell
Albumin
Basement membrane
TJ protein
Resting astrocyte
Cx43
K_{ir}4.1
AQP4
EAAT2
MMP
Resting microglia
Reactive gliosis
3
Reactive astrocyte
TGFβ
TGFβR1
Reactive microglia
4
• Immunomodulators
• ECM-remodelling enzymes
• Neurotrophic factors
• Growth factors
KCC2
K^+
NKCC1
Glu
Cl^-
$GABA_AR$
Neuron
NMDAR
AMPAR
Hyperexcitability
5

◀ Fig. 1 | **Interplay between CNS insult, reactive gliosis and seizures.** (1) Various CNS insults such as stroke, infection, trauma, tumours and hypoxia can cause blood–brain barrier (BBB) damage. (2) The resulting increased permeability of the BBB promotes extravasation of serum albumin, peripheral immune cells (macrophages, monocytes, B cells and T cells) and chemokines into the brain parenchyma, where, along with direct CNS insult, they induce reactive gliosis. (3) Reactive gliosis refers to morphological (hypertrophy of the cell bodies and processes) and physiological changes (changes in the expression and functions of many glial proteins) in glial cells in response to various CNS injuries. (4) Reactive glia release extracellular matrix (ECM)-remodelling enzymes such as matrix metalloproteinases (MMPs), neurotrophic factors such as brain-derived neurotrophic factor (BDNF), growth factors such as transforming growth factor-β (TGFβ) and immunomodulators such as cytokines that modulate the expression and function of receptors, transporters, enzymes and other molecules implicated in the functions of excitatory and inhibitory neurotransmission by a variety of mechanisms. MMPs can aggravate BBB impairment by damaging the tight junction (TJ) connections and promote the infiltration of serum proteins in the brain. In addition to TGFβ, serum albumin is known to activate TGFβ receptor 1 (TGFβR1) signalling in astrocytes by directly interacting with TGFβR1. Accordingly, the membrane expression of inwardly rectifying potassium channel 4.1 (K_{ir}4.1), excitatory amino acid transporter 2 (EAAT2) and the gap junction protein connexin 43 (Cx43) are decreased. Activation of TGFβR1 also changes the trafficking and surface expression of aquaporin 4 (AQP4). As a result, extracellular concentrations of K^+ and glutamate (Glu) increase, which promotes network hyperexcitability. Control of Cl^- concentration is also crucial for regulating hyperexcitability. The K^+–Cl^- cotransporter (KCC2; a Cl^- exporter) and the Na^+–K^+–Cl^- cotransporter 1 (NKCC1; a Cl^- importer) play a crucial role in maintaining a low concentration of intracellular Cl^-, which is essential for maintaining the inhibitory activity of GABAergic neurotransmission. BDNF decreases the membrane expression of KCC2, causing net accumulation of Cl^- intracellularly and reversal of Cl^- flux through GABA type A receptor ($GABA_AR$). Therefore, the activation of $GABA_AR$ paradoxically causes hyperexcitation. Cytokines (for example, tumour necrosis factor (TNF)) released from reactivated glia can increase the postsynaptic density of AMPA receptors (AMPARs) and excitatory neurotransmission. (5) As a consequence, network hyperexcitability and seizures occur owing to impairment in K^+, Cl^- and glutamate buffering and imbalance in excitatory and inhibitory neurotransmission. Importantly, seizures can aggravate the gliotic condition by further increasing the release of neuromodulator molecules, setting the vicious cycle of gliosis and seizures. NMDAR, NMDA receptor.

In individuals with mesial temporal lobe epilepsy (MTLE) and hippocampal sclerosis, downregulation of K_{ir}4.1 has been found most prominently in the perivascular domains of astrocytic end feet[30], and patch clamp studies show a reduction in inwardly rectifying K^+ currents in the astrocytes of sclerotic hippocampus samples from patients with pharmacoresistant TLE[31]. Furthermore, astrocyte-specific deletion of K_{ir}4.1 in mice impairs K^+ uptake and causes ataxia, seizures and premature death[32]. However, even stronger evidence for the role of K_{ir}4.1 in epilepsy comes from quantitative trait loci studies that identified an association between variations in the *KCNJ10* gene that encodes K_{ir}4.1 and seizure susceptibility in humans and mice[33,34]. Note that, in addition to activity-dependent K^+ clearance, K_{ir}4.1 also contributes substantially to setting the resting membrane potential of astrocytes[28], which provides thermodynamic energy for the clearance of glutamate via Na^+-dependent electrogenic glutamate transporters. Indeed, RNAi-mediated knockdown of K_{ir}4.1 decreased both K^+ and glutamate uptake in cultured cortical astrocytes[35]. Taken together, these findings indicate that reactive astrocytes associated with seizure foci show decreased expression and function of K_{ir}4.1, thereby causing dysregulated clearance of extracellular K^+ and glutamate, which both directly contribute to neuronal hyperexcitability (FIG. 1).

Similar to that of K^+, the transmembrane distribution of Cl^- ions is crucial in the maintenance of normal neuronal activity, as the inhibitory action of GABA type A receptors ($GABA_ARs$) is governed by the transmembrane Cl^- gradient. Two transporters, the Na^+–K^+–Cl^- cotransporter (NKCC1) and the K^+–Cl^- cotransporter 2 (KCC2), are responsible for shuttling Cl^- in and out of the cell, respectively. In mature neurons, intracellular Cl^- concentration ($[Cl^-]_i$) is low as it is being shuttled into the ECS via KCC2 (REF.[36]). Loss-of-function mutations in KCC2 are known to cause an increased risk of epilepsy in humans[37], and human and animal studies show reduced expression of KCC2 in the brain during epileptogenesis[37]. Glutamate is one of among several factors that can affect the expression and function of KCC2. Excessive levels of glutamate, present during seizures, activate NMDA receptors (NMDARs) and can cause dephosphorylation of the KCC2 protein via protein phosphatase 1 and, in turn, decrease membrane expression of KCC2 (REF.[38]). As a result, the efflux of Cl^- into the ECS decreases, $[Cl^-]_i$ increases and results in a depolarizing shift in the reversal potential of $GABA_AR$, reducing its inhibitory activity and hence contributing to hyperexcitability[36]. As astrocytes are crucially important in maintaining the extracellular levels of glutamate, it is likely that astrocyte dysfunction in the epileptic condition may contribute to hyperexcitability via elevated levels of glutamate and impaired $GABA_AR$-mediated inhibition (FIG. 1). In addition to glutamate, reactive glia, especially microglia, are known to increase the synthesis and release of brain-derived neurotrophic factor (BDNF), which has been shown likewise to reduce the membrane expression of KCC2 via TrkB-mediated signalling[39].

Ion movement across the cell membrane is accompanied by obligate transmembrane water flux to maintain osmotic balance. As a consequence, neuronal activity is directly associated with water flux, which changes the composition and size of the ECS. Water transport across the membrane occurs through channels known as aquaporins (AQPs). AQP4 is highly expressed by astrocytes, particularly in the neuropil and their end-foot processes where they connect with the blood vessels[40]. Changes in AQP4 expression have been observed in cases of animal and human epilepsy (reviewed in REF.[41]), and elimination of AQP4 by genetic deletion or by RNAi impairs water transport in astrocytes[42,43]. AQP4 is anchored to the astrocytic membrane through a dystrophin-associated protein complex composed of α-syntrophin or dystrophin. Mice with genetic deletions of α-syntrophin or dystrophin show reduced expression of AQP4 in the astrocyte end feet around the blood vessels[44,45] and present with severe seizures when challenged by hyperthermia or pentylenetetrazole. Given that AQP4 and K_{ir}4.1 colocalize in astrocyte end feet[46] and that the flux of ions and water through them changes the size of the ECS, it is commonly suggested that AQP4 functions in concert with K_{ir}4.1 for both K^+ and water homeostasis. This is important, as changing the volume of the ECS alone is sufficient to alter seizure susceptibility and epileptogenesis[47]. Indeed, loss or mislocalization of AQP4 expression in the perivascular end feet of astrocytes in *Aqp4*$^{-/-}$ or *Snta1*$^{-/-}$ mice is associated with decreased clearance of synaptic K^+, impaired K^+ buffering and severe and prolonged seizures[48,49]. Taken together,

Pentylenetetrazole
A convulsant that acts by directly antagonizing GABA type A receptor-mediated inhibitory neurotransmission and therefore is often used experimentally to induce seizures in animals.

