



HAL
open science

Role of astrocyte purinergic signaling in epilepsy

Ljiljana Nikolic, Paola Nobili, Weida Shen, Etienne Audinat

► **To cite this version:**

Ljiljana Nikolic, Paola Nobili, Weida Shen, Etienne Audinat. Role of astrocyte purinergic signaling in epilepsy. *Glia*, 2019, 10.1002/glia.23747 . hal-02400360

HAL Id: hal-02400360

<https://hal.science/hal-02400360>

Submitted on 20 Nov 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Role of astrocyte purinergic signalling in epilepsy

Running title: Astrocyte purinergic signalling in epilepsy

Ljiljana Nikolic¹, Paola Nobili², Weida Shen³, Etienne Audinat⁴

¹ Institute for Biological Research *Siniša Stanković*, University of Belgrade, Serbia

² Neuroscience Institute CNR, Milano, Italy

³ Zhejiang University City College, Hangzhou, Zhejiang, China

⁴ Institute for Functional Genomics (IGF), University of Montpellier, CNRS, INSERM, Montpellier, France

Correspondence: Etienne Audinat

e-mail address: etienne.audinat@igf.cnrs.fr

Institut de Génomique Fonctionnelle, CNRS UMR 5203, INSERM U1191

Université de Montpellier, 141 rue de la Cardonille, 34094 Montpellier Cedex 5, France

Acknowledgments

Work in Audinat Lab is supported by grants from the Fédération pour la Recherche sur le Cerveau (FRC) and the European Commission (EC-H2020 MSCA-ITN EU-GliaPhD No. 722053). Ljiljana Nikolic is supported by Ministry of Education, Science and Technological Development of Republic of Serbia (grant no. 173027).

Conflict of Interest Statement

The authors declare no conflict of interest.

Word count:

Total: 13560 / Abstract: 109 / Introduction: 674 / Main text: 6425

Number of Figures: 1

35 **Abstract**

36 Epilepsy is characterized by unpredictable recurrent seizures resulting from
37 hypersynchronous discharges from neuron assemblies. Increasing evidence indicates that
38 aberrant astrocyte signalling to neurons plays an important role in driving the network
39 hyperexcitability. Purinergic signalling is central in neuron-glia and glia-glia interactions
40 and dysfunctions in communication pathways involving purinergic receptors have been
41 reported in various CNS pathologies, such as Alzheimer disease, stroke, major
42 depression, schizophrenia, and epilepsy. In the present review we will first discuss the
43 mechanisms by which astrocytes influence neuronal activity. We will then review in more
44 details recent evidence indicating that dysregulation of astrocyte purinergic signalling
45 actively contributes to the appearance of abnormal neuronal activity in epilepsy.

46
47 **Keywords:** calcium signalling, cytokine, microglia, gliotransmission, inflammation;

48
49 **Main points**

- 50 • Purinergic signalling is a key signaling pathway in glia-glia and neuron-glia
51 interactions.
- 52 • Purine-driven gliotransmitter release from astrocytes perturbs excitation/inhibition
53 balance and favours the appearance of epileptiform activity.

54

55 **Introduction**

56 Our knowledge of astrocyte functions evolved tremendously during the last decades,
57 adding to the classical view of supportive cells for neurons the concept that astrocytes
58 integrate neuronal and synaptic activities and, in turn, directly influence these activities
59 through the release of gliotransmitters. In physiological conditions, these “metabolic” and
60 “information processing” functions usually operate on a time scale ranging from
61 milliseconds to hours or even days, when considering brain development and hormonal
62 cycles, and are fundamental for brain functions, including cognitive functions (Araque et
63 al., 2014; Attwell et al., 2010; Dallérac & Rouach, 2016; Khakh & Sofroniew, 2015;
64 MacDonald, Robb, Morrissey, Beall, & Ellacott, 2019; Pellerin et al., 2007; Rusakov, Bard,
65 Stewart, & Henneberger, 2014; Mirko Santello, Toni, & Volterra, 2019; Theodosis, Poulain,
66 & Oliet, 2008). The continuous and progressive discovery of key roles played by
67 astrocytes in regulating brain functions has also advanced our understanding of the roles
68 of these cells in the pathogenesis of neurological and psychiatric diseases, including
69 Alzheimer’s disease, cerebral ischemia, multiple sclerosis, Huntington’s disease, major
70 depressive disorder and epilepsy (Cresto, Pillet, Billuart, & Rouach, 2019; Halassa, Fellin,
71 & Haydon, 2007; Patel, Tewari, Chaunsali, & Sontheimer, 2019; Rossi & Volterra, 2009;
72 Steinhäuser, Grunnet, & Carmignoto, 2016; Verkhratsky & Parpura, 2016).

