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Glial Mechanisms of Inflammation during Seizures

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Abstract: It is now clearly established that microglia and astrocytes become reactive in brain regions experiencing seizures both in human and experimental epilepsies. The expression of these reactive phenotypes lead to the dysregulation of the physiological functions normally fulfilled by these glial cells and to the acquisition of inflammatory properties through which they influence the activity and the fate of brain cells, including neurons, glia and cells of the blood vessels. In this chapter, we review how dysregulation of astrocyte and microglia physiological functions and the emergence of specific reactive states impact epilepsy progression.

Keywords: astrocyte, microglia, temporal lobe epilepsy, cytokine, purinergic signaling, gliotransmission, inflammation

Introduction

Glia of the central nervous system (CNS) mainly comprises astrocytes, cells of the oligodendrocyte lineage and microglia. Beside their well-known supportive or metabolic functions for neurons, it is now clear that glial cells also dynamically interact with neurons to integrate neuronal activity and, in turn, directly influence this activity. In the case of astrocytes and microglia, this glial modulation of neuronal activity can operate through the release of mediators, either classical transmitters such as glutamate, GABA, D-serine and ATP or cytokines and growth factors. In physiological conditions, “metabolic” and “information processing” functions of glia operate on a time scale ranging from milliseconds to hours or even days, when considering brain development and hormonal cycles, and are fundamental for brain functions, including cognitive functions (Pellerin et al., 2007; Theodosis et al., 2008; Attwell et al., 2010; Araque et al., 2014; Khakh and Sofroniew, 2015; Dallérac and Rouach, 2016; Mosser et al., 2017; Salter and Stevens, 2017; Allen and Lyons, 2018; Thion et al., 2018; Santello et al., 2019; Vainchtein and Molofsky, 2020). In pathological conditions, microglia and astrocytes develop reactive phenotypes that result in i) an impairment of their homeostatic functions and ii) the up-regulation of “immune” functions through which microglia and astrocytes orchestrate the central inflammatory reaction. This remodelling of glial phenotype modifies their interactions with neurons but also with cells of the blood vessels (pericytes and endothelial cells), thereby leading to impaired neuronal activity and BBB functions (Halassa et al., 2007; Rossi and Volterra, 2009; Salter and Beggs, 2014; Gorter et al., 2015; Steinhäuser et al., 2016; Verkhratsky and Parpura, 2016; Wolf et al., 2017; Volk, 2017; Ising and Heneka, 2018; Cresto et al., 2019; Patel et al., 2019).

The relationships between inflammation, gliosis, seizure and epilepsy emerged at the beginning of this century from clinical and experimental observations. The best documented evidence for a direct causality between inflammation and human epilepsy comes from Rasmussen encephalitis in which brain inflammation of unknown origin triggers focal motor seizure (Varadkar and Cross, 2015). However, Rasmussen encephalitis remains an extreme condition which does not directly relate to other forms of epilepsy. 20 years later, there is a general agreement that brain inflammation represents a hallmark of seizure and epilepsy (Bauer et al., 2017; Terrone et al., 2017) but whether reducing inflammation has any clinical benefit for

patients remains an unanswered question. Another key question is whether inflammation and gliosis contribute to epileptogenesis and/or to the genesis of spontaneous recurrent seizures (ictogenesis). In other words, are these processes involving primarily non-neuronal cells a cause or a consequence of aberrant neuronal network activities? Answering these key questions is difficult for different reasons. First, experimental models of epileptogenesis leading to chronic seizures, even when considering only models of temporal lobe epilepsy (TLE), are diverse and often not strictly comparable, and the evolution of the immune response during the progression of the disease (acute versus chronic) remains poorly characterized. Second, investigating central immune response in human is mostly limited to brain resections obtained from patients undergoing surgical removal of epileptic foci since non-invasive approaches such as PET imaging of inflammatory markers remain rather crude.

In this chapter, we will first review the roles of astrocytes and microglia in homeostatic conditions before discussing the notion of gliosis or glial remodelling in non-homeostatic conditions, its link with inflammation, and how astrocytes and microglia influence the pathogenesis of epilepsies, focusing mostly on temporal lobe epilepsy (TLE).

1. Astrocytes and microglia in homeostatic conditions

1.1. Astrocytes

Homeostasis of K^+ and water

In physiological conditions astrocytes contribute to the maintenance of ion homeostasis and, in particular, regulate extracellular K^+ ($[K^+]_0$). The accumulation of K^+ in the extracellular space leads to sustained neuronal depolarization and hyperexcitability (Heinemann and Dieter Lux, 1977; Walz, 2000). Astrocytes control $[K^+]_0$ by two mechanisms: K^+ uptake and spatial buffering. The K^+ uptake is mediated through Na^+/K^+ pumps, $Na^+/K^+/Cl^-$ cotransporters and the large expression of inward-rectifier potassium (Kir) 4.1 channels; it also relies on the very negative resting potential of astrocytes (Ransom et al., 2000; D'Ambrosio et al., 2002; Kofuji and Newman, 2004; Seifert et al., 2009). Remarkably, mice lacking Kir4.1 channels on

astrocytes were impaired for K+ and glutamate uptake and were more susceptible to seizures (Djukic et al., 2007; Chever et al., 2010).

Control of ion homeostasis by astrocytes relies also on spatial buffering (Orkand et al., 1966), which is mediated by gap junctions (GJ), channels composed of connexin (Cx) 43 and Cx30 (Nagy and Rash, 2000). These channels allow the formation of a functional network between astrocytes (syncytium) and the diffusion of K^+ following the electrochemical gradient: K^+ is taken up by astrocytes where neuronal activity is high, diffuses through the syncytium and is released at remote sites with lower $[K^+]_0$. In line with the spatial buffering concept, the physiological functioning of astroglial networks should have an anti-epileptic role, and indeed, a reduced threshold for generating epileptic activity was found in mice with coupling-deficient astrocytes (Wallraff et al., 2006; Pannasch et al., 2011).

Another important function in which astrocytes are involved is the regulation of extracellular space (ECS) volume and fluid osmolarity through aquaporin 4 (AQP4). This channel mediates transmembrane water movements according to osmotic gradients and that is expressed by astrocytes mostly at perisynaptic processes and perivascular end-feet (Schwartzkroin et al., 1998). Regulating water flux near synapses and blood vessels may be linked to K^+ homeostasis (Papadopoulos and Verkman, 2013).

Neurotransmitter buffering

Another crucial role for astrocytes that directly impacts on neuronal excitability is neurotransmitter buffering. The removal of neurotransmitters, such as glutamate and GABA, from the synaptic cleft is crucial for maintaining a correct functioning of synapses and an appropriate balance between excitation and inhibition (E/I balance). Alterations in these buffering mechanisms could lead to neuronal hyper-excitability and excitotoxicity (Choi, 1988; Meldrum, 1994). The astrocytic buffering of neurotransmitters is mediated by uptake through transporters and their metabolism through the glutamine – glutamate – GABA cycle (Eid et al., 2016). Glutamate and GABA reuptake relies on specific transporters expressed by astrocytes: EAAT1 and EAAT2 (also known as GLAST and GLT1) for glutamate, and GAT1 and GAT3 for GABA. Once internalized in astrocytes, glutamate is converted into glutamine by glutamine synthetase (GS), while GABA enters the Krebs cycle which produces

alpha ketoglutarate, glutamate and glutamine. Glutamine produced in astrocytes is then released, taken up by neurons and used as a precursor for both glutamate and GABA synthesis.

Adenosine kinase (ADK) is another important astrocytic enzyme involved in E/I balance. By phosphorylating adenosine to adenosine-monophosphate (AMP) it exerts a key role in regulating extracellular levels of adenosine (Studer et al., 2006; Etherington et al., 2009), adenosine being known to dampen neuronal activity and its release to exert anti-convulsant effects (Lado and Moshé, 2008; Cunha, 2016).

Gliotransmission

The concept of the “tripartite synapse” emerged from experiments demonstrating that astrocytes are able to sense neurotransmitters released into the synaptic cleft by presynaptic neurons but also can release gliotransmitters that regulate neuronal and synaptic activities (Auld and Robitaille, 2003; Perea et al., 2009).

Several classical neurotransmitters have been also identified as gliotransmitters, among which glutamate, D-serine, ATP, adenosine and GABA have been the most studied for their modulation of neuronal excitability and of the activity of excitatory and inhibitory synapses (Angulo et al., 2008; Araque et al., 2014; Harada et al., 2016; Petrelli and Bezzi, 2016; Papouin et al., 2017). An increase in intracellular Ca^{2+} is often crucial for the release of gliotransmitters (Araque et al., 2014; Rusakov et al., 2014; Volterra et al., 2014; Khakh and Sofroniew, 2015; Bazargani and Attwell, 2016) that can be mediated through exocytotic or non-exocytotic pathways (Hamilton and Attwell, 2010; Verkhratsky et al., 2016). Yet, gliotransmitters can be released also through Ca^{2+} independent mechanisms involving connexin hemichannels or pannexin (PANX) channels (Abudara et al., 2018), exchangers such as the cysteine-glutamate antiporter, or reversal of neurotransmitter transporters (Malarkey and Parpura, 2008). It should be noted, however, that *in vivo* studies on gliotransmission are still sparse and that the mechanisms and the functional consequences of gliotransmission are still matters of debates and controversies. These issues have been discussed recently in several reviews (Araque et al., 2014; Rusakov et al., 2014; Khakh and Sofroniew, 2015; Bazargani and Attwell, 2016; Panatier and Robitaille, 2016;

Papouin et al., 2017; Dallérac et al., 2018; Fiacco and McCarthy, 2018; Savtchouk and Volterra, 2018).

Interactions with blood vessels

Another important aspect of astrocyte function that is affected by gliosis is their close interaction with brain vasculature. Astrocytes contact blood vessels with their endfeet and in physiological conditions contribute to the maintenance of the blood-brain barrier (BBB) structure by increasing the expression of tight junction proteins occludin and claudin-5 and by inhibiting the expression of chemokines and leukocyte adhesion molecules, which would favor BBB disruption (Andreone et al., 2015; Zhao et al., 2015).

Astrocytes have been also proposed to control cerebral blood flow according to neuronal activity and contribute to power neurons by supplying energy substrate needed to sustain synaptic activity (Giaume et al., 2010; Pellerin and Magistretti, 2012; Nortley and Attwell, 2017; Nippert et al., 2018).

1.2. Microglia

Microglial cells are the resident macrophages of the CNS and unlike the other mononuclear macrophages associated with the CNS (i.e. perivascular, meningeal and choroid plexus macrophages) they are in direct contact with other cells and with synapses in the CNS parenchyma (Tremblay et al., 2011; Kettenmann et al., 2013; Schafer et al., 2013; Wu et al., 2013; Nayak et al., 2014; Prinz and Priller, 2014). In line with their immune cell identity, microglial cells have been primarily studied in pathological conditions and, until recently, our knowledge on these cells was mostly derived from analyses of CNS pathologies. It is now clear, however, that these immune cells also interact with their neighboring partners of the CNS parenchyma and fulfill specific functions in homeostatic conditions.