Table 1 | **Glial mechanisms that contribute to ictogenesis and epileptogenesis**

Glial factor and/or pathway	Function	Biochemical changes in epilepsy	Mechanism of hyperexcitability	Refs
$K_{ir}4.1$	• Clearance of extracellular K^+ • Set resting membrane potential	• Downregulated in animal models and in human epileptic tissues • Prominent reduction in perivascular astrocytic end feet	• Increase in extracellular K^+ • Impaired clearance of extracellular glutamate	28,29,35
AQP4	• Transmembrane water transport • Colocalized in perivascular astrocyte end feet and functions in concert with $K_{ir}4.1$	• Differential regulation in epileptic tissue • Loss or mislocalization in perivascular astrocyte end feet	• Decreased clearance of extracellular K^+ • Impaired K^+ buffering and GJ coupling	25,46
Connexins (GJ proteins)	Ionic and biochemical coupling between CNS cells	• Differential regulation in epileptic tissue • Functional consequences of changes in astrocytic GJ coupling remain inconclusive	Impaired spatial redistribution of K^+ and glutamate	29,50,51
NKCC1	Moves Cl^- into the cell	Increased expression	Impaired GABAergic inhibition due to increased intracellular Cl^- and depolarizing shift in the reversal potential of $GABA_AR$	NA
KCC2	Moves Cl^- into the extracellular space	Decreased expression	Impaired GABAergic inhibition due to increased intracellular Cl^- and depolarizing shift in the reversal potential of $GABA_AR$	NA
GLT1 or EAAT2	Clearance of extracellular glutamate	Results of expression studies in human epileptic tissues inconsistent	Increased extracellular glutamate	25,57
GS	• Expressed in astrocytes • Key enzyme in the glutamate–glutamine cycle • Sequesters glutamate by condensing it with ammonia to form glutamine	• Reduced expression and enzymatic activity in hippocampus of individuals with MTLE • Downregulated in gliotic tissue in mice	• Decreased neuronal GABA synthesis due to disruption in astrocytic supply of glutamine • Impaired GABAergic inhibition	17,66,67
Adenosine kinase	Phosphorylates adenosine (endogenous anticonvulsant) and terminates its action	Increased expression	Decreased level of adenosine	95
Gliotransmitters	Regulation of neurotransmission and synaptic plasticity	Increased release of glutamate and ATP	Increased activation of glutamatergic and purinergic signalling	91,134
BBB	Astrocyte end feet support the maintenance of BBB integrity	• Extravasation of albumin and peripheral immune cells into the brain following BBB disruptions • Increased albumin–TGFβ signalling in astrocytes	• Decreased expression of $K_{ir}4.1$, glutamate transporters and GJ proteins • Impaired K^+ and glutamate buffering • Increased neuroinflammation	123,125,135

AQP4, aquaporin 4; BBB, blood–brain barrier; EAAT2, excitatory amino acid transporter 2; $GABA_AR$, GABA type A receptor; GJ, gap junction; GLT1, glutamate transporter 1; GS, glutamine synthetase; KCC2, K^+–Cl^- cotransporter; $K_{ir}4.1$, inwardly rectifying potassium channel 4.1; NA, not available; NKCC1, Na^+–K^+–Cl^- cotransporter 1; MTLE, mesial temporal lobe epilepsy; TGFβ, transforming growth factor-β.

increasing evidence suggests that the colocalization of $K_{ir}4.1$ and AQP4 at astrocytic end feet on blood vessels is important to mediate effective K^+ and water clearance into blood vessels and that functional loss of either $K_{ir}4.1$ or AQP4 enhances seizure susceptibility.

Similar to neuronal networks, astrocytes are connected into a syncytial network formed by GJ channels composed of connexin proteins (Cxs)[50]. GJ coupling enables intercellular movement of ions (ionic coupling) and small molecules (biochemical coupling) between astrocytes as well as between astrocytes, neurons and the vasculature[50]. Several important functions have been attributed to astrocytic GJ coupling, including spatial buffering of K^+ and glutamate, trafficking and delivery of energetic metabolites from blood vessels to neurons, intercellular propagation of Ca^{2+} waves and regulation of cell volume (reviewed in REF.[29]). Naturally, any change in the GJ coupling could impact any one of these astrocytic functions and may contribute, in turn, to epilepsy. At the expression level, both increases and decreases in transcripts and Cxs have been reported in animal models and in humans with epilepsy (reviewed in REFS[50,51]). However, whether these changes translate into changes in function is not clear. Post-translational modifications of Cxs can alter trafficking of Cxs, assembly and subcellular localization of the GJs, as well as gating of the GJ channels[52]. Functional studies of coupling between astrocytes of people with MTLE with hippocampus sclerosis found a complete absence of functional GJ coupling in the sclerotic hippocampus in tissue from 75 individuals, yet abundant coupling was present in the nonsclerotic hippocampal specimens[53]. A reduction in astrocytic coupling may cause hyperexcitability owing to impaired spatial buffering of K^+ and glutamate,

whereas reduced coupling may also restrict the supply of energy metabolites and hinder the propagation of Ca^{2+} waves, thereby dampening the initiation and synchronization of neuronal activity. Pharmacological approaches have also been used to investigate the role of astrocytic GJ coupling in the pathophysiology of epilepsy and generally report that the GJ inhibitors reduce seizures[51]; however, one study found a proconvulsive effect[54]. Note however that the GJ inhibitors cannot differentiate between astrocytic and neuronal Cx isoforms, making it difficult to ascribe an unequivocal role to astrocytic GJs from such studies[51]. In conclusion, an abundance of evidence suggests changes in astrocytic GJ expression and function, but ascribing a clear mechanistic role to these changes as drivers of epileptogenesis remains elusive.

Neurotransmitters and energy metabolites

Increased extracellular glutamate is a biochemical hallmark of animal and human epileptic tissues and is believed to cause neurotoxicity and seizures[55,56]. One of the most widely accepted roles of astrocytes in normal brain physiology is the clearance of neuronally released glutamate[57]. This is accomplished via one of two Na^+-coupled electrogenic excitatory amino acid transporters, EAAT1 and EAAT2, that couple the import of one glutamate with three Na^+ ions and the release of one K^+ ion (REF.[58]). EAAT1 is primarily expressed early during development, whereas EAAT2 is the principal astrocyte-specific glutamate transporter in the adult brain. The rodent versions of EAAT1 and EAAT2 are known as glutamate aspartate transporter (GLAST) and glutamate transporter 1 (GLT1), respectively. Rapid astrocytic clearance of excess synaptic glutamate following synaptic transmission terminates its action, controls excitatory neurotransmission and prevents accidental activation of extrasynaptic NMDARs that are coupled to proapoptotic pathways. Once taken up by astrocytes, glutamate must be sequestered and degraded because the glutamate transporters are bidirectional and increased glutamate concentration within astrocytes will eventually cause its release into synapses. Some of the intracellular glutamate is degraded by conversion via glutamate dehydrogenase into α-ketoglutarate, which is then fuelled into the tricarboxylic acid (TCA) cycle, and thereby glutamate serves as an energy source[57]. A large portion of the remaining intracellular glutamate is converted to glutamine via the glutamine synthetase (GS) pathway and released into the interstitial space where adjacent neurons import it for glutamate synthesis via a phosphate-activated glutaminase (PAG). This is commonly dubbed the glutamate–glutamine cycle[57] (FIG. 2). In excitatory neurons, glutamate is packaged into secretory vesicles to be released into the synapse, whereas in GABAergic inhibitory neurons, glutamate is converted into GABA by glutamate decarboxylase (GAD). Hence, astrocytes are in the supply chain for both vesicular glutamate and GABA, and interfering with the glutamate–glutamine cycle at any stage will crucially impact extracellular glutamate levels, GABA and glutamate supply and synaptic function and thus may be causally associated with epileptogenesis.