73 Epilepsy is a pathological condition affecting more than 1% of the population worldwide
74 (Beghi, 2016; Hesdorffer et al., 2011). It is characterized by repeated and recurrent
75 seizures altering physiological brain functioning and being responsible for the development
76 of comorbidities such as neurological deficits. According to the International League
77 Against Epilepsy, epilepsies should be classified as focal, generalized or combined focal
78 and generalized, with six, non-exclusive, subgroups of etiology (structural, genetic,
79 infectious, metabolic, immune or unknown), selected because of their potential therapeutic
80 consequences (Scheffer et al., 2017). Many of these epilepsies result from an acute insult
81 (e.g. stroke, trauma, infection) that triggers progressive modifications of the brain that lead
82 to the occurrence of spontaneous recurrent seizures. Epileptogenesis corresponds to the
83 gradual remodelling of a formerly healthy brain into a spontaneously seizing one. The
84 latent phase separating the initial insult from the appearance of the first spontaneous
85 seizures can last months or even years. It is followed by the chronic phase during which
86 recurrent spontaneous seizures occur. For a long time, it has been thought that alteration
87 in the excitation / inhibition balance, which is at the basis of seizure generation, was the

88 only mechanism involved in the pathogenesis of epilepsy. Consequently, anti-epileptic
89 drugs (AEDs) developed in the last decades mostly aimed at correcting this perturbed
90 balance between excitatory and inhibitory neurotransmission. These pharmacological
91 interventions can, in the best scenario, control seizures but they cannot cure the disease.
92 Furthermore, about one third of the patients are refractory to pharmacological treatments
93 and this ratio reaches 2/3 of the patients when considering mesial temporal lobe epilepsy
94 (MTLE), the most frequent and severe form of focal epilepsy in adults (Asadi-Pooya,
95 Stewart, Abrams, & Sharan, 2017). There is therefore an obvious and urgent need for the
96 development of new AEDs. In this context, the increasing evidence that brain functions
97 and dysfunctions rely on an integrated interplay between neurons and glial cells, and in
98 particular astrocytes, opens new routes for the identification of promising non-neuronal
99 targets that could lead to the development of new AEDs. Dysfunction of several astrocyte
100 signalling pathways can contribute to hyperexcitation of neuronal networks and
101 progression of seizures (Steinhauser and Boison, 2012; Devinsky et al., 2013; Patel et al
102 2019). In particular, purinergic signalling is a key signalling pathway in glia-glia and
103 neuron-glia interactions and recent evidence indicates that this pathway contributes to the
104 deregulation of neuronal activity in epilepsy (Engel, Alves, Sheedy, & Henshall, 2016;
105 Rassendren & Audinat, 2016).

106 In a first section, we will briefly review the physiological functions of astrocytes that can
107 impact on neuronal and synaptic activities, highlighting astrocyte dysfunctions that have
108 been identified in pathological conditions and in particular in epilepsy. In the following
109 sections, we will discuss more extensively how deregulation of ATP signalling in astrocytes
110 can contribute to epileptogenesis and ictogenesis (*i.e.* the acute process generating
111 seizures).