Never resting microglia

During the last 15 years, the development of new tools for functional analysis and of new animal models has completely changed our view of microglia functions in physiological conditions. In 2005, two *in vivo* studies using two-photon microscopy in anesthetized knock-in CX3CR1-eGFP mice reported that microglial cells in

physiological conditions are not resting, as previously thought, but are permanently extending and retracting their thin processes (Davalos et al., 2005; Nimmerjahn et al., 2005). This motility of microglial processes may help to detect more rapidly danger signals appearing in the parenchyma. These two articles actually showed that in response to laser lesions or to local applications of ATP, which is considered as an alarm signal (Rodrigues et al., 2015), microglial cells rapidly extend their processes toward the lesion or the source of ATP, which acts here as a chemoattractant. Although other signaling pathways may control the oriented motility of microglia processes, purinergic P2Y12 receptors and adenosine A2A or P2Y13 receptors are instrumental for rapid process extension and retraction, respectively, occurring right after an insult to the CNS parenchyma (Haynes et al., 2006; Orr et al., 2009; Milior et al., 2020).

Monitoring and modulating neuronal activity

Following these initial observations, other groups showed that during their surveillance of the parenchyma, which involves a two-pore domain K^+ channel (Madry et al., 2018), microglial cell processes contact synapses. The occurrence and duration of these contacts are regulated by neuronal activity and can influence the activity and the fate of the synapses (Wake et al., 2009; Tremblay et al., 2010; Li et al., 2012; Dissing-Olesen et al., 2014; Eyo et al., 2014; Pfeiffer et al., 2016; Sipe et al., 2016). Interestingly, microglial cells promote the formation of new spines in the cortex of animals engaged in a motor learning task (Parkhurst et al., 2013). Parallel studies also showed that mobilization of microglia specific pathways could lead to the modulation of synaptic activity. For instance the fractalkine signaling pathway regulates excitatory synaptic transmission and plasticity in the hippocampus (Rogers et al., 2011; Scianni et al., 2013), microglial Toll Like Receptor (TLR) 4 signaling controls glutamate release probability of presynaptic fibers indirectly through the mobilization of astrocytes (Pascual et al., 2012) and microglial P2Y12 receptors are necessary for synaptic plasticity during the critical period of ocular dominance (Sipe et al., 2016). A recent study also revealed the existence of contact between microglial processes and neuronal soma. These junctions, which can be rapidly induced by neuronal activation, are organized in specialized nanodomains enriched in Kv2.1 potassium channels, mitochondria and vesicular nucleotide transporter (Vnut) containing vesicles on the neuronal side and P2Y12 on the microglial side (Cserép et

al., 2020). These junctions could therefore inform microglia on the excitability and metabolic state of the neuron. Curiously, these junctions, which are observed in a very large proportion (90%) of neurons in different regions of the CNS, had not been noticed before. Altogether, these influential studies have led to the notion that microglial cells monitor but also regulate synaptic and neuronal activity in the healthy brain.

Microglia diversity

Heterogeneity of microglia in physiological conditions was first evidenced during CNS development when their phenotype is constantly evolving from amoeboid cells, when entering the CNS parenchyma at embryonic stages, to fully ramified cells at juvenile stages. Remarkably, at a given stage of development, microglial cells with different morphological and functional phenotypes are differentially distributed in different areas of the CNS. This reflects the dynamics of microglia colonization of the brain and their exceptional ability to adapt to their environment, which explains the tight coupling between their own maturation and the maturation of other components of the CNS (Mosser et al., 2017). This variety of phenotypes underlies a variety of microglia functions during development that have been reviewed recently (Mosser et al., 2017; Paolicelli and Ferretti, 2017; Thion et al., 2018). More recent studies have also revealed the existence of different microglia populations in different regions of the CNS (Sierra et al., 2010; Baalman et al., 2015; De Biase et al., 2017; Stowell et al., 2018). Interestingly, microglia repopulating the CNS after a transient depletion in adult mice regain the specific phenotype that they normally express in a given brain area (De Biase et al., 2017), indicating that at any given time microglial cells retain the ability to adapt their phenotype to local environmental cues.

2. Astrocytes and microglia reactivity

The role of astrocytes and microglia in pathologies of the CNS is widely associated with reactive phenotypes developed by these cells in response to a rupture in CNS homeostasis. The acquisition of these reactive phenotypes, called gliosis, is specific of each glial cell type but is also dependent on the nature, duration and location of the pathological triggers. Because glial cells have homeostatic

functions in non-pathological conditions (see above), profound changes of their phenotype upon appearance of pathological conditions have two consequences: i) the loss of their homeostatic influence and ii) the emergence of new functions associated with the acquisition of reactive, inflammatory phenotype.

2.1. Astrogliosis in epilepsy

Astrogliosis is particularly evident in epileptic syndromes associated with hippocampal sclerosis (HS) that is characterized by a glial scar (Thom, 2014) and this reactivity of astroctytes is commonly revealed by the up-regulation of GFAP expression. Astrogliosis, however, involves many more changes at the molecular, cellular and functional levels (Dossi et al., 2018b), and these changes can precedes the GFAP up-regulation (see below). The degree of alterations observed in reactive astrocytes varies according to the type of insult inducing the reaction and to the possibility of reversion of the damage (Sofroniew, 2009; Anderson et al., 2014). Transcriptomic analysis of reactive astrocytes has disclosed the occurrence of two broad phenotypes: a pro-inflammatory phenotype A1 having detrimental effects (Zamanian et al., 2012; Lian et al., 2015; Liddelow et al., 2017) and an immune-suppressive phenotype A2 with protective functions (Anderson et al., 2016; Liddelow and Barres, 2017). Interestingly, astrocyte A1 phenotype can be induced by the co-release of IL-1α, TNFα and C1q from reactive microglia (Liddelow et al., 2017). Although the specific phenotype of astrocytes in different forms of epilepsy has not yet been firmly established, different studies rather point toward a deleterious A1 phenotype. For example, in epilepsy models, the release of Interleukin-1beta (IL-1β) and High Mobility Group Box1 by astrocytes induces the activation of IL1R/TLR signaling pathways that favors the induction and maintenance of seizure activity (Maroso et al., 2010; Vezzani et al., 2011). On the other hand, mechanisms that oppose to this deleterious astrocyte phenotype have been described (Iyer et al., 2012; van Scheppingen et al., 2018; Korotkov et al., 2020) and transforming growth factor-beta (TGF-β) signaling, which is activated in astrocytes during epilepsy, reverts A1 phenotype *in vitro* (Liddelow et al., 2017). However, in most cases the population of astrocytes has been considered as a whole without taking into consideration a possible diversity of the astrocyte reactivity (*e.g.* lesion vs peri-lesional area).

A still debated question is whether astrocyte reactivity is a cause or a consequence of seizures. In favor of a causative role, astrocyte-specific deletion of genes (tuberous sclerosis complex 1 or Beta 1 integrin) or mutation of an astrocyte-specific gene (GFAP) induce astrogliosis and the occurrence of spontaneous seizures (Ortinski et al., 2010; Messing et al., 2012; Robel et al., 2015). On the other hand, there were no sign of astrogliosis in non-lesional epileptogenic cortical areas of patients with focal cortical dysplasia (Rossini et al., 2017). Moreover, in a guinea pig model of temporal lobe epilepsy with hippocampal sclerosis (HS-MTLE; unilateral, intra-hippocampal kainate), astrogliosis and cell damage were observed only in the injected, and not in the contralateral, hippocampus despite the occurrence of epileptiform activities in both hippocampi (Noè et al., 2019). The latter two examples suggest dissociation between the occurrence of seizure and astrogliosis, as defined with the classical marker GFAP. However, they do not exclude the contribution of astrocytes to the functional remodeling that occurs during epileptogenesis and to the generation of seizures, especially in view of the many functions of astrocytes by which they influence the excitability of neuronal networks.

Homeostasis of K^+ and water

Different studies, both in humans and animal models, have highlighted a clear association between uncontrolled extracellular K^+ increase and epilepsy (Steinhäuser et al., 2016). In particular, measurements of extracellular K^+, patch-clamp recordings of Kir currents in astrocytes, single-cell RT-PCR and immunostaining of Kir proteins revealed a reduction in Kir-mediated current and a reduction of Kir4.1 expression in surgical specimen from patients suffering from MTLE as well as in rodent models of MTLE (Bordey and Sontheimer, 1998; Heinemann et al., 2000; Hinterkeuser et al., 2000; Kivi et al., 2000; Schröder et al., 2000; Jauch et al., 2002; Heuser et al., 2012). GJ communication within the astrocyte syncytium contributes to K^+ homeostasis (see above) and is regulated by pro-inflammatory cytokines (IL-1β & TNFα) (Meme et al., 2006). Evidence suggests that alterations in astrocyte coupling could be involved in the genesis and progression of epilepsy. In a mouse model of HS-MTLE (unilateral intracortical kainate injection), it was demonstrated that astrocyte coupling is impaired as early as 4 hours after the induction of status epilepticus (SE), a mechanism probably related to the release of IL-1β and TNFα, and thus suggesting that impaired coupling represents a key event in epileptogenesis (Bedner et al., 2015). Moreover,

the authors showed a complete lack of GJ coupling in sclerotic hippocampi of HS-MTLE patients.

Mice lacking AQP4 have a mild ECS volume expansion, an elevated threshold but prolonged duration for electrographic seizures induced *in vivo* by hippocampal electrical stimulation (Binder and Steinhäuser, 2006; Binder et al., 2006). Subsequent analysis of these mice, however, indicated that they also displayed an enhanced gap junctional coupling and an improved spatial $[K^+]_0$ buffering (Strohschein et al., 2011). In human and in animal models of MTLE, there is a loss of astrocytic perivascular AQP4, which precedes the appearance of chronic seizures (Eid et al., 2005; Alvestad et al., 2013). This suggests that perturbed polarized expression of AQP4 during epileptogenesis, together with consequences on $[K^+]_0$ homeostasis, may be of pathophysiological relevance.

Neurotransmitter buffering

Alterations in neurotransmitter transporters are reported in different neurological diseases, including epilepsy (Coulter and Eid, 2012). The involvement of alterations in neurotransmitter homeostasis in epilepsy is demonstrated by an increase in extracellular glutamate levels in the sclerotic hippocampus of MTLE patients (Cavus et al., 2005, 2008). In experimental epilepsy, deletion of GLT1 in astrocytes enhanced responses to sub-convulsive doses of pentylenetetrazol (Tanaka et al., 1997).

Alterations in GABA extracellular levels can also contribute to hyper-excitability and epilepsy. Depending on the epileptic syndrome, both decreased extracellular GABA levels and increased GABA receptor mediated activity can be detrimental for the maintenance of E/I balance. On the one hand, an increase in the expression levels of GAT3 astrocytic GABA transporter in TLE, leading to a reduction of extracellular GABA levels, has been reported just before seizure onset (During and Spencer, 1993; Lee et al., 2006). On the other hand, in absence epilepsy a dysfunction of the astrocytic GABA transporter GAT1 was responsible for the generation of non-convulsive seizures by determining an increase in GABA-A receptor mediated inhibition in thalamocortical neurons (Cope et al., 2009; Pirttimaki et al., 2013).