Indeed, the findings that GLT1-knockout mice exhibit severe spontaneous seizures[59] whereas mice overexpressing glial GLT1 are resistant to pilocarpine-induced epileptogenesis[60] illustrate the importance of GLT1 in maintaining synaptic glutamate homeostasis and seizure threshold. Interestingly, GLT1 expression is modulated by the antibiotic ceftriaxone, which acts as a transcriptional stimulator[61]. Early treatment with ceftriaxone before the onset of epilepsy increased astrocytic expression of GLT1, decreased the extracellular glutamate level and reduced seizure frequency in a mouse model of tuberous sclerosis complex[62]. Another finding yielding a compelling new treatment strategy originated from the finding that GLT1 is downregulated in reactive astrocytes by heat shock protein 90β (HSP90β)-mediated proteasomal degradation[63]. Chronic treatment with an HSP90β inhibitor suppressed kainic-acid-induced seizures[63]. Changes in the functions of GLT1 have also been found prominently in lesional epilepsies[64,65].

Efforts to measure the expression of EAATs in human epileptic tissues have yielded inconsistent results, albeit pathophysiological increases in glutamate can be measured in patients before and during seizures[55], suggesting that astrocytes are unable to clear neuronally released glutamate. Abnormally high levels of extracellular glutamate during ictal and interictal periods and the changes in the expression and function of EAATs in astrocytes are often accompanied by impairment in the function of GS. A significant reduction in the expression and enzymatic activity of GS has been observed in the hippocampus of individuals with MTLE, and this loss of GS activity was particularly pronounced in areas with astrogliosis[66,67]. Continuous infusion of the GS antagonist, methionine sulfoximine (MSO), into the hippocampus of rats caused drastic reduction in GS activity, neuropathological changes similar to MTLE and epilepsy in a majority of the MSO-treated rats, suggesting that a deficiency in astroglial GS activity is a potential molecular mechanism for seizure generation in MTLE[68].

The role of astrogliosis in disrupting the glutamate–glutamine cycle and consequently causing hyperexcitability has been elegantly demonstrated in a mouse model of virally induced reactive gliosis[17]. Here local infection of astrocytes with adeno-associated virus (AAV) led to reactive gliosis in AAV-infected astrocytes, evident by cellular hypertrophy and increased GFAP and vimentin expression. Reactive astrocytes also had decreased expression of GS. Tissue slices isolated from infected animals showed epileptiform hippocampal activity. Importantly, all of these consequences of astrogliosis could be mimicked by inhibiting the glutamate–glutamine cycle and were reversed by providing exogenous glutamine. Similar findings have since been reported in rat spinal dorsal horn neurons[69] and in an astrocyte neuron co-culture system[70], suggesting that a disruption of neuronal GABA synthesis solely by disrupting the astrocytic supply of glutamine can induce the neural hyperexcitability underlying epilepsy.

Astrocytes also serve the crucial function of regulating the energy homeostasis essential for optimal synaptic activity[71]. Clearance of extracellular glutamate

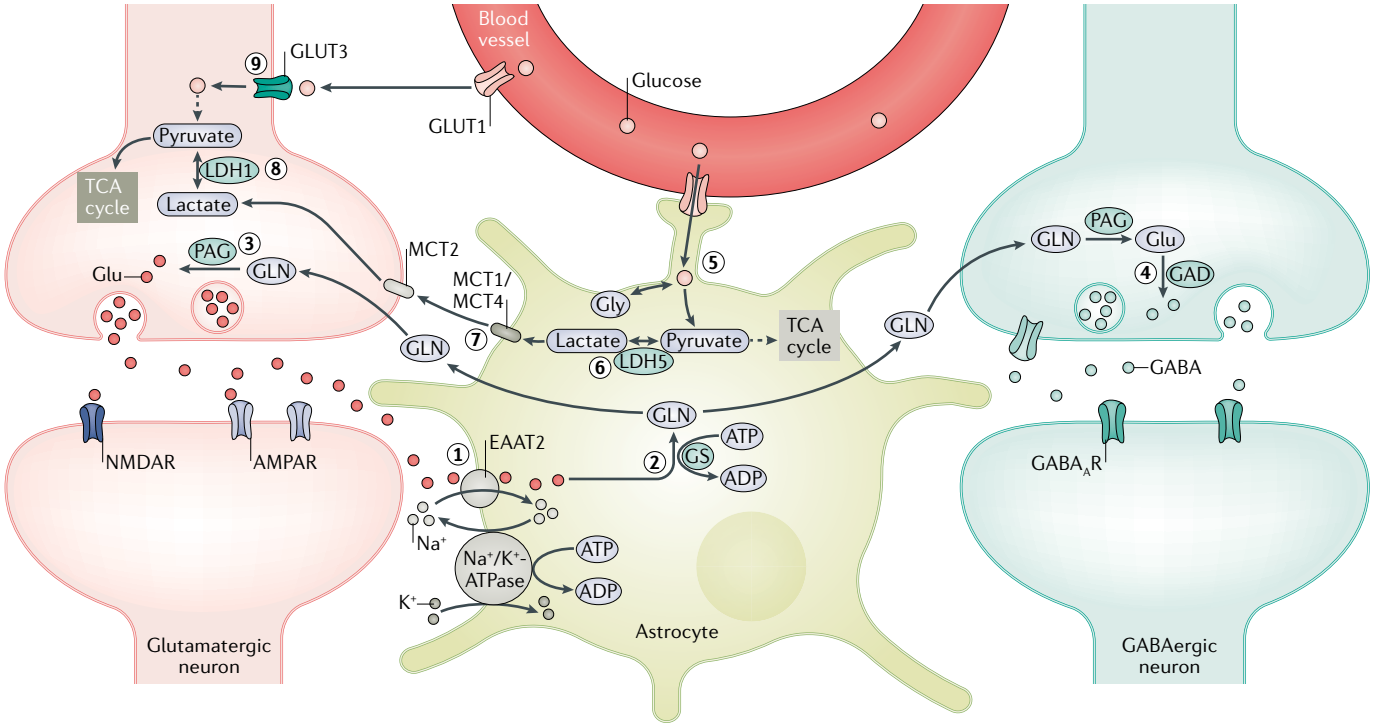


Fig. 2 | **Regulation of synaptic transmitter homeostasis and energy metabolites by astrocytes.** A crucial function of astrocytes is to maintain the optimal level of extracellular glutamate (Glu) through the Glu–glutamine (GLN) cycle. (**1**) Following synaptic transmission, excess extracellular Glu is absorbed by astrocytes through excitatory amino acid transporter 2 (EAAT2). (**2**) A large amount of Glu is converted by GLN synthetase (GS) into GLN, which is shuttled into the surrounding neurons. (**3**) GLN is then metabolized by phosphate-activated glutaminase (PAG) into Glu, which is packaged into synaptic vesicles in the glutamatergic neurons. (**4**) In GABAergic neurons, Glu is further converted by Glu decarboxylase (GAD) into GABA, which is released in the synapse during inhibitory neurotransmission. Rapid clearance of extracellular Glu following increased activity of synaptic transmission exerts an energetic burden on astrocytes owing to the energy-intensive nature of the Glu–GLN cycle, as both EAAT2 and GS require ATP. (**5**) The energy deficit that results stimulates the glycolytic oxidation of glucose imported from the blood through glucose transporter 1 (GLUT1) and is enzymatically derived from glycogen stores in astrocytes to generate energy metabolites — lactate and pyruvate. (**6**) Owing to the limited oxidative capacity of astrocytes, pyruvate is primarily converted into lactate via lactate dehydrogenase 5 (LDH5) and (**7**) shuttled into the surrounding neurons via monocarboxylate transporters (MCTs). (**8**) In neurons, LDH1 catalyses the conversion of lactate into pyruvate, which is subsequently used as an energy source through the tricarboxylic acid (TCA) cycle. This pathway is termed the astrocyte neuron lactate shuttle (ANLS) and is crucial in meeting the energy demands of neurons during high synaptic activity, such as during seizures. (**9**) Glucose is also taken up by neurons from the blood vessels through GLUT3; however, owing to the limited glycolytic capability of neurons and the lack of glycogen stores, neurons derive pyruvate primarily from astrocytes via the ANLS pathway. Disruptions in the expressions and functions of enzymes and transporters implicated in both the Glu–GLN cycle and the ANLS are associated with the development of neuronal hyperexcitability and seizures. AMPAR, AMPA receptor; $GABA_AR$, GABA type A receptor; Gly, glycine; NMDAR, NMDA receptor.