112 **1. Astrocytes: function and dysfunction in health and disease**

113 *1.1. Astrogliosis: an adaptive reactive phenotype*

114 The role of astrocytes in pathologies of the central nervous system (CNS) is widely
115 associated with reactive phenotype developed by astrocytes in response to a rupture in
116 CNS homeostasis. This process called astrogliosis is particularly evident in epileptic
117 syndromes associated with hippocampal sclerosis (HS) that is characterized by a glial scar
118 (Thom, 2014). Astrogliosis involves changes at the molecular, cellular and functional levels
119 (Dossi, Vasile, & Rouach, 2018). The degree of alterations observed in reactive astrocytes

120 varies according to the type of insult inducing the reaction and to the possibility of
121 reversion of the damage (Anderson, Ao, & Sofroniew, 2014; Sofroniew, 2009).
122 Transcriptomic analysis of reactive astrocytes has disclosed the occurrence of two broad
123 phenotypes: a pro-inflammatory phenotype A1 having detrimental effects (Lian et al.,
124 2015; Liddelow et al., 2017; Zamanian et al., 2012) and an immune-suppressive
125 phenotype A2 with protective functions (Anderson et al., 2016; Liddelow & Barres, 2017).
126 Although the specific phenotype of astrocytes in different forms of epilepsy has not yet
127 been firmly established, different studies point rather toward a deleterious A1 phenotype.
128 For example, in epilepsy models, the release of Interleukin-1beta (IL-1 β) and High Mobility
129 Group Box1 by astrocytes induces the activation of IL1R/TLR signalling pathways that
130 favours the induction and maintenance of seizure activity (Maroso et al., 2010; Vezzani,
131 French, Bartfai, & Baram, 2011). However, it should be pointed out that in most cases the
132 population of astrocytes has been considered as a whole without taking into consideration
133 a possible diversity of the astrocyte reactivity (e.g. lesion vs peri-lesional area). One
134 important and still debated question is whether astrocyte reactivity is a cause or a
135 consequence of seizures. In favour of a causative role, astrocyte-specific deletion of genes
136 (tuberous sclerosis complex 1 or Beta 1 integrin) or mutation of an astrocyte-specific gene
137 (GFAP) induce astrogliosis and the occurrence of spontaneous seizures (Messing,
138 Brenner, Feany, Nedergaard, & Goldman, 2012; Ortinski et al., 2010; Robel et al., 2015).
139 On the other hand, there were no sign of astrogliosis in non-lesional epileptogenic cortical
140 areas of patients with focal cortical dysplasia (Rossini et al., 2017). Moreover, in a guinea
141 pig model of temporal lobe epilepsy with hippocampal sclerosis (HS-MTLE; unilateral,
142 intra-hippocampal kainate), astrogliosis and cell damage were observed only in the
143 injected, and not in the contralateral, hippocampus despite the occurrence of epileptiform
144 activities in both hippocampi (Noè et al., 2019). The latter two examples suggest
145 dissociation between the occurrence of seizure and astrogliosis, as seen with the classical
146 marker GFAP. However, they do not exclude the contribution of astrocytes to the
147 functional remodelling that occurs during epileptogenesis and to the generation of
148 seizures, especially in view of the many functions of astrocytes by which they influence the
149 excitability of neuronal networks.

150 Another important aspect of astrocyte function that is affected by gliosis is their close
151 interaction with brain vasculature that they contact with their endfeet. Astrocytes in
152 physiological conditions contribute to the maintenance of blood-brain barrier (BBB)

153 structure by increasing the expression of tight junction proteins occludin and claudin-5 and
154 by inhibiting the expression of chemokines and leukocyte adhesion molecules, which
155 would favour BBB disruption (Andreone, Lacoste, & Gu, 2015; Zhao, Nelson, Betsholtz, &
156 Zlokovic, 2015). Modifications of astrocyte phenotype during astrogliosis could contribute
157 to BBB impairment that occurs in epilepsy through disruption of tight-junction coupling,
158 increased expression of adhesion molecules on endothelial cells determining an increase
159 of pro-inflammatory cytokines and the extravasation of leukocytes in brain parenchyma
160 (Marchi, Granata, & Janigro, 2014; van Vliet, Aronica, & Gorter, 2015). Furthermore, BBB
161 disruption in epilepsy leads to extravasation of serum albumin, which is taken up
162 preferentially by astrocytes and activates them through transforming growth factor beta
163 (Heinemann, Kaufer, & Friedman, 2012). We will now review how dysfunction of several
164 astrocyte properties can contribute to seizure and epilepsy.