Reduction in GS function is involved in the pathogenesis of epilepsy by inducing extracellular glutamate accumulation, as reported in TLE patients (Eid et al., 2004, 2016; van der Hel et al., 2005). Evidence for GS involvement in the pathogenesis of epilepsy is reported also in animal models in which blocking of GS function with the inhibitor methionine sulfoximine was able to induce recurrent seizures, neuronal loss and hippocampal atrophy (Eid et al., 2008; Wang et al., 2009). Considering that glutamine is fundamental also for GABA synthesis in interneurons, GS dysfunction could be related also to impaired inhibitory transmission, as shown in an experimental model of TLE in which the downregulation of GS in astrocytes was responsible for a compromised inhibitory synaptic transmission (Ortinski et al., 2010).

Astrogliosis occurring in epilepsy is known to be associated with increased levels of ADK both in humans and in experimental models (Boison, 2016). The increase in ADK and the consequent reduction of extracellular adenosine levels are responsible for reducing seizure threshold (Boison, 2016).

Altogether these data clearly highlight how crucial is neurotransmitter buffering in order to maintain a functional synaptic activity and avoid the generation of a seizure-prone context.

Gliotransmission

In vitro models of seizure showed that an excitatory loop involving astrocyte Ca^{2+} signaling, glutamate and ATP signaling lowers seizure threshold for neurons (Gomez-Gonzalo et al., 2010). Moreover, Ca^{2+} signals in astrocytes facilitates spread of epileptiform activity *in vivo* (Heuser et al., 2018) and increased astrocyte Ca^{2+} signaling and glutamate release contribute to neuronal cell death in the neocortex *in vivo* after a pilocarpine induced SE (Ding et al., 2007). Astrocyte glutamate release occurs in different forms, one of which generates in neurons slow inward currents (SICs) resulting from the activation of extra-synaptic NMDA receptors and allowing the synchronization of small ensemble of neurons (Parri et al., 2001; Angulo et al., 2004; Fellin et al., 2004). It has been proposed that SICs generated by astrocytes could be at the origin of hypersynchronous excitation of neurons in models of epilepsy (Tian et al., 2005) but further pharmacological experiments clearly showed a dissociation between ictal events and SICs (Fellin et al., 2006).

ATP is an essential mediator of communication between astrocytes and other types brain cells (Butt, 2011). Expression of a dominant-negative SNARE that impairs the vesicular release of ATP from astrocytes (Pascual et al., 2005) delays the onset of recurrent seizures, decrease epileptiform activity and reduce hippocampal damage after a pilocarpine-induced SE (Clasadonte et al., 2013). Release of ATP through the other route constituted by pannexin 1 (PANX1) hemi-channels appears to contribute to ictal discharges in human epileptic brain tissue and in mouse models of MTLE (intra-hippocampal kainate) or SE (systemic kainate) (Santiago et al., 2011; Dossi et al., 2018a). In a rat model of TLE (rapid kindling), activation of P2Y1 receptors induces astrocyte Ca^{2+} hyperactivity that is responsible for an increase in glutamate release from astrocytes. This glutamate release activates neuronal presynaptic mGluR5, which increases the release probability at the CA3-CA1 synapses. Blocking astrocyte Ca^{2+} signalling or inhibiting P2Y1 or mGluR5 receptors relieved this abnormal enhancement of synaptic strength (Álvarez-Ferradas et al., 2015). Similarly, in a mouse model of TLE (unilateral intracortical kainate injection), enhanced astrocyte P2Y1 signalling increased astrocyte glutamate release. However, in the dentate gyrus this glutamate release activated neuronal presynaptic NMDA receptors that enhanced excitatory synaptic transmission in granule cells of the dentate gyrus (Nikolic et al., 2018). Remarkably, this enhanced P2Y1 signalling resulted from a constitutive activation of an autocrine loop involving astrocyte ATP release onto P2Y1 receptors (Shen et al., 2017). This autocrine loop, which can be triggered by TNFα, was up-regulated before the initiation of the chronic stage of this TLE model (Nikolic et al., 2018). It should be remembered, however, that ATP released from astrocytes also activates P2Y1 expressed by inhibitory interneurons (Khakh et al., 2003; Tan et al., 2017) and that its degradation into adenosine inhibits the activity of excitatory pyramidal cells through A1 receptor activation (Shen et al., 2017; Tan et al., 2017).

Altogether, the majority of the above cited observations point toward the conclusion that the actions of astrocyte purinergic signalling determined by the combined effects of ATP, adenosine and glutamate can displace the equilibrium between excitation and inhibition and promote appearance of epileptiform activity. It is important to note that P2Y1 regulation of astrocyte Ca^{2+} signalling described in the context of epilepsy has been also reported in a mouse model of Alzheimer's disease (Delekate et al.,

2014; Reichenbach et al., 2018) and in a model of traumatic brain injury (Choo et al., 2013). In both cases, blocking this pathway had beneficial consequences on outcome of these models. Impaired astrocyte purinergic signalling could thus be a common signature of different CNS pathologies. Yet, additional *in vivo* experiments are needed to evaluate its weight on TLE progression.

Interactions with blood vessels

Modifications of astrocyte phenotype during astrogliosis can contribute to BBB impairment that occurs in epilepsy through disruption of tight-junction coupling, increased expression of adhesion molecules on endothelial cells determining an increase of pro-inflammatory cytokines and the extravasation of leukocytes in brain parenchyma (Marchi et al., 2014; van Vliet et al., 2015). In line with the idea that astrocytes contribute to perturbed BBB integrity is the observation of enhanced Ca^{2+} signaling in astrocyte endfeet during the latent phase of a mouse model of TLE (unilateral intra-cortical kainate; (Szokol et al., 2015)). This reactivity of astrocyte endfeet may be driven by the TNFα-induced endothelin-1 release that occurs after pilocarpine-triggered SE in rats which promotes the production of reactive oxygen species in astrocytes via endothelin receptor-B activation (Kim et al., 2013). Furthermore, BBB disruption in epilepsy leads to extravasation of serum albumin, which activates TGF-β in astrocytes (Heinemann et al., 2012). TGF-β signaling activation in astrocytes then leads to a series of modifications, including impaired K+, water and glutamate buffering capacity (Seiffert et al., 2004), increased excitatory synaptogenesis (Weissberg et al., 2015) and degradation of perineuronal net around inhibitory interneurons (Kim et al., 2017), which may promote hyperexcitability (Terrone et al., 2019). Interestingly, reactive astrocytes are associated with microglia-pericytes clusters along leaky capillaries in human and experimental epilepsies and may contribute to the formation of a perivascular scar at loci of impaired blood-brain barrier (Klement et al., 2018, 2019).

2.2. Microgliosis in epilepsy

Microglial cells are involved in a large number of CNS diseases and very often they are mobilized at early stages of these diseases. This mobilization leads to changes of their phenotype, which allows microglia to react and adapt in a specific manner to the new environment (Hanisch and Kettenmann, 2007; Ransohoff and

Perry, 2009; Shemer et al., 2015). Microglia reactivity was first described as a morphological change: from a small soma with long and thin ramified processes in physiological conditions, they adopt a less ramified morphology with fewer and thicker processes in pathological conditions, which can eventually lead to an amoeboid morphology, without process, in the most severe cases. We know now that these morphological changes are accompanied by important functional modifications including changes in gene expression and motility properties, increased proliferation, phagocytic activity and release of numerous pro- and anti-inflammatory mediators (Hanisch and Kettenmann, 2007; Ransohoff and Perry, 2009; Kierdorf and Prinz, 2013). Yet, microglia activation is not an all-or-none process; it is progressive, depends on the actual pathological conditions and on the context in which the disease appears. There is therefore not a single reactive state but a diversity of phenotypes that are determined by a fine detection of environmental cues and that allows microglial cells to perform specific functions in different pathological conditions. Although microglial morphological alteration is a convenient mean to assess reactivity, it does not reflect the complexity of the functional remodeling of microglia associated to specific pathological context. This is best exemplified for P2Y12 receptors whose expression is either up or down regulated in morphological similar reactive microglia but in different pathological context, status epilepticus or neuropathic pain, respectively (Haynes et al., 2006; Avignone et al., 2008; Tozaki-Saitoh et al., 2008). The diversity of functional remodeling of reactive microglia was further demonstrated by transcriptional analyzes performed in various pathological states. These studies firmly established that in a given pathology microglial reactivity constantly evolves, that there is a diversity of microglial reactive states at a given time of the disease, and that the transcriptional profile adopted by microglia is disease specific (Hirbec et al., 2019). Nevertheless, there is a core of genes that are commonly deregulated across different pathological states and may serve as unambiguous makers of microglial reactivity (Hirbec et al., 2018).

Microglial inflammatory signaling pathways in epilepsy

Recently, human microglial cells of patients suffering from MTLE and who underwent surgery were analyzed in depth, uncovering specific phenotypes in regions of sclerosis (CA1, CA3) versus areas with less neuronal damage (Morin-Brureau et al., 2018). These observations suggested that the repairing interleukin IL-

10 regulates the microglial phenotype in these sclerotic regions. Moreover, this study also provides evidence for an early and transient expression of several cytokines, including CXCL8, IL-1β, after each spontaneous seizure (Morin-Brureau et al., 2018).

Increased expression and production of inflammatory cytokines in the hippocampus is well documented in experimental models of TLE and occurs rapidly following SE (Vezzani et al., 2019). These cytokines can be produced by different cell types, including neurons, astrocytes and microglia. However, in a mouse model of TLE induced by pilocarpine, microglial cells were shown to up-regulate transiently pro-inflammatory cytokines IL-1β, IL-6 and TNFα mRNAs 3 days after SE (Benson et al., 2015). Interestingly, at this stage anti-inflammatory markers arginase-1, chitinase-3-like protein (Ym1), IL-4, but not IL-10, were also up-regulated by microglia (Benson et al., 2015). Because many of these cytokines are known to modulate neuronal excitability and astrocyte signaling, these observations suggests that microglial inflammatory mediators have a complex role during epileptogenesis. Surprisingly, none of these genes were found to be dysregulated in microglia 3 days post-SE in a model of intra-cerebroventricular injection of kainate (Bosco et al., 2018). These conflicting observations are likely due to differences between the two models and to methodological variations (RNASeq of purified microglia versus qPCR of hippocampal tissue).

Toll-like receptor (TLR) signaling could contribute to the production of cytokines and other mediators by microglial cells after SE. Activation of TLR3 and TLR4 receptors *in vitro* induces the production of inflammatory cytokines by microglia (Olson and Miller, 2004). TLR3 deficiency reduces microglia reactivity, the level of proinflammatory cytokines TNFα and interferon β as well as spontaneous recurrent seizures (Gross et al., 2017). Antagonizing TLR4 reduces acute and chronic seizure recurrence in a kainate model of TLE (Maroso et al., 2010). This effect of TLR4 could involve a signaling pathway including microglia, astrocytes and neurons. Pascual and co-workers showed that activation of microglial TLR4 induces the release of ATP by microglia, which activates astrocyte P2Y1 receptors, promoting the release of glutamate by astrocytes that in turn increases excitatory synaptic transmission in CA1 (Pascual et al., 2012).