and its conversion into glutamine are energy-intensive processes. As mentioned above, astrocytes sequester extracellular glutamate via EAATs utilizing the electrochemical gradient of Na^+ as a driving force with a stoichiometry of one glutamate to three Na^+ ions. The transmembrane distribution of Na^+ ions is then re-established by the energy-consuming Na^+/K^+-ATPase. The conversion of glutamate into glutamine by GS also requires ATP. Thus, the glutamate–glutamine cycle imposes an energetic burden on astrocytes that triggers an uptake of glucose from the blood vessel through the glucose transporter GLUT1 expressed on the membranes of both blood capillary endothelial cells and astrocytes. Glucose is also taken up by neurons from the blood vessels through GLUT3. However, the glucose-handling pathways differ between astrocytes and neurons owing to the cell-specific expression of glucose-metabolizing machinery. Glycolytic oxidation of glucose into pyruvate is predominant in astrocytes and can be upregulated, but the utilization of pyruvate through the TCA cycle is limited. By contrast, neurons have a limited capacity for glycolysis but an active TCA cycle and oxidative phosphorylation[71]. In fact, neurons predominantly process glucose through the pentose phosphate pathway to maintain the redox status necessary to counter the oxidative stress that results from their high mitochondrial activity. Glycolytically produced pyruvate in astrocytes is converted into lactate by lactate dehydrogenase 5 (LDH5). Lactate is then shuttled via monocarboxylate transporters (MCTs) to neurons, where it is utilized as an energy substrate via the TCA cycle after being converted into pyruvate by LDH1. This pathway is often called the

astrocyte neuron lactate shuttle (ANLS) (FIG. 2), and its activity has been shown to positively correlate with neuronal activity (reviewed in REF.[72]). Astrocytes (but not neurons) store glycogen as an energy source to meet the high energy demand of neurons during prolonged neuronal activity by augmenting glycolysis and exporting lactate to neurons via the ANLS pathway. Any perturbations in this metabolic pathway may modulate neuronal excitability. Strategies such as a ketogenic diet that switch the energy source of the brain from glucose to ketone bodies by using high-fat and low-carbohydrate diets are often employed successfully in controlling refractory seizures[73]. The ketogenic diet tampers with energy metabolism and is likely modulating the ANLS pathway. A study by Sada et al. demonstrated that substituting glucose with ketone bodies (β-hydroxybutyrate or acetoacetate) hyperpolarized excitatory neurons in mouse brain slices[74]. The stabilizing effects of glucose restriction on neuronal excitability were abrogated by an exogenous provision of lactate, pyruvate and its downstream energy metabolites. It was further shown that pharmacological inhibition of LDH, specifically in astrocytes, decreased the excitability of surrounding excitatory neurons in the hippocampus, suggesting that lactate from astrocytes is a source of ictogenic pyruvate in neurons. Although the downstream mechanisms are not clear, the authors suggested that the activation of K_{ATP} channels could contribute to neuronal hyperpolarization because inhibitors of K_{ATP} channels abolished the effects of LDH inhibition. Importantly, an analogue of stiripentol, a clinically used antiepileptic drug that also inhibits LDH, potently suppressed kainic acid-induced seizures in mice.

Taken together, these findings show that astrocytes serve a pivotal function in supplying neurons with high-energy substrates to meet their energy demand and amino acid precursors for the synthesis of neurotransmitters. Thus, astrocytes are crucial in maintaining normal excitation–inhibition balance. A detailed mechanistic understanding of the role of astrocytes in providing metabolic support to neurons may enable the development of novel metabolic interventions for the treatment of epilepsy.

Synaptic structure and function

Overwhelming evidence suggests that epilepsy is a disease in which neural circuits are chronically altered. As these circuits are formed by synapses between neurons, it is reasonable to consider epilepsy as a disease resulting from impairment in synaptic structure and/or function. Glial cells influence synaptic structure and function by regulating long-term processes such as synaptogenesis, synaptic pruning and synaptic maturation as well as by releasing gliotransmitters.

Synaptic structure. Although a role for glia in the development of the brain has been well recognized for decades, more recent studies have identified astrocytes and microglia as essential players in normal synaptogenesis, and many of the molecules involved in synapse development and activity-dependent pruning have been identified[75]. These include BDNF, cholesterol, thrombospondins (TSPs), oestradiol, protocadherins, integrins, ephrins, glypicans, transforming growth factor-β (TGFβ) and hevin, among others[75]. Some of these factors, for example, TSPs, are primarily expressed in the developing brain, and subsequently their expressions are downregulated. However, after CNS injury, reactive astrocytes secrete excessive TSPs, which have been shown to interact with α2δ1, an auxiliary calcium channel subunit, to promote aberrant synaptogenesis, which can alter the neuronal circuitry to generate epileptiform activity[76]. Interestingly, the anticonvulsant drug gabapentin disrupts the interaction between TSP and α2δ1, attenuating epileptiform activity following epileptogenic freeze lesion[77], suggesting that reactive astrocyte-triggered aberrant synaptogenesis might be a potential mechanism of network hyperexcitability in neurodevelopment-disorder-associated epilepsy.

Glial cells also regulate critical signalling pathways associated with synaptic pruning. For example, during the development of retinothalamic circuitry, axons from the retinal ganglion cells undergo eye-specific segregation through elimination of overlapping inputs by microglia and astrocytes in an activity-dependent manner[78]. Astrocytes induce the expression of complement component C1q at synapses that, in turn, activates the complement pathway to stimulate microglia for phagocytosing the synapses[79]. Mice deficient in C1q exhibit impaired synaptic pruning, enhanced synaptic connectivity and eventually epileptic activity[80]. Recent studies suggest that astrocytes not only trigger phagocytosis via microglia but also exhibit phagocytic activity themselves under physiological and pathological conditions[81]. The complement components are upregulated in rodent models of epilepsy and may revitalize the synaptic-pruning functions of microglia during adolescence or adulthood[82].

Astrocyte-released molecules are also important for the regulation of synaptic strength. For example, glial-derived tumour necrosis factor (TNF) regulates trafficking of glutamate and $GABA_A$Rs onto postsynaptic membranes, cholesterol enhances presynaptic neurotransmitter release and glypican 4 and glypican 6 increase the number of AMPA receptors (AMPARs) at postsynaptic sites[75]. Presynaptic and postsynaptic differentiation and regulation of $GABA_A$R expression at developing inhibitory synapses can also be modulated by astrocyte-released molecules[83]; however, their molecular characterization is yet to be done.

Although there is little direct evidence of seizures resulting from impaired glia-mediated regulation of synaptogenesis and pruning, a few studies have reported aberrant synaptogenesis and synaptic pruning in neurodevelopmental disorders that are associated with epilepsy[78]. For example, astrocytes contribute to abnormal dendritic morphology and the reduced synapse density commonly found in fragile X syndrome, which is tightly associated with epileptic seizures[81,84]. Similarly, microglia-induced disruptions in neuronal circuits due to impaired synaptic pruning can contribute to seizures in the mouse model of Rett syndrome[85]. Finally, impaired synaptic pruning is a major driving force for the development of TLE in individuals with a mutation in the

gene *LGI1*, encoding leucine-rich glioma-inactivated protein 1 (REF.[78]).