165 1.2. *Homeostasis: keeping an eye on K^+ and water*

166 One of the most important physiological functions of astrocytes is the maintenance of ion
167 homeostasis and, in particular, the regulation of extracellular K^+ [K^+]_o. The accumulation of
168 K^+ in the extracellular space can lead to sustained neuronal depolarization and
169 hyperexcitability (Heinemann & Dieter Lux, 1977; Walz, 2000). Astrocytes control [K^+]_o by
170 two mechanisms: K^+ uptake and spatial buffering. The K^+ uptake is mediated through
171 Na^+/K^+ pumps, $Na^+/K^+/Cl^-$ cotransporters and the large expression of inward-rectifier
172 potassium (Kir) 4.1 channels and relies also on the very negative resting potential of
173 astrocytes (D'Ambrosio, Gordon, & Winn, 2002; Kofuji & Newman, 2004; Ransom,
174 Ransom, & Sontheimer, 2000; Seifert et al., 2009). Remarkably, mice lacking Kir4.1
175 channels on astrocytes were impaired for K^+ and glutamate uptake and were more
176 susceptible to seizures (Chever, Djukic, McCarthy, & Amzica, 2010; Djukic, Casper,
177 Philpot, Chin, & McCarthy, 2007). Altered expression of astrocyte Kir4.1 channels has
178 been reported for Alzheimer's disease (Wilcock, Vitek, & Colton, 2009), major depressive
179 disorder (MDD; (Medina et al., 2016) and for Huntington's disease (Tong et al., 2014).
180 Different studies, both in humans and animal models, have highlighted a clear association
181 between uncontrolled extracellular K^+ increase and epilepsy (Steinhäuser et al., 2016). In
182 particular, measurements of extracellular K^+ , patch-clamp recordings of Kir currents in
183 astrocytes, single-cell RT-PCR and immunostaining of Kir proteins revealed a reduction in
184 Kir-mediated current and a reduction of Kir4.1 expression in surgical specimen from
185 patients suffering from MTLE and in rodent models of MTLE (Bordey & Sontheimer, 1998;

186 Heinemann et al., 2000; Heuser et al., 2012; Hinterkeuser et al., 2000; Jauch, Windmüller,
187 Lehmann, Heinemann, & Gabriel, 2002; Kivi et al., 2000; Schröder et al., 2000).

188 Control of ion homeostasis by astrocytes relies also on spatial buffering (Orkand, Nicholls,
189 & Kuffler, 1966), which is mediated by gap junctions (GJ), channels composed of connexin
190 (Cx) 43) and Cx30 (Nagy & Rash, 2000). These channels allow the formation of a
191 functional network between astrocytes (syncytium) and the diffusion of K^+ following the
192 electrochemical gradient: K^+ is taken up by astrocytes where neuronal activity is high,
193 diffuses through the syncytium and is released at remote sites with lower $[K^+]_0$. In line with
194 the spatial buffering concept, the physiological functioning of astroglial networks should
195 have an anti-epileptic role, and indeed, a reduced threshold for generating epileptic activity
196 was found in mice with coupling-deficient astrocytes (Pannasch et al., 2011; Wallraff et al.,
197 2006). Furthermore, evidence suggests that alterations in astrocyte coupling could be
198 involved in the genesis and progression of epilepsy. Using a mouse model of HS-MTLE
199 (unilateral intracortical kainate), it has been demonstrated that astrocyte coupling is
200 impaired as early as 4 hours after the induction of status epilepticus (SE), a mechanism
201 probably related to the release of pro-inflammatory cytokines and thus suggesting that
202 impaired coupling represents a key event in epileptogenesis (Bedner et al., 2015).
203 Moreover, the authors showed a complete lack of GJ coupling in sclerotic hippocampi of
204 HS-MTLE patients.