Stromal cell-derived factor-1 (SDF-1 or CXCL12) and its receptor CXCR4 also seem to play a role in TLE. Rats treated with a CXCR4 antagonist decreased spontaneous seizures and aberrant adult neurogenesis in a kainate model (Song et al., 2016). Interestingly, CXCR4 is upregulated in hippocampal microglia 3 days post kainate-induced SE (Bosco et al., 2018). Again, some of these effects could involve both microglia and astrocytes as SDF-1 has been shown to trigger the release of TNFα by microglia that stimulates astrocyte glutamate release (Bezzi et al., 2001).

Fractalkine (CX3CL1) is a neuronal chemokine that exclusively signals to microglia, which are the only CNS cells expressing the fractalkine receptor CX3CR1. This signaling pathway is thought to act as an Off signal that opposes microglial reactivity (Cardona et al., 2006; Biber et al., 2007; Limatola and Ransohoff, 2014). Increased expression of fractalkine has been reported in the hippocampus and temporal neocortex of TLE patients and in a pilocarpine rat model (Xu et al., 2012). Fractalkine application was shown to restore inhibitory GABAergic currents in excitatory neurons in slices of TLE patients (Roseti et al., 2013). Moreover, fractalkine signaling favors microglial process convergence toward neuronal axons and dendrites that increases after pilocarpine or kainate induced SE, and CX3CR1 deficiency worsens seizure phenotype (Eyo et al., 2016). Interestingly, deficiency of P2Y12 receptors, which drive microglial process motility, also worsens kainate-induced seizure behaviors (Eyo et al., 2014).

Accumulating evidence favors a role of microglial purinergic receptors in experimental models of seizure. The transcripts of the 4 main microglial purinergic receptors, *i.e*. P2X4, P2X7, P2Y6 and P2Y12, are upregulated in the hippocampus in a mouse model of kainate-induced SE. The membrane currents induced by the activation of P2X7, P2Y6, P2Y12 and the microglial process motility toward an agonist of P2Y12 are increased after SE (Avignone et al., 2008). In the same model, P2X4 proteins are upregulated in reactive microglia and P2X4 deficiency reduces several aspects of microglial reactivity, the upregulation of several pro-inflammatory mediators and the neuronal death induced in the hippocampus by SE (Ulmann et al., 2013). However, while microglial P2X4 receptor has been involved in BDNF-mediated disinhibition and generation of local hyperexcitability in the spinal cord (Beggs et al., 2012), this receptor is also upregulated in neurons and it is still not

known whether P2X4 expressed by neurons, microglia or both contribute to inflammatory state post SE.

P2X7 receptors are prominently expressed in different immune cells, are one of the main trigger of IL1-ß processing and contribute to TNF-α production. In the brain, P2X7 is expressed in homeostatic microglia but its expression increases after SE. For these reasons the potential involvement of P2X7 in epilepsy has been early investigated in different animal models using either pharmacological or genetic approaches to modulate the receptor activity. However, opposite results were reported depending on the way SE was triggered. In the model of pilocarpine-evoked SE, inhibition of P2X7 activity using either specific antagonists or P2X7-deficient mice increases seizure-susceptibility after pilocarpine injection, an effect that may be linked to PANX1 since siRNA-mediated down regulation of PANX1 also reduced the threshold of seizure apparition (Kim and Kang, 2011). On the contrary, in a model of intra-amygdala injection of KA, P2X7 activity seems to be pro-convulsive since P2X7-deficient mice show reduced electrographic seizure during SE and pre-treatment with P2X7 antagonists reduced behavioral seizures, neuronal cell death and IL1-ß increase in the hippocampus. In this model, microRNA-22 repress the expression of P2X7 and thus has anti-convulsive effects (Jimenez-Mateos et al., 2015). Whether this regulation is also present in human is unknown. The reasons for these opposite results are not elucidated. In both studies the same pharmacological agents and the same strain of P2X7-deficient mice were used. However the two experimental models of SE present notable differences and it is possible that in the peripheral administration of pilocarpine might trigger mechanisms which are not present in the alternate model. Importantly, adhesion of circulating leukocytes, which express P2X7, on endothelial cerebral blood vessels cells is thought to be an initial and mandatory step of SE induction in the pilocarpine model (Fabene et al., 2008). This adhesion might depend on P2X7 activity and therefore impaired by P2X7 antagonists. Recent studies using P2X7 antagonist that crosses the BBB showed in rats that administration of this molecule three months after systemic kainate injections decreased the severity of spontaneous recurrent seizures (Amhaoul et al., 2016). Although the seizure frequency remained unaltered, this study further strength the idea that blocking P2X7 may have beneficial effects in regulating seizure during the acute but also the chronic phases of the disease.

While rodent studies support that P2X7 may represent a potential therapeutic target to reduce the burden of seizures, experimental data in human are still lacking. Particularly, all clinical trials testing the effectiveness of P2X7 antagonists in rheumatoid arthritis have failed to demonstrate any beneficial effect (Keystone et al., 2012), despite the clear demonstration of the involvement of this receptor in animal models of the disease. Nevertheless, in human specimen from patients suffering with Focal Cortical Dysplasia (FCD), P2X7 mRNA and protein expression was increased compared to control samples. This was accompanied with an increased immunoreactivity against IL1-ß in microglia but also in dysmorphic neurons and balloon cells, which are characteristic cells of FCD (Wei et al., 2016). Other studies also reported an increase of P2X7 expression in the brain of epileptic patients, however because the specificity of the antibodies are questionable, these results have to be interpreted with caution.

Complex contribution of microglia in epilepsy

The above described studies indicate that targeting different signaling pathways dysregulated (mostly upregulated) in microglia can have either pro- or anti-convulsive effects. There are certainly concerns regarding the cell-type specificity of some of these manipulations but if we consider the most microglia-specific receptors that have been studied, *i.e*. CX3CR1 and P2Y12, it is interesting to note that microglial cells seem to have anti-convulsive actions. This is in agreement with different studies supporting the idea that, in pathological conditions, homeostatic interactions between microglia and neurons have rather an inhibiting effect on neuronal activities. This was initially described in the Zebrafish embryo where microglial processes are attracted by active neurons, in an ATP-dependent manner, and the resulting contacts lead to a reduction of both spontaneous and evoked neuronal activities (Li et al., 2012). Another study reports dynamic interactions of microglial processes with the axonal initial segment in conditions of neuronal hyperexcitability leading to a reversal of the depolarization (Kato et al., 2016). Interestingly, recent experiments of microglia depletion in mouse models of epilepsy also support such a calming effect of microglia on neuronal activity. Using 3 different genetic strategies to deplete microglia, Wu and collaborators showed that the absence of microglia aggravates both acute and chronic seizures in kainate mouse models of SE or TLE (Wu et al., 2020). In another study on seizure progression in a

mouse model of viral encephalitis, pharmacological depletion of microglia using the antagonist of the Csf1 receptor PLX5622, maximum seizure occurrence was reached more rapidly in microglia depleted mice but the overall seizure incidence was not affected (Waltl et al., 2018). Depletion of microglia with PLX5622 was also shown to induce seizures in a mouse model of low level picornavirus infection of the CNS (Sanchez et al., 2019). In contrast, the use of another Csf1 receptor antagonist, PLX3397, had anti-epileptic effects in mouse models of pilocarpine or intra-hippocampal kainate (Srivastava et al., 2018). However, that study used a much lower concentration of PLX3397 than the one required to deplete microglia (Elmore et al., 2014) and the authors indeed showed that microglia were not depleted (Srivastava et al., 2018). These observations suggest that inhibition of microglial Csf1 receptors have anti-seizure effects whereas microglial depletion worsens seizures.

The signaling pathway through which microglia cells inhibit active neurons in physiological or epileptic conditions remains to be identified. Converging evidence, however, indicates that P2Y12 receptors are central in this calming effect. Indeed, pharmacological or genetic inhibition of P2Y12 receptors result in aggravated neuronal death following transient ischemia (Cserép et al., 2020) or worsened seizure (Eyo et al., 2014; Mo et al., 2019). While P2Y12 expression can be up regulated in specific reactive states associated with neuropathic pain (Inoue and Tsuda, 2012) or shortly after SE (Avignone et al., 2008), sustained microglia reactivity is often associated with total repression of P2Y12 expression (Hirbec et al., 2019). Such a switch in the expression pattern of P2Y12 may contribute to the opposite effects of microglia during seizure progression but many other pathways may also be involved. Interestingly, elevating mTOR signaling in mouse microglia induces a microglial reactive state without induction of pro-inflammatory cytokines and leads to the appearance of spontaneous recurrent seizures (Zhao et al., 2018). This study suggests that the loss of homeostatic functions of microglia may be more important than the acquisition of an inflammatory phenotype in promoting the appearance of seizures. However, the genetic strategy used in that study does not allow excluding the possibility that border-associated macrophages residing in the meninges, the choroid plexus, and the perivascular spaces or even some CX3CR1 expressing monocytes contribute to the observed effect.

Finally, the spatio-temporal dynamics of microglial reactivity within different parts of the epileptic hippocampus (*e.g.* sclerotic vs non-sclerotic regions) generate a diversity of microglial phenotypes that need to be characterized to understand the complex roles of these immune cells in regulating the activity and fate of neurons and other brain cells during epileptogenesis and at a chronic stage of the disease.

Concluding remarks

In the CNS glial cells have homeostatic functions that support and also finely tune neuronal and synaptic activities. Yet, in pathological conditions where homeostasis is durably lost, glial cells become reactive. These reactive states evolve along disease conditions and can be deleterious or protective. In the context of epilepsy, glial reactivity occurs only in brain areas experiencing seizures and increasing evidence indicates that this reactivity contributes to disease progression. Major challenges in the field aim at identifying the triggers, the time course and evolution of glial reactivity. However, better characterizing the consequences of the loss of glial homeostatic functions is certainly of equal importance. The urgent need of new therapeutic strategies will benefit from a better understanding of the signaling pathways through which glial cells regulate or dysregulate other brain cells and thereby contribute to the progression of different forms of epilepsy.

References

Abudara V, Retamal MA, Del Rio R, Orellana JA (2018) Synaptic Functions of Hemichannels and Pannexons: A Double-Edged Sword. Front Mol Neurosci 11.

Allen NJ, Lyons DA (2018) Glia as architects of central nervous system formation and function. Science (80-) 362:181–185.

Álvarez-Ferradas C, Morales JC, Wellmann M, Nualart F, Roncagliolo M, Fuenzalida M, Bonansco C (2015) Enhanced astroglial Ca2+ signaling increases excitatory synaptic strength in the epileptic brain. Glia 63:1507–1521.

Alvestad S, Hammer J, Hoddevik EH, Skare Ø, Sonnewald U, Amiry-Moghaddam M, Ottersen OP (2013) Mislocalization of AQP4 precedes chronic seizures in the kainate model of temporal lobe epilepsy. Epilepsy Res 105:30–41.

Amhaoul H, Ali I, Mola M, Van Eetveldt A, Szewczyk K, Missault S, Bielen K, Kumar-Singh S, Rech J, Lord B, Ceusters M, Bhattacharya A, Dedeurwaerdere S (2016) P2X7 receptor antagonism reduces the severity of spontaneous seizures in a chronic model of temporal lobe epilepsy. Neuropharmacology 105:175–185.