Gliotransmission and epilepsy. A number of astrocyte-released molecules including glutamate, ATP and adenosine act as neurotransmitters and neuromodulators of neurons in a process called gliotransmission. As each of these neuromodulators can potently induce neuronal excitability[86], gliotransmission may contribute to epileptogenesis. Astrocyte-released glutamate exerts a multitude of effects on neurons including increased neuronal excitability and synchronization of activity, synaptic plasticity, regulation of GABAergic neurotransmission and generation of slow inward currents in neurons[87]. However, glutamate release from astrocytes is typically induced experimentally by photolysis of caged calcium or inositol 1,4,5-trisphosphate (IP_3) infusion. Although these stimulations induce slow inward currents that cause transient depolarizations reminiscent of paroxysmal depolarizations[86,88], the physiological relevance of such astrocyte stimulation has been questioned[89]. Nevertheless, as these neuronal paroxysmal depolarizations occur entirely in response to astrocytic calcium signalling and precede neuronal hyperactivity, it illustrates that, at least in principle, astrocytic glutamate release is capable of inducing neuronal hyperexcitability.

More compelling evidence for glutamate being the driver of epilepsy exists for malignant glia. Astrocyte-derived tumours (that is, gliomas) are notorious for causing seizures, with >70% of people presenting with seizures before diagnosis. Approximately one-third of all these individuals develop tumour-associated epilepsy, which is often pharmacoresistant. Both in tumour-bearing mice and humans, glutamate is released from the tumour as a result of the elevated expression of the system Xc, a cystine–glutamate antiporter encoded by the *SLC7A11* gene, which is upregulated in a majority of individuals with glioma[90]. This transporter is primarily responsible for the synthesis of the cellular antioxidant glutathione (GSH) from the uptake of cystine. However, the obligatory release of glutamate leads to chronic elevation of extracellular glutamate, which induces excitotoxicity, in turn, on the surrounding principal neurons[91]. In addition to glutamate-mediated excitotoxicity, matrix metalloproteinases (MMPs) released by the glioma cause preferential death of GABAergic interneurons by disintegrating the protective coat of the perineuronal nets (PNNs) around them[92]. The resulting increase in excitatory drive and decrease in inhibitory drive eventually lead to spontaneous electrographic and behavioural seizures[91,93,94].

Astrocytes release ATP and express a wide range of ion channels and G protein-coupled receptors activated by extracellular nucleotides and their cleavage products by ectonucleotidases[95]. The pro-hyperexcitability consequences of the purinergic signalling are mainly effectuated by astrogliosis, release of glutamate and pro-inflammatory factors, remodelling of the ECM and synaptogenesis. By contrast, ATP-mediated suppression of synaptic activity is largely mediated by its cleavage product adenosine and a subset of ATP receptors, eventually reducing excitatory drive either by enhancing inhibitory neurotransmission[96] or by suppressing excitatory neurotransmission[97]. Adenosine activates presynaptic and postsynaptic $G\alpha_{i/o}$ protein-coupled adenosine A1 receptors (A1Rs), and studies in humans with TLE and rodent models of epilepsy have shown downregulation of A1Rs[95]. At the presynaptic terminal, activation of A1Rs reduces calcium influx by inhibiting N-type calcium channels, thereby curtailing excitatory synaptic transmission. Moreover, miniature events at excitatory synapses are also decreased by presynaptic A1R-mediated reduced sensitivity of the neurotransmitter release machinery[98]. At postsynaptic terminals, A1R activation decreases neuronal excitability by enhancing the outflow of potassium through G protein-coupled K_{ir} channels[95].

Adenosine has been widely accepted as an endogenous anticonvulsant owing to its A1R-mediated synaptic inhibition and neuroprotective effects. Moreover, the physiological relevance of adenosine has been evident by an increase in the level of adenosine triggered by seizure activity[99]. Additional studies on the anticonvulsive effects of adenosine have elucidated an interesting role of the astrocyte-based adenosine cycle in acquired epilepsies. Astrocyte-released ATP is rapidly degraded into adenosine in the ECS and subsequently cleared by astrocytic nucleoside transporters and phosphorylated by adenosine kinase (ADK) predominantly expressed in astrocytes[95]. As ADK determines extracellular adenosine levels, any alteration in ADK expression can limit the adenosine levels and accordingly curtail its anticonvulsive and neuroprotective functions. A possible ADK dysfunction in epilepsy has been seen in epilepsies of animals and humans, which show robust overexpression of ADK in reactive astrocytes[100], suggesting adenosine deficiency as a contributing factor to seizures. Furthermore, ADK overexpression is sufficient to trigger seizures even in the absence of astrogliosis or any other epileptogenic event[95]. Importantly, these findings support a therapeutic approach through adenosine augmentation using either A1R agonists or ADK inhibitors, which are reported to reduce seizures in a wide range of epilepsy models, including those resistant to conventional antiepileptic drugs[95].

In essence, glial cells release a wide range of substances and gliotransmitters to shape the synaptic structure and function with high fidelity, and the disorders with defective synaptic structure and function, including epilepsy, are highly associated with the dysfunctional glial cells.

ECM remodelling and BBB integrity

Although most epilepsy research studies have focused on physiological changes in neurons and glia, emerging literature now suggests that the ECM into which neurons and glia are embedded may significantly influence neuronal excitability and indeed contribute to epilepsy (TABLE 2). Both glia and neurons release a heterogeneous mixture of proteins, glycoproteins and proteoglycans that form an amorphous interstitial matrix filling the ECS and highly specialized lattice-like assemblies known as PNNs (BOX 1) ensheathing mainly fast-spiking

Table 2 | **Extracellular matrix remodelling in epileptogenesis**

ECM component and/or remodelling molecules	Source	Functions	Pathology and/or epileptogenic effects	Refs
CSPG and/or CS–GAGs	Neurons, astrocytes and radial glia	• Ionic homeostasis • Synaptic stabilization and plasticity • Neuroprotection • Regulate localization of receptors on membrane	• Altered after CNS injury and epilepsy • Reactive glia-secreted CSPGs form glial scar and inhibit axon regeneration • Epileptiform activity, altered chloride homeostasis and decreased inhibition on CSPGs and/or PNN disruption	110,118, 133,136
HSPGs and/or glypicans	Astrocytes, neurons and endothelial cells	Synaptogenesis and synaptic maturation	• Downregulated in epilepsy models • Unaltered in individuals with TLE • Upregulated after CNS injury	136–138
Tenascin C	Astrocytes, neurons, radial glial and epithelial cells	• Synaptic plasticity • Regulates astrocyte progenitor proliferation and astroglial maturation	• Upregulated in seizure models associated with gliosis and axonal sprouting • Excessive release after injury and/or seizure • Impaired LTP, astrogliosis and low density of PV^+ interneurons with tenascin C deficiency	12,103, 139,140
Tenascin R	Neurons and oligodendrocytes	• Synaptic plasticity • Functional modulation of sodium channels	• Upregulated after injury, lesion and epilepsy • Abnormal PNN formation, impaired LTP, gliosis and decreased excitatory synapses and axonal conduction velocity on tenascin R deficiency	12,103, 140–142
HA	Neurons and astrocytes	• Regulation of extracellular space • Cell migration and signalling	• Upregulated in individuals with TLE • Epileptiform activity and impaired LTP with HA depletion • Spontaneous seizures with HA deficiency	103,108, 109,136
MMPs	Astrocytes, endothelial cells, neurons, microglia and oligodendrocytes	• ECM remodelling and cell migration • Synaptic morphology regulation	• Upregulated in CNS injury and seizure • Causes aberrant synaptogenesis associated with epileptic seizures • Decreased seizure sensitivity in MMP9-knockout mice • Increased seizure sensitivity in MMP-overexpressing mice	12,105,106, 143,144
ADAMTs	Neurons, astrocytes, microglia, monocytes and macrophages	• Proteoglycanase activity • Regulation of neural plasticity and regeneration • Inflammatory and antiangiogenic actions	• Upregulated in reactive astrocytes after seizure and CNS injury • Proteolytic cleavage of reelin • Deficiency causes reduction in synaptic proteins and an increase in neurocan	103,145–147
tPA	Neurons and microglia	• Serine proteases activating plasmin and MMPs • Regulation of neuronal development and survival and synaptic function • Modulation of synaptic plasticity	• Upregulation in epilepsy • Mossy fibre sprouting during epileptogenesis • Reduced synaptic plasticity and delayed seizure progression in knockout mice	103,146,148
TSPs	Astrocytes and macrophages	• Excitatory synaptogenesis • Modulation of cellular signalling, cell adhesion and migration	• Upregulated in reactive astrocytes after injury • Astrogliosis and decreased number of excitatory synapses with TSP deficiency	12,140
Reelin	Cajal–Retzius cells and interneurons	• Synaptogenesis and cortical lamination • Integration of DGCs	• Impaired formation of DGCs in individuals with TLE and rodent models of epilepsy with reelin loss • Autosomal dominant TLE in heterozygous mutations • DGC dispersion, neuronal heterotopia and enhanced seizure susceptibility in knockout mice	103,149
Hevin	Vasculature cells, astrocytes, radial glia and neurons	• Excitatory synaptogenesis • Cortical lamination • Synaptic plasticity	• Increased perisynaptic and decreased somatic expression in epilepsy • Upregulated in reactive astrocytes after seizures • Altered synaptic plasticity and glutamate receptor expression in knockout mice	15,103,140
SPARC	Radial glia, vasculature cells, astrocytes and microglia	• Synaptogenesis and cortical lamination • Inhibition of hevin synaptogenic action • Synaptic maturation	• Upregulated in reactive astrocytes after seizures • Expressed by reactive human astrocytes near tumours and correlated with epilepsy • Anxiety and antidepressant-like behaviours, increase in excitatory synapse function and impaired LTP in SPARC-knockout mice	140,150