205 Another important function in which astrocytes are involved is the regulation of
206 extracellular space (ECS) volume and fluid osmolarity through aquaporin 4 (AQP4), a
207 channel mediating transmembrane water movements according to osmotic gradients and
208 that is expressed by astrocytes mostly at perisynaptic processes and perivascular end-feet
209 (Schwartzkroin, Baraban, & Hochman, 1998). Regulating water flux near synapses and
210 blood vessels may be linked to K^+ homeostasis (Papadopoulos & Verkman, 2013). Mice
211 lacking AQP4 have a mild ECS volume expansion, an elevated threshold but prolonged
212 duration for electrographic seizures induced *in vivo* by hippocampal electrical stimulation
213 (Binder & Steinhäuser, 2006; Binder et al., 2006). Subsequent analysis of these mice,
214 however, indicated that they also displayed an enhanced gap junctional coupling and an
215 improved spatial $[K^+]_0$ buffering (Strohschein et al., 2011). In human and in animal models
216 of MTLE, there is a loss of astrocytic perivascular AQP4, which precedes the appearance
217 of chronic seizures (Alvestad et al., 2013; Eid et al., 2005). This suggests that perturbed

218 polarized expression of AQP4 during epileptogenesis, together with consequences on
219 $[K^+]_0$ homeostasis, may be of pathophysiological relevance.

220 *1.3. Neurotransmitter buffering: maintaining the equilibrium for glutamate, GABA*
221 *and purines*

222 A crucial role for astrocytes that can also impact on neuronal excitability is
223 neurotransmitter buffering. Indeed, the removal of neurotransmitters, such as glutamate
224 and GABA, from the synaptic cleft is crucial for maintaining a correct functioning of
225 synapses and an appropriate balance between excitation and inhibition. Alterations in
226 these buffering mechanisms could lead to neuronal hyper-excitability and excitotoxicity
227 (Choi, 1988; Meldrum, 1994). The astrocytic buffering is mediated by neurotransmitter
228 uptake through transporters and their metabolism through the glutamine – glutamate –
229 GABA cycle (Tore Eid, Behar, Dhaher, Bumanglag, & Lee, 2012). Glutamate and GABA
230 reuptake relies on specific transporters expressed by astrocytes: EAAT1 and EAAT2 (also
231 known as GLAST and GLT1) for glutamate, and GAT1 and GAT3 for GABA. Once
232 internalized in astrocytes, glutamate is converted into glutamine by glutamine synthetase
233 (GS), while GABA enters the TCA cycle which produces alpha ketoglutarate, glutamate
234 and glutamine. Glutamine produced in astrocytes is then released, up taken by neurons
235 and used as a precursor for both glutamate and GABA synthesis.

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

242 Alterations in GABA extracellular levels can also contribute to hyper-excitability and
243 epilepsy. Depending on the epileptic syndrome, both decreased extracellular GABA levels
244 and increased GABA receptor mediated activity can be detrimental for the maintenance of
245 excitation / inhibition balance. For example, in TLE an increase in the expression levels of
246 GAT3 astrocytic GABA transporter, leading to a reduction of extracellular GABA levels,
247 has been reported just before seizure onset (During & Spencer, 1993; Lee et al., 2006).
248 On the other hand, in absence epilepsy a dysfunction of the astrocytic GABA transporter
249 GAT1 was responsible for the generation of non-convulsive seizures by determining an

250 increase in GABA-A receptor mediated inhibition in thalamocortical neurons (Cope et al.,
251 2009; Pirrtimaki, Parri, & Crunelli, 2013).