Anderson MA, Ao Y, Sofroniew M V. (2014) Heterogeneity of reactive astrocytes. Neurosci Lett 565:23–29.

Anderson MA, Burda JE, Ren Y, Ao Y, O'Shea TM, Kawaguchi R, Coppola G, Khakh BS, Deming TJ, Sofroniew M V. (2016) Astrocyte scar formation aids central nervous system axon regeneration. Nature 532:195–200.

Andreone BJ, Lacoste B, Gu C (2015) Neuronal and Vascular Interactions. Annu Rev Neurosci 38:25–46.

Angulo MC, Kozlov AS, Charpak S, Audinat E (2004) Glutamate released from glial cells synchronizes neuronal activity in the hippocampus. J Neurosci 24:6920–6927.

Angulo MC, Le Meur K, Kozlov AS, Charpak S, Audinat E (2008) GABA, a forgotten gliotransmitter. Prog Neurobiol 86:297–303.

Araque A, Carmignoto G, Haydon PG, Oliet SH, Robitaille R, Volterra A (2014)

Gliotransmitters travel in time and space. Neuron 81:728–739.

Attwell D, Buchan AM, Charpak S, Lauritzen M, Macvicar BA, Newman EA (2010) Glial and neuronal control of brain blood flow. Nature 468:232–243.

Auld DS, Robitaille R (2003) Glial cells and neurotransmission: an inclusive view of synaptic function. Neuron 40:389–400.

Avignone E, Ulmann L, Levavasseur F, Rassendren F, Audinat E (2008) Status Epilepticus Induces a Particular Microglial Activation State Characterized by Enhanced Purinergic Signaling. J Neurosci 28:9133–9144.

Baalman K, Marin MA, Ho TS-Y, Godoy M, Cherian L, Robertson C, Rasband MN (2015) Axon initial segment-associated microglia. J Neurosci 35:2283–2292.

Bauer J, Becker AJ, Elyaman W, Peltola J, Rüegg S, Titulaer MJ, Varley JA, Beghi E (2017) Innate and adaptive immunity in human epilepsies. Epilepsia 58:57–68.

Bazargani N, Attwell D (2016) Astrocyte calcium signaling: the third wave. Nat Neurosci 19:182–189.

Bedner P, Dupper A, Huttmann K, Muller J, Herde MK, Dublin P, Deshpande T, Schramm J, Haussler U, Haas CA, Henneberger C, Theis M, Steinhauser C (2015) Astrocyte uncoupling as a cause of human temporal lobe epilepsy. Brain 138:1208–1222.

Beggs S, Trang T, Salter MW (2012) P2X4R+ microglia drive neuropathic pain. Nat Neurosci 15:1068–1073.

Benson MJ, Manzanero S, Borges K (2015) Complex alterations in microglial M1/M2 markers during the development of epilepsy in two mouse models. Epilepsia 56:895–905.

Bezzi P, Domercq M, Brambilla L, Galli R, Schols D, De Clercq E, Vescovi A, Bagetta G, Kollias G, Meldolesi J, Volterra A (2001) CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by microglia triggers neurotoxicity. NatNeurosci 4:702–710.

Biber K, Neumann H, Inoue K, Boddeke HW (2007) Neuronal “On” and “Off” signals

control microglia. Trends Neurosci 30:596–602.

Binder DK, Steinhäuser C (2006) Functional changes in astroglial cells in epilepsy. Glia 54:358–368.

Binder DK, Yao X, Zador Z, Sick TJ, Verkman AS, Manley GT (2006) Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4 water channels. Glia 53:631–636.

Boison D (2016) Adenosinergic signaling in epilepsy. Neuropharmacology 104:131–139.

Bordey A, Sontheimer H (1998) Properties of human glial cells associated with epileptic seizure foci. Epilepsy Res 32:286–303.

Bosco DB, Zheng J, Xu Z, Peng J, Eyo UB, Tang K, Yan C, Huang J, Feng L, Wu G, Richardson JR, Wang H, Wu LJ (2018) RNAseq analysis of hippocampal microglia after kainic acid-induced seizures. Mol Brain 11:34.

Butt AM (2011) ATP: A ubiquitous gliotransmitter integrating neuron–glial networks. Semin Cell Dev Biol 22:205–213.

Cardona AE, Pioro EP, Sasse ME, Kostenko V, Cardona SM, Dijkstra IM, Huang D, Kidd G, Dombrowski S, Dutta R, Lee JC, Cook DN, Jung S, Lira SA, Littman DR, Ransohoff RM (2006) Control of microglial neurotoxicity by the fractalkine receptor. NatNeurosci 9:917–924.

Cavus I, Kasoff WS, Cassaday MP, Jacob R, Gueorguieva R, Sherwin RS, Krystal JH, Spencer DD, Abi-Saab WM (2005) Extracellular metabolites in the cortex and hippocampus of epileptic patients. Ann Neurol 57:226–235.

Cavus I, Pan JW, Hetherington HP, Abi-Saab W, Zaveri HP, Vives KP, Krystal JH, Spencer SS, Spencer DD (2008) Decreased hippocampal volume on MRI is associated with increased extracellular glutamate in epilepsy patients. Epilepsia 49:1358–1366.

Chever O, Djukic B, McCarthy KD, Amzica F (2010) Implication of Kir4.1 channel in excess potassium clearance: an in vivo study on anesthetized glial-conditional Kir4.1 knock-out mice. J Neurosci 30:15769–15777.

Choi DW (1988) Glutamate neurotoxicity and diseases of the nervous system. Neuron 1:623–634.

Choo AM, Miller WJ, Chen Y-C, Nibley P, Patel TP, Goletiani C, Morrison B, Kutzing MK, Firestein BL, Sul J-Y, Haydon PG, Meaney DF (2013) Antagonism of purinergic signalling improves recovery from traumatic brain injury. Brain 136:65–80.

Clasadonte J, Dong J, Hines DJ, Haydon PG (2013) Astrocyte control of synaptic NMDA receptors contributes to the progressive development of temporal lobe epilepsy. Proc Natl Acad Sci U S A 110:17540–17545.

Cope DW, Di GG, Fyson SJ, Orban G, Errington AC, Lorincz ML, Gould TM, Carter DA, Crunelli V (2009) Enhanced tonic GABAA inhibition in typical absence epilepsy. NatMed 15:1392–1398.

Coulter DA, Eid T (2012) Astrocytic regulation of glutamate homeostasis in epilepsy. Glia 60:1215–1226.

Cresto N, Pillet L-E, Billuart P, Rouach N (2019) Do Astrocytes Play a Role in Intellectual Disabilities? Trends Neurosci 42:518–527.

Cserép C et al. (2020) Microglia monitor and protect neuronal function through specialized somatic purinergic junctions. Science (80-) 367:528–537.

Cunha RA (2016) How does adenosine control neuronal dysfunction and neurodegeneration? J Neurochem 139:1019–1055.

D'Ambrosio R, Gordon DS, Winn HR (2002) Differential role of KIR channel and Na(+)/K(+)-pump in the regulation of extracellular K(+) in rat hippocampus. J Neurophysiol 87:87–102.

Dallérac G, Rouach N (2016) Astrocytes as new targets to improve cognitive functions. Prog Neurobiol 144:48–67.

Dallérac G, Zapata J, Rouach N (2018) Versatile control of synaptic circuits by astrocytes: where, when and how? Nat Rev Neurosci 19:729–743.

Davalos D, Grutzendler J, Yang G, Kim J V, Zuo Y, Jung S, Littman DR, Dustin ML,

Gan WB (2005) ATP mediates rapid microglial response to local brain injury in vivo. NatNeurosci 8:752–758.

De Biase LM, Schuebel KE, Fusfeld ZH, Jair K, Hawes IA, Cimbro R, Zhang HY, Liu QR, Shen H, Xi ZX, Goldman D, Bonci A (2017) Local Cues Establish and Maintain Region-Specific Phenotypes of Basal Ganglia Microglia. Neuron 95:341-356.e6.

Delekate A, Füchtemeier M, Schumacher T, Ulbrich C, Foddis M, Petzold GC (2014) Metabotropic P2Y1 receptor signalling mediates astrocytic hyperactivity in vivo in an Alzheimer's disease mouse model. Nat Commun 5:5422.

Ding S, Fellin T, Zhu Y, Lee SY, Auberson YP, Meaney DF, Coulter DA, Carmignoto G, Haydon PG (2007) Enhanced astrocytic Ca2+ signals contribute to neuronal excitotoxicity after status epilepticus. J Neurosci 27:10674–10684.

Dissing-Olesen L, LeDue JM, Rungta RL, Hefendehl JK, Choi HB, MacVicar BA (2014) Activation of neuronal NMDA receptors triggers transient ATP-mediated microglial process outgrowth. J Neurosci 34:10511–10527.

Djukic B, Casper KB, Philpot BD, Chin LS, McCarthy KD (2007) Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. J Neurosci 27:11354–11365.

Dossi E, Blauwblomme T, Moulard J, Chever O, Vasile F, Guinard E, Le Bert M, Couillin I, Pallud J, Capelle L, Huberfeld G, Rouach N (2018a) Pannexin-1 channels contribute to seizure generation in human epileptic brain tissue and in a mouse model of epilepsy. Sci Transl Med 10:eaar3796.

Dossi E, Vasile F, Rouach N (2018b) Human astrocytes in the diseased brain. Brain Res Bull 136:139–156.

During MJ, Spencer DD (1993) Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet 341:1607–1610 Available at: http://www.ncbi.nlm.nih.gov/pubmed/8099987.

Eid T, Ghosh A, Wang Y, Beckstrom H, Zaveri HP, Lee T-SW, Lai JCK, Malthankar-

Phatak GH, de Lanerolle NC (2008) Recurrent seizures and brain pathology after inhibition of glutamine synthetase in the hippocampus in rats. Brain 131:2061–2070.

Eid T, Gruenbaum SE, Dhaher R, Lee T-SW, Zhou Y, Danbolt NC (2016) The Glutamate–Glutamine Cycle in Epilepsy. In: Advances in neurobiology, pp 351–400.

Eid T, Lee T-SW, Thomas MJ, Amiry-Moghaddam M, Bjørnsen LP, Spencer DD, Agre P, Ottersen OP, de Lanerolle NC (2005) Loss of perivascular aquaporin 4 may underlie deficient water and K+ homeostasis in the human epileptogenic hippocampus. Proc Natl Acad Sci U S A 102:1193–1198.

Eid T, Thomas MJ, Spencer DD, Rundén-Pran E, Lai JCK, Malthankar G V, Kim JH, Danbolt NC, Ottersen OP, de Lanerolle NC (2004) Loss of glutamine synthetase in the human epileptogenic hippocampus: possible mechanism for raised extracellular glutamate in mesial temporal lobe epilepsy. Lancet (London, England) 363:28–37.

Elmore MR, Najafi AR, Koike MA, Dagher NN, Spangenberg EE, Rice RA, Kitazawa M, Matusow B, Nguyen H, West BL, Green KN (2014) Colony-stimulating factor 1 receptor signaling is necessary for microglia viability, unmasking a microglia progenitor cell in the adult brain. Neuron 82:380–397.