ADAMTs, a disintegrin and metalloproteinase with thrombospondin motifs; CS–GAG, chondroitin sulfate–glycosaminoglycan; CSPG, chondroitin sulfate proteoglycan; DGC, dentate granule cell; ECM, extracellular matrix; HA, hyaluronic acid; HSPG, heparin sulfate proteoglycan; LTP, long-term potentiation; MMP, matrix metalloproteinase; PNN, perineuronal net; PV^+, parvalbumin positive; SPARC, secreted protein acidic and rich in cysteine; TLE, temporal lobe epilepsy; tPA, tissue plasminogen activator; TSP, thrombospondin.

Box 1 | **Perineuronal nets**

A specialized extracellular matrix (ECM) structure, termed the perineuronal net (PNN), has fascinated histologists since its discovery in the 1900s. PNNs are formed by lattice-like assemblies of chondroitin sulfate proteoglycans (CSPGs), hyaluronic acid (HA), tenascin R and link proteins and are tightly applied to the cell somas, apical dendrites and axon initial segments[101]. The chemical constituents of PNNs interact with receptors on the cell membrane and harbour various growth factors and guidance molecules, thereby controlling several cellular processes. For instance, semaphorin 3A, an axon guidance molecule, is anchored to the PNN via chondroitin sulfate E, and fibroblast growth factor 2 binds to the phosphacan core protein of PNN–CSPG[132]. PNNs are believed to serve a wide range of functions, including synaptic stabilization, neuroprotection and ionic buffering[101]. Their importance in CNS function is most well documented during the development of the visual system, for which the establishment of ocular dominance columns occurs through the stabilization of synapses in the visual cortex by PNNs[133]. The enzymatic destruction of PNNs dissolves the ocular dominance, enabling renewed synaptic plasticity, suggesting that the PNNs do not permit synaptic plasticity.

GABAergic interneurons[101]. These molecules collectively constitute the ECM, which also includes a highly organized basement membrane surrounding the blood vessels and the entire pial surface. The ECM primarily provides support for the cellular arrangement; however, interactions with membrane receptors including integrins, osteopontin and CD44 suggest that their physiological role may be much more complex[101].

ECM remodelling in epilepsy. The ECM is subject to remodelling by a number of matrix-remodelling enzymes such as MMPs and tissue inhibitors of metalloproteinases (TIMPs) that are released by neurons and glial cells. During the course of development, substantial ECM remodelling transforms the immature brain ECM that supports structural and functional plasticity into mature brain ECM, which restricts the structural remodelling of neuronal network, axonal outgrowth and synaptogenesis and hence is nonpermissive to synaptic plasticity[101]. Pathological changes in the remodelling of the ECM and PNNs that can potentially alter synaptic circuits in a maladaptive way have been observed in animal models of epilepsy and in human epileptic tissues[102–104] (FIGS 3,4). As the biochemical changes in the ECM and PNN molecules correlate positively with upregulated levels of MMPs and tissue plasminogen activator (tPA)[104–106], it is likely that the abnormal enzymatic activity causes the degradation of these structures. Indeed, at least in the rodent models, PNN breakdown and seizure activity can be inhibited by treatment with doxycycline, which is a broad-spectrum inhibitor of MMPs[107].

A major unanswered question is whether ECM remodelling is a cause or consequence of seizures. A partial answer is provided by a recent animal study in which the genetic deficiency of hyaluronic acid (HA), a component of PNNs, was sufficient to cause behavioural and electrographic seizures[108]. Deficiency of one of the major PNN components, tenascin C, increases the number of GFAP-expressing astrocytes and increases seizure susceptibility in mice, whereas depletion of another PNN component, tenascin R, induces aggravated astrogliosis, dysregulation of synaptic plasticity and perisomatic GABAergic inhibition[103]. In addition, in vivo enzymatic breakdown of the ECM using hyaluronidase causes electrographic-seizure-like events[109], and the disruption of the PNNs using chondroitinase increases the frequency of myoclonic seizures[110]. These studies suggest that an intact ECM suppresses development of seizures and that disruptions in the ECM alone may be sufficient for at least some seizure activity. TABLE 2 presents a summary of the most common ECM components, enzymes involved in their remodelling and the effects of ECM remodelling on epileptogenesis.

How might the ECM composition and/or the structural integrity of PNNs affect neuronal excitability? There are several ways in which ECM molecules can specifically regulate the localization, mobility and function of ion channels and neurotransmitter receptors, namely, by regulating channels and receptors in the membrane, by acting as buffers for extracellular ions and ligands or by altering the intrinsic properties of neurons. For example, HA has been shown to regulate the activity of voltage-activated calcium channels[111], whereas brevican[112] and tenasin[113] regulate the localization of voltage-gated potassium and sodium channels, respectively. The ECM molecules may also modulate synaptic plasticity by limiting the lateral mobility of receptors as shown for AMPAR[114]. Furthermore, experimental disruption of the ECM decreases the excitability of fast-spiking interneurons and thus inhibitory drive[115].

One of the salient features of the ECM constituents is a very high density of negative charges contained in their CSPG side chains[116]. As these are attached to a protein backbone, they resemble stationary charges with little diffusional mobility in the ECS and complete impermeability to the neuronal membrane. When intact, stationary charges may act as a buffering system for extracellular cations, including Ca^{2+} (REF.[117]). Moreover, they may affect the transmembrane chloride gradient and thereby the excitatory or inhibitory nature of GABAergic neurotransmission[118]. Indeed, Cl^- measurements in hippocampal slices using a genetically encoded Cl^- sensor show an increase in $[Cl^-]_i$ upon digestion of the ECM using chondroitinase. This, in turn, is sufficient to facilitate cellular epileptiform activity[118].