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

262 Another important astrocytic enzyme involved in the balance between excitation and
263 inhibition is adenosine kinase (ADK). By phosphorylating adenosine to adenosine-
264 monophosphate (AMP) it exerts a key role in regulating extracellular levels of adenosine
265 (Etherington et al., 2009; Studer et al., 2006). Since adenosine is known to dampen
266 neuronal activity, its release exerts anti-convulsant effects (Cunha, 2016; Lado & Moshé,
267 2008). Astroglialosis occurring in epilepsy is known to be associated with increased levels of
268 ADK both in humans and in experimental models (Boison, 2016). The increase in ADK and
269 the consequent reduction of extracellular adenosine levels are responsible for reducing
270 seizure threshold (Boison, 2016).

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

273 *1.4. Gliotransmission: sensing and modulating the surrounding environment*

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

278 Several classical neurotransmitters have been also identified as gliotransmitters, among
279 which glutamate, D-serine, ATP, adenosine and GABA have been the most studied
280 (Angulo, Le Meur, Kozlov, Charpak, & Audinat, 2008; Araque et al., 2014; Harada,
281 Kamiya, & Tsuboi, 2016; Papouin, Dunphy, Tolman, Foley, & Haydon, 2017; Petrelli &

282 Bezzi, 2016). An increase in intracellular Ca^{2+} is often crucial for the release of
283 gliotransmitters (Araque et al., 2014; Bazargani & Attwell, 2016; Khakh & Sofroniew, 2015;
284 Rusakov et al., 2014; Volterra, Liaudet, & Savtchouk, 2014) that can be mediated through
285 exocytotic or non-exocytotic pathways (Hamilton & Attwell, 2010; A. Verkhratsky, Matteoli,
286 Parpura, Mothet, & Zorec, 2016) but gliotransmitters can be released also through Ca^{2+}
287 independent mechanisms involving connexin hemichannels or pannexin channels
288 (Abudara, Retamal, Del Rio, & Orellana, 2018), plasma membrane ion channels like P2X7
289 receptors, exchangers such as the cysteine-glutamate antiporter, or reversal of
290 neurotransmitter transporters (Malarkey & Parpura, 2008). It should be noted, however,
291 that the existence, the possible mechanisms of gliotransmission and the functional
292 consequences of the release of gliotransmitters are still matters of debates and
293 controversies and that *in vivo* studies on gliotransmission are still sparse. These issues
294 have been discussed recently in several reviews (Araque et al., 2014; Bazargani & Attwell,
295 2016; Dallérac, Zapata, & Rouach, 2018; Fiacco & McCarthy, 2018; Khakh & Sofroniew,
296 2015; Panatier & Robitaille, 2016; Papouin, Dunphy, Tolman, Foley, et al., 2017; Rusakov
297 et al., 2014; Savtchouk & Volterra, 2018). We will therefore only summarize here findings
298 on hippocampal gliotransmitters other than ATP that are relevant in the context of
299 epilepsy.

300 Following the pioneer work of Parri et al (Parri, Gould, & Crunelli, 2001) in the thalamus,
301 we and others showed that astrocytes in acute slices of the hippocampus release
302 glutamate that induces slow inward currents (SICs) in CA1 pyramidal cells due to the
303 activation of extrasynaptic NMDA receptors (Angulo, Kozlov, Charpak, & Audinat, 2004;
304 Fellin et al., 2004; G Perea & Araque, 2005). SICs generated by a single astrocyte have
305 the capacity to synchronize a small ensemble of neurons whose soma or dendrites are
306 within the morphological domain of this astrocyte. It was later on proposed that this form of
307 gliotransmission could be at the origin of hypersynchronous excitation of neurons in
308 models of epilepsy (Kang, Xu, Xu, Nedergaard, & Kang, 2005; Tian et al., 2005). However,
309 although Ca^{2+} signalling in astrocytes and SICs frequency are enhanced during ictal
310 events triggered in acute hippocampal slices, blocking action potentials with tetrodotoxin
311 blocks ictal events but not SICs whereas blocking NMDA receptors blocks SICs but not
312 ictal events (Fellin, Gomez-Gonzalo, Gobbo, Carmignoto, & Haydon, 2006). Yet, astrocyte
313 glutamate also contribute to set the extracellular concentration of ambient glutamate that
314 tonically activate NMDA receptors of CA1 neurons (Cavelier & Attwell, 2005; Jabaudon et