Etherington L-A V., Patterson GE, Meechan L, Boison D, Irving AJ, Dale N, Frenguelli BG (2009) Astrocytic adenosine kinase regulates basal synaptic adenosine levels and seizure activity but not activity-dependent adenosine release in the hippocampus. Neuropharmacology 56:429–437.

Eyo UB, Peng J, Murugan M, Mo M, Lalani A, Xie P, Xu P, Margolis DJ, Wu LJ (2016) Regulation of physical microglia–neuron interactions by fractalkine signaling after status epilepticus. eNeuro 3.

Eyo UB, Peng JY, Swiatkowski P, Mukherjee A, Bispo A, Wu LJ (2014) Neuronal hyperactivity recruits microglial processes via neuronal NMDA receptors and microglial P2Y12 receptors after status epilepticus. J Neurosci 34:10528–10540.

Fabene PF et al. (2008) A role for leukocyte-endothelial adhesion mechanisms in

epilepsy. NatMed 14:1377–1383.

Fellin T, Gomez-Gonzalo M, Gobbo S, Carmignoto G, Haydon PG (2006) Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. J Neurosci 26:9312–9322.

Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G (2004) Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. Neuron 43:729–743.

Fiacco TA, McCarthy KD (2018) Multiple Lines of Evidence Indicate That Gliotransmission Does Not Occur under Physiological Conditions. J Neurosci 38:3–13.

Giaume C, Koulakoff A, Roux L, Holcman D, Rouach N (2010) Astroglial networks: A step further in neuroglial and gliovascular interactions. Nat Rev Neurosci 11:87–99.

Gomez-Gonzalo M et al. (2010) An excitatory loop with astrocytes contributes to drive neurons to seizure threshold. PLoS Biol 8:e1000352.

Gorter JA, Van Vliet EA, Aronica E (2015) Status epilepticus, blood-brain barrier disruption, inflammation, and epileptogenesis. Epilepsy Behav.

Gross A, Benninger F, Madar R, Illouz T, Griffioen K, Steiner I, Offen D, Okun E (2017) Toll-like receptor 3 deficiency decreases epileptogenesis in a pilocarpine model of SE-induced epilepsy in mice. Epilepsia 58:586–596.

Halassa MM, Fellin T, Haydon PG (2007) The tripartite synapse: roles for gliotransmission in health and disease. Trends Mol Med 13:54–63.

Hamilton NB, Attwell D (2010) Do astrocytes really exocytose neurotransmitters? NatRevNeurosci 11:227–238.

Hanisch UK, Kettenmann H (2007) Microglia: active sensor and versatile effector cells in the normal and pathologic brain. NatNeurosci 10:1387–1394.

Harada K, Kamiya T, Tsuboi T (2016) Gliotransmitter Release from Astrocytes: Functional, Developmental, and Pathological Implications in the Brain. Front

Neurosci 9.

Haynes SE, Hollopeter G, Yang G, Kurpius D, Dailey ME, Gan WB, Julius D (2006) The P2Y12 receptor regulates microglial activation by extracellular nucleotides. NatNeurosci 9:1512–1519.

Heinemann U, Dieter Lux H (1977) Ceiling of stimulus induced rises in extracellular potassium concentration in the cerebral cortex of cat. Brain Res 120:231–249.

Heinemann U, Gabriel S, Jauch R, Schulze K, Kivi A, Eilers A, Kovacs R, Lehmann TN (2000) Alterations of glial cell function in temporal lobe epilepsy. Epilepsia 41 Suppl 6:S185-9.

Heinemann U, Kaufer D, Friedman A (2012) Blood-brain barrier dysfunction, TGFbeta signaling, and astrocyte dysfunction in epilepsy. Glia 60:1251–1257.

Heuser K, Eid T, Lauritzen F, Thoren AE, Vindedal GF, Taubøll E, Gjerstad L, Spencer DD, Ottersen OP, Nagelhus EA, Lanerolle NC de (2012) Loss of Perivascular Kir4.1 Potassium Channels in the Sclerotic Hippocampus of Patients With Mesial Temporal Lobe Epilepsy. J Neuropathol Exp Neurol 71:814–825.

Heuser K, Nome CG, Pettersen KH, Åbjørsbråten KS, Jensen V, Tang W, Sprengel R, Taubøll E, Nagelhus EA, Enger R (2018) Ca2+ Signals in Astrocytes Facilitate Spread of Epileptiform Activity. Cereb Cortex 28:4036–4048.

Hinterkeuser S, Schröder W, Hager G, Seifert G, Blümcke I, Elger CE, Schramm J, Steinhäuser C (2000) Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. Eur J Neurosci 12:2087–2096.

Hirbec H, Marmai C, Duroux-Richard I, Roubert C, Esclangon A, Croze S, Lachuer J, Peyroutou R, Rassendren F (2018) The microglial reaction signature revealed by RNAseq from individual mice. Glia 66(5):971-986.

Hirbec H, Rassendren F, Audinat E (2019) Microglia Reactivity: Heterogeneous Pathological Phenotypes. :41–55.

Inoue K, Tsuda M (2012) Purinergic systems, neuropathic pain and the role of

microglia. Exp Neurol 234:293–301.

Ising C, Heneka MT (2018) Functional and structural damage of neurons by innate immune mechanisms during neurodegeneration review-Article. Cell Death Dis 9(2):120.

Iyer A, Zurolo E, Prabowo A, Fluiter K, Spliet WGM, van Rijen PC, Gorter JA, Aronica E (2012) MicroRNA-146a: A Key Regulator of Astrocyte-Mediated Inflammatory Response. PLoS One 7:e44789.

Jauch R, Windmüller O, Lehmann T-N, Heinemann U, Gabriel S (2002) Effects of barium, furosemide, ouabaine and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS) on ionophoretically-induced changes in extracellular potassium concentration in hippocampal slices from rats and from patients with epilepsy. Brain Res 925:18–27.

Jimenez-Mateos EM et al. (2015) microRNA targeting of the P2X7 purinoceptor opposes a contralateral epileptogenic focus in the hippocampus. Sci Rep 5:17486.

Kato G, Inada H, Wake H, Akiyoshi R, Miyamoto A, Eto K, Ishikawa T, Moorhouse AJ, Strassman AM, Nabekura J (2016) Microglial contact prevents excess depolarization and rescues neurons from excitotoxicity. eNeuro 3:9133–9144.

Kettenmann H, Kirchhoff F, Verkhratsky A (2013) Microglia: new roles for the synaptic stripper. Neuron 77:10–18.

Keystone EC, Wang MM, Layton M, Hollis S, McInnes IB (2012) Clinical evaluation of the efficacy of the P2X7 purinergic receptor antagonist AZD9056 on the signs and symptoms of rheumatoid arthritis in patients with active disease despite treatment with methotrexate or sulphasalazine. Ann Rheum Dis 71:1630–1635.

Khakh BS, Gittermann D, Cockayne DA, Jones A (2003) ATP modulation of excitatory synapses onto interneurons. J Neurosci 23:7426–7437.

Khakh BS, Sofroniew M V (2015) Diversity of astrocyte functions and phenotypes in neural circuits. Nat Neurosci 18:942–952.

Kierdorf K, Prinz M (2013) Factors regulating microglia activation. Front Cell Neurosci

7:44.

Kim JE, Kang TC (2011) The P2X7 receptor-pannexin-1 complex decreases muscarinic acetylcholine receptor-mediated seizure susceptibility in mice. J Clin Invest 121:2037–2047.

Kim JE, Ryu HJ, Kang TC (2013) Status Epilepticus Induces Vasogenic Edema via Tumor Necrosis Factor-α/ Endothelin-1-Mediated Two Different Pathways. PLoS One 8:e74458.

Kim SY, Senatorov V V, Morrissey CS, Lippmann K, Vazquez O, Milikovsky DZ, Gu F, Parada I, Prince DA, Becker AJ, Heinemann U, Friedman A, Kaufer D (2017) TGFβ signaling is associated with changes in inflammatory gene expression and perineuronal net degradation around inhibitory neurons following various neurological insults. Sci Rep 7:7711.

Kivi A, Lehmann TN, Kovacs R, Eilers A, Jauch R, Meencke HJ, von DA, Heinemann U, Gabriel S (2000) Effects of barium on stimulus-induced rises of [K+]o in human epileptic non-sclerotic and sclerotic hippocampal area CA1. EurJ Neurosci 12:2039–2048.

Klement W, Blaquiere M, Zub E, deBock F, Boux F, Barbier E, Audinat E, Lerner-Natoli M, Marchi N (2019) A pericyte-glia scarring develops at the leaky capillaries in the hippocampus during seizure activity. Epilepsia 60:1399–1411.

Klement W, Garbelli R, Zub E, Rossini L, Tassi L, Girard B, Blaquiere M, Bertaso F, Perroy J, de Bock F, Marchi N (2018) Seizure progression and inflammatory mediators promote pericytosis and pericyte-microglia clustering at the cerebrovasculature. Neurobiol Dis 113:70–81.

Kofuji P, Newman EA (2004) Potassium buffering in the central nervous system. Neuroscience 129:1043–1054.

Korotkov A, Broekaart DWM, Banchaewa L, Pustjens B, van Scheppingen J, Anink JJ, Baayen JC, Idema S, Gorter JA, van Vliet EA, Aronica E (2020) microRNA-132 is overexpressed in glia in temporal lobe epilepsy and reduces the expression of pro-epileptogenic factors in human cultured astrocytes. Glia 68:60–75.

Lado FA, Moshé SL (2008) How do seizures stop? Epilepsia 49:1651–1664.

Lee T-S, Bjørnsen LP, Paz C, Kim JH, Spencer SS, Spencer DD, Eid T, de Lanerolle NC (2006) GAT1 and GAT3 expression are differently localized in the human epileptogenic hippocampus. Acta Neuropathol 111:351–363.

Li Y, Du XF, Liu CS, Wen ZL, Du JL (2012) Reciprocal regulation between resting microglial dynamics and neuronal activity in vivo. DevCell 23:1189–1202.

Lian H, Yang L, Cole A, Sun L, Chiang AC-A, Fowler SW, Shim DJ, Rodriguez-Rivera J, Taglialatela G, Jankowsky JL, Lu H-C, Zheng H (2015) NFκB-activated astroglial release of complement C3 compromises neuronal morphology and function associated with Alzheimer's disease. Neuron 85:101–115.

Liddelow SA et al. (2017) Neurotoxic reactive astrocytes are induced by activated microglia. Nature 541:481–487.

Liddelow SA, Barres BA (2017) Reactive Astrocytes: Production, Function, and Therapeutic Potential. Immunity 46:957–967.

Limatola C, Ransohoff RM (2014) Modulating neurotoxicity through CX3CL1/CX3CR1 signaling. Front Cell Neurosci 8:229.

Madry C, Kyrargyri V, Arancibia-Cárcamo IL, Jolivet R, Kohsaka S, Bryan RM, Attwell D (2018) Microglial Ramification, Surveillance, and Interleukin-1β Release Are Regulated by the Two-Pore Domain K+Channel THIK-1. Neuron 97(2):299-312.e6.

Malarkey EB, Parpura V (2008) Mechanisms of glutamate release from astrocytes. Neurochem Int 52:142–154.