We recently reported an additional mechanism whereby PNNs may regulate neuronal firing and contribute to epilepsy[92]. We propose that by virtue of the high-density negative charges, the PNNs increase the charge separation distance across the neuronal membrane, thereby decreasing the effective capacitance of the cell. The reduced capacitance decreases the time constant of the action potential and enhances the maximal achievable spike frequency. As PNNs are most prominent on fast-spiking parvalbumin-expressing GABAergic interneurons, we propose that the PNNs act akin to the myelin sheath on axons to facilitate rapid firing of action potentials at the axon hillock and more rapid transmission of electrical signals along the apical

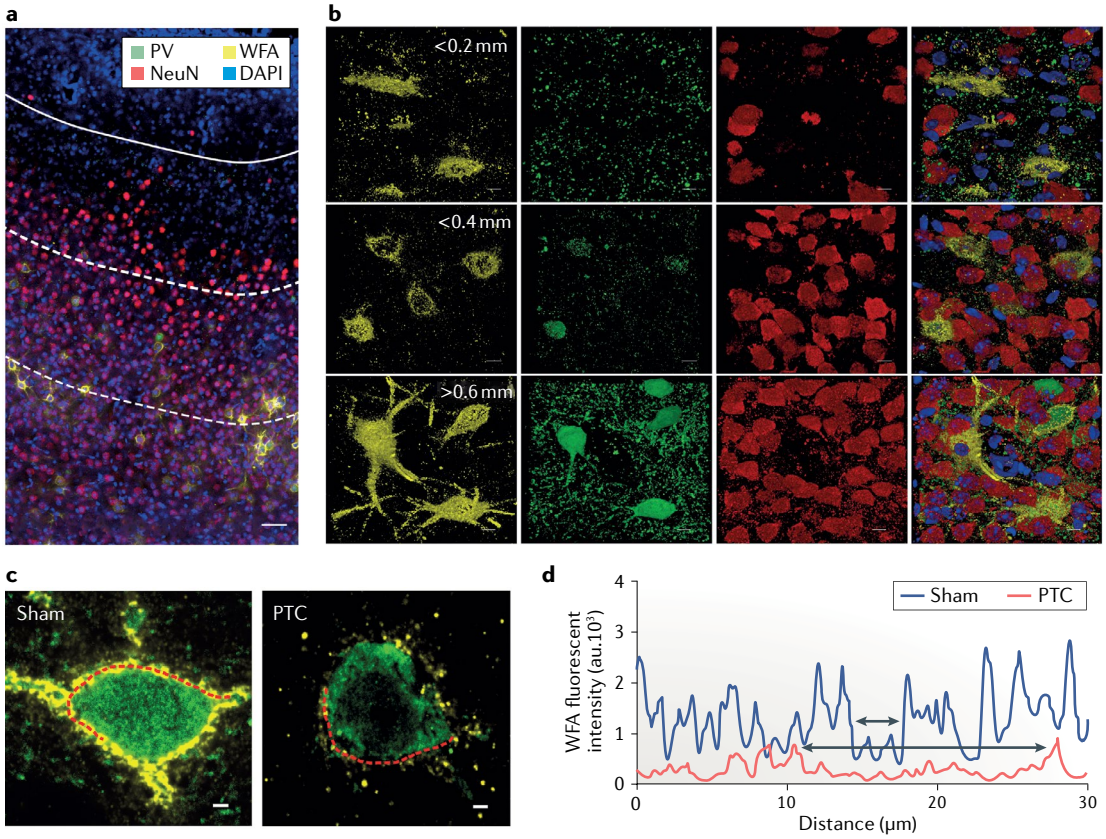


Fig. 3 | **Disintegration of perineuronal nets in glioma-associated epilepsy. a**,**b** | Immunohistochemical staining of the cerebral cortex from a glioma-implanted mouse (part **a**) shows 4′,6-diamidino-2-phenylindole (DAPI)-stained nuclei (blue), Wisteria floribunda agglutinin (WFA)-stained perineuronal nets (PNNs; yellow), parvalbumin-positive (PV^+) interneurons (green) and NeuN-stained neurons (red) (scale = 50 μm). The presence of glioma is identified by densely packed DAPI-stained cells indicated in the upper part of the image demarcated by a white solid line (tumour border). The distance between the tumour border and each of the white dotted lines below is ~0.2 mm. Magnified individual images of PNNs, PV^+ interneurons and $NeuN^+$ neurons and their merged images corresponding to their distance from the tumour border are shown on the right (upper panels <0.2 mm, middle panels 0.2–0.4 mm and lower panels 0.4–0.6 mm; scale = 5 μm). **c** | Confocal images of PV^+ interneurons (green) show disintegrated architecture of PNNs (yellow) in the peritumoural cortex (PTC) and are compared to sham-treated mouse cortex (scale = 2 μm). **d** | The WFA fluorescence intensity of a line drawn along the periphery of a PV^+ interneuron in the peritumoural cortex (left panel of part **c**) and sham (right panel of part **c**) shows many high-intensity WFA peaks in sham compared with those in the peritumoural cortex. Upper and lower two-headed arrows within the two nearest WFA peaks indicate the size of a hole in the PNN from the sham and peritumoural cortex, respectively. au, arbitrary units. Adapted from REF.[92], Springer Nature Limited.

dendrites and somas. Indeed, in a study of tumour-associated epilepsy, we find that MMPs released from the tumour cells degrade the PNNs, which increases the membrane capacitance of GABAergic interneurons, thereby slowing their firing frequency. We show that peritumoural hyperexcitability can be induced as a result and that the PNN disruptions alone can phenocopy the presence of the tumour (FIG. 4).

As MMPs are commonly released after CNS infection, stroke or trauma, it is reasonable to hypothesize that similar degradation of PNNs may occur in association with other forms of acquired epilepsy, particularly those associated with reactive gliosis in which astrocytes are known to secrete the components and the remodelling enzymes of the ECM[12,106,119]. An excessive release of CSPGs from reactive astrocytes has been documented in spinal cord injury in which the newly formed ECM retards axonal sprouting[120]. In addition, the polarized expression of astrocyte membrane proteins including $K_{ir}4.1$, AQP4 and GLT1 is controlled by the ECM molecules; hence, disruptions of the ECM can dramatically alter the functions of astrocytes and contribute to epileptogenesis[120–122].

BBB integrity in epilepsy. The basement membrane is another component of the ECM that is primarily known for the maintenance of BBB integrity[101]. However, it is also subject to degradation by MMPs, as is the case in cerebral ischaemia. The resulting leakiness of BBB may contribute directly to epileptogenesis. Acquired forms of epilepsies are invariably present with an impaired BBB, and the breakdown of the BBB contributes to the progression of epilepsy[7]. BBB disruption exposes the brain to invading immune system cells and serum components, most importantly albumin[7,123]. Albumin alone can trigger neuronal hyperexcitability by activating