315 al., 1999; Le Meur, Galante, Angulo, & Audinat, 2007) and regulate synaptic glutamate
316 release through the activation of presynaptic metabotropic glutamate receptors or NMDA
317 receptors in CA1 and in the dentate gyrus, respectively (Jourdain et al., 2007; Gertrudis
318 Perea & Araque, 2007). In the context of epilepsy, *in vitro* models of seizure showed that
319 an excitatory loop involving astrocyte Ca^{2+} signalling, glutamate and ATP signalling lowers
320 seizure threshold for neurons (Gomez-Gonzalo et al., 2010). Moreover, increased
321 astrocyte Ca^{2+} signalling and glutamate release contribute to neuronal cell death in the
322 neocortex *in vivo* after a pilocarpine induced SE (Ding et al., 2007).

323 Finally, it should be noted also that astrocyte Ca^{2+} signalling is modulated by inhibitory
324 interneurons through GABA signalling and the activation of astrocyte GABA-B receptors
325 and GAT-3 transporters (Mariotti, Losi, Sessolo, Marcon, & Carmignoto, 2016; Matos et
326 al., 2018; Mederos et al., 2019) but also through the release of somatostatin by specific
327 interneurons (Mariotti et al., 2018). This interneuron-induced astrocyte Ca^{2+} signalling
328 differentially modulates inhibitory synaptic transmission originating from different types of
329 interneurons (Matos et al., 2018; Mederos & Perea, 2019). Knowing the importance of
330 neuronal inhibitory networks in different forms of epilepsy, such as temporal lobe or focal
331 cortical epilepsies (Trevelyan, Muldoon, Merricks, Racca, & Staley, 2015) changes in the
332 activity or survival of inhibitory interneurons can lead to impaired astrocyte signalling that
333 may be relevant in the context of these epilepsies.

334

335 **2. Astrocyte release of ATP (mechanisms of release)**

336 ATP plays an important role in physiological and pathological conditions, via activating
337 various receptors on glia and neurons (Illes & Verkhratsky, 2016)(Fig. 1). Under
338 physiological conditions, the concentration of ATP in extracellular space is very low.
339 However, it can dramatically increase under pathological conditions such as trauma,
340 ischemia/hypoxia, inflammation or excessive neuronal activity (Engel et al., 2016; Franke,
341 Verkhratsky, Burnstock, & Illes, 2012; Rassendren & Audinat, 2016; Rodrigues, Tome, &
342 Cunha, 2015). The sustained increase of extracellular ATP concentration under
343 pathological conditions indicates that mechanisms regulating ATP release exist.

344 As for other gliotransmitters (see section 1.4), a variety of molecular mechanisms of ATP
345 release from astrocytes have been suggested, including calcium-dependent exocytosis
346 and opening of large conductance channels such as hemichannels and anion channels