Marchi N, Granata T, Janigro D (2014) Inflammatory pathways of seizure disorders. Trends Neurosci 37:55–65.

Maroso M, Balosso S, Ravizza T, Liu J, Aronica E, Iyer AM, Rossetti C, Molteni M, Casalgrandi M, Manfredi AA, Bianchi ME, Vezzani A (2010) Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis and can be targeted to reduce seizures. NatMed 16:413–419.

Meldrum BS (1994) The role of glutamate in epilepsy and other CNS disorders. Neurology 44:S14-23.

Meme W, Calvo C-FF, Froger N, Ezan P, Amigou E, Koulakoff A, Giaume C, Même W, Calvo C-FF, Froger N, Ezan P, Amigou E, Koulakoff A, Giaume C (2006) Proinflammatory cytokines released from microglia inhibit gap junctions in astrocytes: potentiation by beta-amyloid. FASEB J 20:494–496.

Messing A, Brenner M, Feany MB, Nedergaard M, Goldman JE (2012) Alexander Disease. J Neurosci 32:5017–5023.

Milior G, Morin-Brureau M, Chali F, Le Duigou C, Savary E, Huberfeld G, Rouach N, Pallud J, Capelle L, Navarro V, Mathon B, Clemenceau S, Miles R (2020) Distinct P2Y Receptors Mediate Extension and Retraction of Microglial Processes in Epileptic and Peritumoral Human Tissue. J Neurosci 40:1373–1388.

Mo M, Eyo UB, Xie M, Peng J, Bosco DB, Umpierre AD, Zhu X, Tian DS, Xu P, Wu LJ (2019) Microglial P2Y12 Receptor Regulates Seizure-Induced Neurogenesis and Immature Neuronal Projections. J Neurosci 39:9453–9464.

Morin-Brureau M, Milior G, Royer J, Chali F, Le Duigou C, Savary E, Blugeon C, Jourdren L, Akbar D, Dupont S, Navarro V, Baulac M, Bielle F, Mathon B, Clemenceau S, Miles R (2018) Microglial phenotypes in the human epileptic temporal lobe. Brain 141(12):3343-3360.

Mosser CA, Baptista S, Arnoux I, Audinat E (2017) Microglia in CNS development: Shaping the brain for the future. Prog Neurobiol 149–150:1–20.

Nagy JI, Rash JE (2000) Connexins and gap junctions of astrocytes and oligodendrocytes in the CNS. Brain Res Brain Res Rev 32:29–44.

Nayak D, Roth TL, McGavern DB (2014) Microglia development and function. AnnuRevImmunol 32:367–402.

Nikolic L, Shen W, Nobili P, Virenque A, Ulmann L, Audinat E (2018) Blocking TNFα-driven astrocyte purinergic signaling restores normal synaptic activity during epileptogenesis. Glia 66:2673–2683.

Nimmerjahn A, Kirchhoff F, Helmchen F (2005) Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. Science (80-) 308:1314–1318.

Nippert AR, Biesecker KR, Newman EA (2018) Mechanisms Mediating Functional Hyperemia in the Brain. Neuroscientist 24:73–83.

Noè F, Cattalini A, Vila Verde D, Alessi C, Colciaghi F, Figini M, Zucca I, Curtis M (2019) Epileptiform activity contralateral to unilateral hippocampal sclerosis does not cause the expression of brain damage markers. Epilepsia 60:epi.15611.

Nortley R, Attwell D (2017) Control of brain energy supply by astrocytes. Curr Opin Neurobiol 47:80–85.

Olson JK, Miller SD (2004) Microglia Initiate Central Nervous System Innate and Adaptive Immune Responses through Multiple TLRs. J Immunol 173:3916–3924.

Orkand RK, Nicholls JG, Kuffler SW (1966) Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. J Neurophysiol 29:788–806.

Orr AG, Orr AL, Li XJ, Gross RE, Traynelis SF (2009) Adenosine A(2A) receptor mediates microglial process retraction. NatNeurosci 12:872–878.

Ortinski PI, Dong J, Mungenast A, Yue C, Takano H, Watson DJ, Haydon PG, Coulter DA (2010) Selective induction of astrocytic gliosis generates deficits in neuronal inhibition. Nat Neurosci 13:584–591.

Panatier A, Robitaille R (2016) Astrocytic mGluR5 and the tripartite synapse. Neuroscience 323:29–34.

Pannasch U, Vargova L, Reingruber J, Ezan P, Holcman D, Giaume C, Sykova E, Rouach N (2011) Astroglial networks scale synaptic activity and plasticity. ProcNatlAcadSciUSA 108:8467–8472.

Paolicelli RC, Ferretti MT (2017) Function and Dysfunction of Microglia during Brain Development: Consequences for Synapses and Neural Circuits. Front Synaptic Neurosci 9:9.

Papadopoulos MC, Verkman AS (2013) Aquaporin water channels in the nervous

system. Nat Rev Neurosci 14:265–277.

Papouin T, Dunphy J, Tolman M, Foley JC, Haydon PG (2017) Astrocytic control of synaptic function. Philos Trans R Soc B Biol Sci 372:20160154.

Parkhurst CN, Yang G, Ninan I, Savas JN, Yates III JR, Lafaille JJ, Hempstead BL, Littman DR, Gan WB (2013) Microglia Promote Learning-Dependent Synapse Formation through Brain-Derived Neurotrophic Factor. Cell 155:1596–1609.

Parri HR, Gould TM, Crunelli V (2001) Spontaneous astrocytic Ca2+ oscillations in situ drive NMDAR-mediated neuronal excitation. NatNeurosci 4:803–812.

Pascual O, Ben Achour S, Rostaing P, Triller A, Bessis A (2012) Microglia activation triggers astrocyte-mediated modulation of excitatory neurotransmission. Proc Natl Acad Sci 109:E197–E205.

Pascual O, Casper KB, Kubera C, Zhang J, Revilla-Sanchez R, Sul J-YY, Takano H, Moss SJ, McCarthy K, Haydon PG (2005) Astrocytic Purinergic Signaling Coordinates Synaptic Networks. Science (80-) 310:113–116.

Patel DC, Tewari BP, Chaunsali L, Sontheimer H (2019) Neuron–glia interactions in the pathophysiology of epilepsy. Nat Rev Neurosci 20:282–297.

Pellerin L, Bouzier-Sore A-K, Aubert A, Serres S, Merle M, Costalat R, Magistretti PJ (2007) Activity-dependent regulation of energy metabolism by astrocytes: An update. Glia 55:1251–1262.

Pellerin L, Magistretti PJ (2012) Sweet sixteen for ANLS. J Cereb Blood Flow Metab 32:1152–1166.

Perea G, Navarrete M, Araque A (2009) Tripartite synapses: astrocytes process and control synaptic information. Trends Neurosci 32:421–431.

Petrelli F, Bezzi P (2016) Novel insights into gliotransmitters. Curr Opin Pharmacol 26:138–145.

Pfeiffer T, Avignone E, Nägerl UV (2016) Induction of hippocampal long-term potentiation increases the morphological dynamics of microglial processes and prolongs their contacts with dendritic spines. Sci Rep 6:32422.

Pirttimaki T, Parri HR, Crunelli V (2013) Astrocytic GABA transporter GAT-1 dysfunction in experimental absence seizures. J Physiol 591:823–833.

Prinz M, Priller J (2014) Microglia and brain macrophages in the molecular age: from origin to neuropsychiatric disease. NatRevNeurosci 15:300–312.

Ransohoff RM, Perry VH (2009) Microglial Physiology: Unique Stimuli, Specialized Responses. Annu Rev Immunol 27:119–145.

Ransom CB, Ransom BR, Sontheimer H (2000) Activity-dependent extracellular K+ accumulation in rat optic nerve: the role of glial and axonal Na+ pumps. J Physiol 522 Pt 3:427–442.

Reichenbach N, Delekate A, Breithausen B, Keppler K, Poll S, Schulte T, Peter J, Plescher M, Hansen JN, Blank N, Keller A, Fuhrmann M, Henneberger C, Halle A, Petzold GC (2018) P2Y1 receptor blockade normalizes network dysfunction and cognition in an Alzheimer's disease model. J Exp Med 215:1649–1663.

Robel S, Buckingham SC, Boni JL, Campbell SL, Danbolt NC, Riedemann T, Sutor B, Sontheimer H (2015) Reactive Astrogliosis Causes the Development of Spontaneous Seizures. J Neurosci 35:3330–3345.

Rodrigues RJ, Tome AR, Cunha RA (2015) ATP as a multi-target danger signal in the brain. Front Neurosci 9:148.

Rogers JT, Morganti JM, Bachstetter AD, Hudson CE, Peters MM, Grimmig BA, Weeber EJ, Bickford PC, Gemma C (2011) CX3CR1 deficiency leads to impairment of hippocampal cognitive function and synaptic plasticity. J Neurosci 31:16241–16250.

Roseti C, Fucile S, Lauro C, Martinello K, Bertollini C, Esposito V, Mascia A, Catalano M, Aronica E, Limatola C, Palma E (2013) Fractalkine/CX3CL1 modulates GABAA currents in human temporal lobe epilepsy. Epilepsia 54:1834–1844.

Rossi D, Volterra A (2009) Astrocytic dysfunction: Insights on the role in neurodegeneration. Brain Res Bull 80:224–232.

Rossini L, Garbelli R, Gnatkovsky V, Didato G, Villani F, Spreafico R, Deleo F, Lo

Russo G, Tringali G, Gozzo F, Tassi L, de Curtis M (2017) Seizure activity per se does not induce tissue damage markers in human neocortical focal epilepsy. Ann Neurol 82:331–341.

Rusakov DA, Bard L, Stewart MG, Henneberger C (2014) Diversity of astroglial functions alludes to subcellular specialisation. Trends Neurosci 37:228–242.

Salter MW, Beggs S (2014) Sublime microglia: expanding roles for the guardians of the CNS. Cell 158:15–24.

Salter MW, Stevens B (2017) Microglia emerge as central players in brain disease. Nat Med 23(9):1018-1027.

Sanchez JMS, DePaula-Silva AB, Doty DJ, Truong A, Libbey JE, Fujinami RS (2019) Microglial cell depletion is fatal with low level picornavirus infection of the central nervous system. J Neurovirol 25:415–421.

Santello M, Toni N, Volterra A (2019) Astrocyte function from information processing to cognition and cognitive impairment. Nat Neurosci 22:154–166.

Santiago MF, Veliskova J, Patel NK, Lutz SE, Caille D, Charollais A, Meda P, Scemes E (2011) Targeting Pannexin1 Improves Seizure Outcome Barnes S, ed. PLoS One 6:e25178.

Savtchouk I, Volterra A (2018) Gliotransmission: Beyond Black-and-White. J Neurosci 38:14–25.

Schafer DP, Lehrman EK, Stevens B (2013) The “quad-partite” synapse: Microglia-synapse interactions in the developing and mature CNS. Glia 61:24–36.

Schröder W, Hinterkeuser S, Seifert G, Schramm J, Jabs R, Wilkin GP, Steinhäuser C, Schroder W, Hinterkeuser S, Seifert G, Schramm J, Jabs R, Wilkin GP, Steinhauser C (2000) Functional and molecular properties of human astrocytes in acute hippocampal slices obtained from patients with temporal lobe epilepsy. Epilepsia 41 Suppl 6:S181–S184.