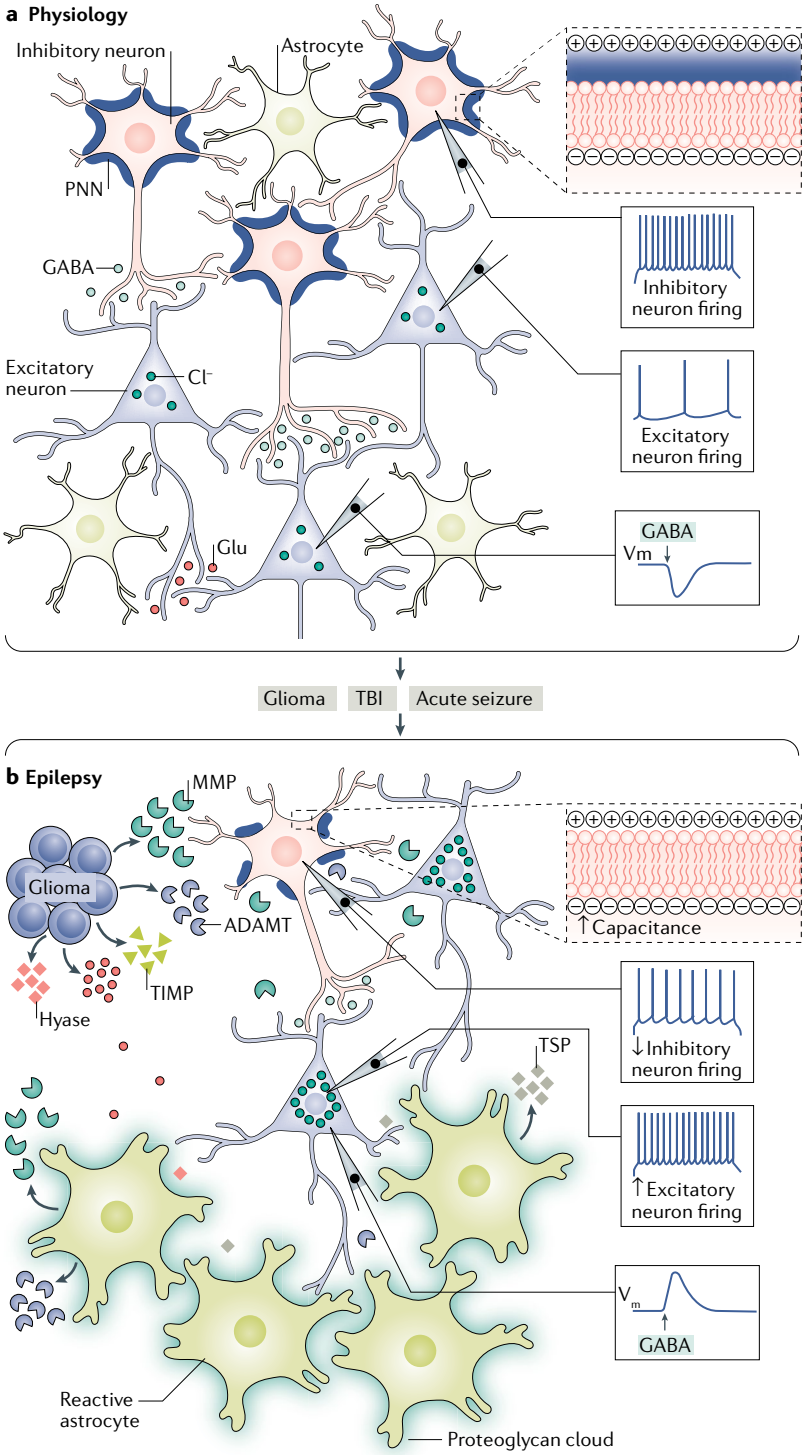


Fig. 4 | **Changes in the extracellular matrix as contributors to epilepsy. a** | Under physiological conditions, the extracellular space is filled with a dense amorphous interstitial matrix and highly organized perineuronal nets (PNNs) surrounding parvalbumin-positive (PV^+) inhibitory interneurons. The presence of intact PNNs around the cell reduces the effective membrane capacitance of the cell by increasing the charge separation between intracellular and extracellular compartments (upper right panel). This enables interneurons to sustain high firing frequencies to maintain a steady-state inhibitory drive by releasing GABA onto the excitatory cells, which helps to maintain optimal firing of the excitatory neurons. Intact structure of the extracellular matrix (ECM) also helps to maintain a low concentration of intracellular chloride; therefore, activation of the GABA type A receptor ($GABA_AR$) causes hyperpolarization owing to the chloride influx. **b** | Alterations in the ECM that occur during several pathological conditions such as glioma, traumatic brain injury (TBI) and acute seizures can contribute to the development of epilepsy. Glioma cells release glutamate (Glu) and ECM-remodelling molecules, including matrix metalloproteinases (MMPs), tissue inhibitors of metalloproteinases (TIMPs), hyaluronidase (hyase) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTs). These ECM-remodelling molecules cause degradation of the PNNs around inhibitory interneurons and thinning of the interstitial matrix. The loss of PNNs increases the membrane capacitance of inhibitory interneurons by decreasing the charge separation between intracellular and extracellular compartments (upper right panel), thereby slowing their firing frequency and, as a consequence, reducing their GABA release and lowering inhibition of excitatory neurons. Hence, the firing of excitatory neurons increases, contributing to network hyperexcitability. Glioma cells also cause selective loss of PV^+ inhibitory interneurons owing to Glu excitotoxicity further lowering the inhibitory drive, thereby leading to epileptiform activity. Impairment in the ECM structure also alters chloride homeostasis, which results in an increased intracellular concentration of chloride and a depolarizing shift in the activation of the $GABA_AR$. In a manner similar to that of glioma, following TBI and acute seizures, reactive astrocytes become the primary source of the ECM-remodelling molecules (MMPs and ADAMTs), synaptogenic molecules (thrombospondins (TSPs)) and proteoglycans, which can contribute to epileptogenesis by degrading PNNs and the interstitial matrix, thinning the interstitial matrix and causing aberrant synaptogenesis. V_m, membrane potential.

TGFβ receptors in astrocytes, which results in reduced expression of K_{ir} channels, glutamate transporters and GJ proteins[124] (FIG. 1). TGFβ also changes the trafficking and surface expression of AQP4 (REF.[125]). Together, this results in impaired K^+ and glutamate buffering, which is likely to contribute to neuronal hyperexcitability. Activation of TGFβ signalling also induces aberrant neurogenesis, increased excitatory synaptogenesis and the release of pro-inflammatory molecules from astrocytes that cause excitotoxic damage to neurons and other surrounding cells[126]. Extravasation of albumin in the brain following BBB damage may also contribute to the development of pharmacoresistance to antiseizure drugs in refractory epilepsy by binding to antiseizure drugs in the brain and thereby decreasing their bioavailability and efficacy[127]. In addition, activation of TGFβ signalling in astrocytes following BBB disruption initiates transcriptional activation of various ECM-remodelling molecules, which potentially break the PNNs, thereby affecting the inhibitory activity of GABAergic interneurons and inducing the excitatory synaptogenesis contributing to epileptogenesis[12,128]. The critical contribution of TGFβ signalling in dysregulating astrocyte functions during BBB-disrupting injuries and epileptogenesis is supported by the findings that losartan, a US Food

and Drug Administration-approved drug that inhibits TGFβ signalling, decreased the number of rats developing albumin-induced chronic seizures and inhibited epileptogenesis in CNS vascular injury-induced models of epilepsy[129]. The molecular mechanisms of BBB disruption during epileptogenesis are not clearly understood. In this regard, it has been proposed that MMPs critically contribute to the breakdown of the BBB by digesting tight junctions and basement membrane proteins[130,131]. Recently, it has been suggested that glutamate released during seizures increases the levels of MMP2 and MMP9 in the BBB capillaries via NMDARs and cytosolic phospholipase A2-mediated signalling, which promotes, in turn, reduction in the number of tight junction proteins and BBB leakage[109].

Taken together, disruption of the physiochemical properties of the ECM, basement membrane and PNNs by ECM-remodelling molecules may significantly contribute to epileptogenesis by altering the membrane expression or function of ion channels and receptors; by disrupting glutamate, potassium and chloride homeostasis; or by changing the intrinsic firing properties of fast-spiking GABAergic interneurons.

Summary and conclusions

An important and rapidly growing body of scientific evidence, some of which is discussed above, leads us to suggest that the neuronal microenvironment contributes meaningfully to the aetiology of epilepsy. This microenvironment is dynamic and greatly influenced by glial cells. There remains little doubt that glia contribute to ictogenesis and epileptogenesis, and the many ways in which this occurs are summarized in TABLE 1. Glial cells are not just passive bystanders providing structural and tropic support to neurons but can actively participate in neuronal physiology. Although in some examples their contribution has been unequivocally demonstrated, many important questions regarding specific changes in glia and their contribution to the onset and progression of epilepsy remain, as summarized below.

Gliosis is a defining histological feature in epileptic tissue. Is it a cause or consequence of epilepsy? Although some studies have provided evidence that gliosis is a driving factor of epileptogenesis, additional studies are needed to test the causal relationship between gliosis and epileptogenesis under different experimental conditions.

Are the functional changes that occur in gliotic tissue restricted to the affected brain region, or does gliosis also alter distant brain regions?

Do glial cells preferentially contribute to lesional epilepsies, or is there a more generalizable glial dysfunction present across all forms of epilepsy?

Epilepsy is a chronic disorder, yet neuro–glial interactions are highly dynamic. Do these interactions evolve during epileptogenesis? Do they change with initiation and termination of seizures?

Current biochemical and pathophysiological analyses of human epileptic tissues examine the end point of disease and fail to address the dynamics of epileptogenesis. Are there any biomarkers released from glia that may predict disease progression?

Activated glia are important to resolve neuropathological conditions following CNS insult. However, uncontrolled, severe, reactive gliosis may contribute notably to the neuropathology associated with epilepsy. Are there factors that can turn off activated glia? Could such factors be harnessed to develop antiepileptic therapies?

Can strategies aimed at maintaining the integrity of the BBB control seizures? To what extent does the infiltration of blood serum proteins and peripheral immune cells influence neuroglial functions in the brain?

Glial cells secrete a wide range of ECM-modifying molecules that critically influence neuronal functions. To what extent do these molecules affect the neuronal environment such that they tip the excitation–inhibition balance? Might such changes be common among many forms of epilepsy?

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