Schwartzkroin PA, Baraban SC, Hochman DW (1998) Osmolarity, ionic flux, and changes in brain excitability. Epilepsy Res 32:275–285.

Scianni M, Antonilli L, Chece G, Cristalli G, Di Castro MA, Limatola C, Maggi L (2013) Fractalkine (CX3CL1) enhances hippocampal N-methyl-D-aspartate receptor (NMDAR) function via D-serine and adenosine receptor type A2 (A2AR) activity. JNeuroinflammation 10:108.

Seifert G, Hüttmann K, Binder DK, Hartmann C, Wyczynski A, Neusch C, Steinhäuser C (2009) Analysis of astroglial K+ channel expression in the developing hippocampus reveals a predominant role of the Kir4.1 subunit. J Neurosci 29:7474–7488.

Seiffert E, Dreier JP, Ivens S, Bechmann I, Tomkins O, Heinemann U, Friedman A (2004) Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. J Neurosci 24:7829–7836.

Shemer A, Erny D, Jung S, Prinz M (2015) Microglia Plasticity During Health and Disease: An Immunological Perspective. Trends Immunol 36:614–624.

Shen W, Nikolic L, Meunier C, Pfrieger F, Audinat E (2017) An autocrine purinergic signaling controls astrocyte-induced neuronal excitation. Sci Rep 7:11280.

Sierra A, Encinas JM, Deudero JJ, Chancey JH, Enikolopov G, Overstreet-Wadiche LS, Tsirka SE, Maletic-Savatic M (2010) Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. Cell Stem Cell 7:483–495.

Sipe GO, Lowery RL, Tremblay ME, Kelly EA, Lamantia CE, Majewska AK (2016) Microglial P2Y12 is necessary for synaptic plasticity in mouse visual cortex. NatCommun 7:10905.

Sofroniew M V. (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638–647.

Song C, Xu W, Zhang X, Wang S, Zhu G, Xiao T, Zhao M, Zhao C (2016) CXCR4 Antagonist AMD3100 Suppresses the Long-Term Abnormal Structural Changes of Newborn Neurons in the Intraventricular Kainic Acid Model of Epilepsy. Mol Neurobiol 53:1518–1532.

Srivastava PK et al. (2018) A systems-level framework for drug discovery identifies

Csf1R as an anti-epileptic drug target. Nat Commun 9:3561.

Steinhäuser C, Grunnet M, Carmignoto G (2016) Crucial role of astrocytes in temporal lobe epilepsy. Neuroscience 323:157–169.

Stowell RD, Wong EL, Batchelor HN, Mendes MS, Lamantia CE, Whitelaw BS, Majewska AK (2018) Cerebellar microglia are dynamically unique and survey Purkinje neurons in vivo. Dev Neurobiol 78:627–644.

Strohschein S, Hüttmann K, Gabriel S, Binder DK, Heinemann U, Steinhäuser C (2011) Impact of aquaporin-4 channels on K+ buffering and gap junction coupling in the hippocampus. Glia 59:973–980.

Studer FE, Fedele DE, Marowsky A, Schwerdel C, Wernli K, Vogt K, Fritschy JM, Boison D (2006) Shift of adenosine kinase expression from neurons to astrocytes during postnatal development suggests dual functionality of the enzyme. Neuroscience 142:125–137.

Tan Z, Liu Y, Xi W, Lou H, Zhu L, Guo Z, Mei L, Duan S (2017) Glia-derived ATP inversely regulates excitability of pyramidal and CCK-positive neurons. Nat Commun 8:13772.

Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, Iwama H, Nishikawa T, Ichihara N, Kikuchi T, Okuyama S, Kawashima N, Hori S, Takimoto M, Wada K (1997) Epilepsy and Exacerbation of Brain Injury in Mice Lacking the Glutamate Transporter GLT-1. Science (80-) 276:1699–1702.

Terrone G, Balosso S, Pauletti A, Ravizza T, Vezzani A (2019) Inflammation and reactive oxygen species as disease modifiers in epilepsy. Neuropharmacology:107742.

Terrone G, Salamone A, Vezzani A (2017) Inflammation and Epilepsy: Preclinical Findings and Potential Clinical Translation. Curr Pharm Des 23:5569–5576.

Theodosis DT, Poulain DA, Oliet SHR (2008) Activity-Dependent Structural and Functional Plasticity of Astrocyte-Neuron Interactions. Physiol Rev 88:983–1008.

Thion MS, Ginhoux F, Garel S (2018) Microglia and early brain development: An intimate journey. Science 362:185–189.

Thom M (2014) Review: Hippocampal sclerosis in epilepsy: a neuropathology review. Neuropathol Appl Neurobiol 40:520–543.

Tian GF, Azmi H, Takano T, Xu Q, Peng W, Lin J, Oberheim N, Lou N, Wang X, Zielke HR, Kang J, Nedergaard M (2005) An astrocytic basis of epilepsy. NatMed 11:973–981.

Tozaki-Saitoh H, Tsuda M, Miyata H, Ueda K, Kohsaka S, Inoue K (2008) P2Y12 receptors in spinal microglia are required for neuropathic pain after peripheral nerve injury. J Neurosci 28:4949–4956.

Tremblay ME, Lowery RL, Majewska AK (2010) Microglial interactions with synapses are modulated by visual experience. PLoSBiol 8:e1000527.

Tremblay ME, Stevens B, Sierra A, Wake H, Bessis A, Nimmerjahn A (2011) The role of microglia in the healthy brain. J Neurosci 31:16064–16069.

Ulmann L, Levavasseur F, Avignone E, Peyroutou R, Hirbec H, Audinat E, Rassendren F (2013) Involvement of P2X4 receptors in hippocampal microglial activation after *status epilepticus*. Glia 61:1306–1319.

Vainchtein ID, Molofsky A V (2020) Astrocytes and Microglia : In Sickness and in Health. Trends Neurosci:1–11.

van der Hel WS, Notenboom RGE, Bos IWM, van Rijen PC, van Veelen CWM, de Graan PNE (2005) Reduced glutamine synthetase in hippocampal areas with neuron loss in temporal lobe epilepsy. Neurology 64:326–333.

van Scheppingen J, Mills JD, Zimmer TS, Broekaart DWM, Iori V, Bongaarts A, Anink JJ, Iyer AM, Korotkov A, Jansen FE, van Hecke W, Spliet WG, van Rijen PC, Baayen JC, Vezzani A, van Vliet EA, Aronica E (2018) miR147b: A novel key regulator of interleukin 1 beta-mediated inflammation in human astrocytes. Glia 66:1082–1097.

van Vliet EA, Aronica E, Gorter JA (2015) Blood–brain barrier dysfunction, seizures and epilepsy. Semin Cell Dev Biol 38:26–34.

Varadkar S, Cross JH (2015) Rasmussen syndrome and other inflammatory epilepsies. Semin Neurol 35:259–268.

Verkhratsky A, Matteoli M, Parpura V, Mothet J-P, Zorec R (2016) Astrocytes as secretory cells of the central nervous system: idiosyncrasies of vesicular secretion. EMBO J 35:239–257.

Verkhratsky A, Parpura V (2016) Astrogliopathology in neurological, neurodevelopmental and psychiatric disorders. Neurobiol Dis 85:254–261.

Vezzani A, Balosso S, Ravizza T (2019) Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy. Nat Rev Neurol 15:459–472.

Vezzani A, French J, Bartfai T, Baram TZ (2011) The role of inflammation in epilepsy. Nat Rev Neurol 7:31–40.

Volk DW (2017) Role of microglia disturbances and immune-related marker abnormalities in cortical circuitry dysfunction in schizophrenia. Neurobiol Dis 99:58–65.

Volterra A, Liaudet N, Savtchouk I (2014) Astrocyte Ca2+ signalling: an unexpected complexity. Nat Rev Neurosci 15:327–335.

Wake H, Moorhouse AJ, Jinno S, Kohsaka S, Nabekura J (2009) Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. J Neurosci 29:3974–3980.

Wallraff A, Kohling R, Heinemann U, Theis M, Willecke K, Steinhauser C (2006) The impact of astrocytic gap junctional coupling on potassium buffering in the hippocampus. J Neurosci 26:5438–5447.

Waltl I, Käufer C, Gerhauser I, Chhatbar C, Ghita L, Kalinke U, Löscher W (2018) Microglia have a protective role in viral encephalitis-induced seizure development and hippocampal damage. Brain Behav Immun 74:186–204.

Walz W (2000) Role of astrocytes in the clearance of excess extracellular potassium. Neurochem Int 36:291–300.

Wang Y, Zaveri HP, Lee T-SW, Eid T (2009) The development of recurrent seizures after continuous intrahippocampal infusion of methionine sulfoximine in rats: a video-intracranial electroencephalographic study. Exp Neurol 220:293–302.

Wei Y-J, Guo W, Sun F-J, Fu W-L, Zheng D-H, Chen X, Li S, Zang Z-L, Zhang C-Q, Liu S-Y, Yang H (2016) Increased Expression and Cellular Localization of P2X7R in Cortical Lesions of Patients With Focal Cortical Dysplasia. J Neuropathol Exp Neurol 75:61–68.

Weissberg I, Wood L, Kamintsky L, Vazquez O, Milikovsky DZ, Alexander A, Oppenheim H, Ardizzone C, Becker A, Frigerio F, Vezzani A, Buckwalter MS, Huguenard JR, Friedman A, Kaufer D (2015) Albumin induces excitatory synaptogenesis through astrocytic TGF-β/ALK5 signaling in a model of acquired epilepsy following blood-brain barrier dysfunction. Neurobiol Dis 78:115–125.

Wolf SA, Boddeke HWGM, Kettenmann H (2017) Microglia in Physiology and Disease. Annu Rev Physiol 79:619–643.

Wu LJ, Stevens B, Duan S, MacVicar BA (2013) Microglia in neuronal circuits. Neural Plast 2013:586426.

Wu W, Li Y, Wei Y, Bosco DB, Xie M, Zhao MG, Richardson JR, Wu LJ (2020) Microglial depletion aggravates the severity of acute and chronic seizures in mice. Brain Behav Immun PMID: 32621847.

Xu Y, Zeng K, Han Y, Wang L, Chen D, Xi Z, Wang H, Wang X, Chen G (2012) Altered expression of CX3CL1 in patients with epilepsy and in a rat model. Am J Pathol 180:1950–1962.

Zamanian JL, Xu L, Foo LC, Nouri N, Zhou L, Giffard RG, Barres BA (2012) Genomic Analysis of Reactive Astrogliosis. J Neurosci 32:6391–6410.

Zhao X, Liao Y, Morgan S, Mathur R, Feustel P, Mazurkiewicz J, Qian J, Chang J, Mathern GW, Adamo MA, Ritaccio AL, Gruenthal M, Zhu X, Huang Y (2018) Noninflammatory Changes of Microglia Are Sufficient to Cause Epilepsy. Cell Rep 22:2080–2093.

Zhao Z, Nelson AR, Betsholtz C, Zlokovic B V (2015) Establishment and Dysfunction of the Blood-Brain Barrier. Cell 163:1064–1078.