347 (Araque et al., 2014; Engel et al., 2016; Hamilton & Attwell, 2010; Harada et al., 2016;
348 Rassendren & Audinat, 2016; Xiong, Sun, Teng, Jin, & Zhou, 2018). Regarding vesicular
349 release, ATP can be packaged into vesicles and co-released with other transmitters
350 (Zimmermann, 1994). A vesicular nucleotide transporter (VNUT) concentrating ATP into
351 vesicles has been identified (Sawada et al., 2008) and is also expressed in non-neuronal
352 cells where it localizes with vesicles of the regulatory secretory pathway (Lazarowski,
353 2012). Moreover, VNUT and fluorescent ATP co-labelled lysosomes in astrocytes have
354 been observed and several groups have shown that astrocytes release ATP by lysosome
355 exocytosis (Oya et al., 2013; Verderio et al., 2012; Zhang et al., 2007). Verderio and co-
356 workers further identified that vesicle-associated membrane protein-7 (VAMP7), which is
357 insensitive to the tetanus toxin, mediated lysosome exocytosis and contributed to ATP
358 release from astrocytes (Verderio et al., 2012). Different functions of astrocyte vesicular
359 release of ATP have been proposed. In cultured astrocytes, ATP vesicular release is the
360 predominant cause of intercellular calcium waves (Bowser & Khakh, 2007). *In vivo*,
361 brainstem astrocytes were shown to control breathing through a pH-dependent vesicular
362 release of ATP (Gourine et al., 2010; Kasymov et al., 2013). Moreover, the use of
363 transgenic mice expressing a dominant-negative cytoplasmic SNARE domain of the
364 synaptobrevin-2 protein specifically in astrocytes (dnSNARE mice) provided evidence
365 indicating that ATP vesicular release from astrocytes regulates synaptic strength and
366 plasticity in the hippocampus (Pascual et al., 2005), sleep homeostasis in the
367 hypothalamus (Halassa et al., 2009), inhibition and induction of long-term potentiation in
368 the neocortex (Lalo et al., 2014; Rasooli-Nejad, Palygin, Lalo, & Pankratov, 2014).
369 Although the specificity of the astrocyte expression of the transgene in these mice has
370 been questioned (Fujita et al., 2014), a more recent report has excluded any neuronal
371 expression in the hippocampus (Papouin, Dunphy, Tolman, Dineley, & Haydon, 2017).
372 Overall, these studies indicate that astrocytes have the capability to release ATP from
373 synaptic-like vesicles and that this process regulates the physiology of different neuronal
374 networks.

375 Additional mechanisms contributing to the release of ATP from astrocytes corresponds to
376 the so-called conductive pathways allowing direct exchange between the cytoplasmic
377 compartment and the extracellular space through large pores (Lazarowski, 2012;
378 Rassendren & Audinat, 2016). The best documented of these pathways is ATP
379 permeation through hemichannels, either constituted by pannexins or by connexins.

380 Although these two families of proteins have similar membrane topology (*i.e.* 4
381 transmembrane domains, 2 extracellular and 1 intracellular loop, along with intracellular N-
382 and C- terminal tails), they have no amino acid sequence homology and differ in their
383 activation mode. Several studies have shown that pannexin-1 (PANX1) can release ATP in
384 physiological conditions in response to the activation of different pathways, including
385 purinergic receptor activation (Bao, Locovei, & Dahl, 2004; Pelegrin & Surprenant, 2006).
386 Since PANX1 is widely expressed and can be activated at membrane potentials in the
387 physiological range, it is considered as the major cause of ATP efflux through
388 hemichannels. However, the positive feedback loop of ATP-induced ATP release may also
389 constitute a danger signal under pathological conditions (Rodrigues et al., 2015) and it
390 should be noted that the activity of PANX1 channels is much higher in acute slices (human
391 and mice) experiencing ictal discharges than in slices without ictal discharges (Dossi,
392 Blauwblomme, et al., 2018). Conditions leading to ATP efflux through hemiconnexins
393 require low extracellular calcium concentration and strong depolarization, indicating that
394 this pathway operates mostly in pathological conditions. The results obtained from
395 pannexin- and connexin-deficient mice have led to somewhat controversial interpretations
396 (Bedner, Steinhauser, & Theis, 2012; Hanstein et al., 2013) but they nevertheless support
397 the idea that hemichannels contribute to the release of ATP, particularly in pathological
398 conditions.

399 Anion channels have long been suspected to mediate ATP release from astrocytes
400 (Kimelberg, MacVicar, & Sontheimer, 2006). In cultured astrocytes, ATP release through
401 Maxi-anion channels may play a role in glutamate-induced release of ATP (Zhao, Gu, Liu,
402 Zhang, & Liu, 2017) whereas volume-regulated anion channels (VRAC) contribute to the
403 propagation of calcium waves (Fujii, Maekawa, & Morita, 2017) through an autocrine
404 mechanism (Akita, Fedorovich, & Okada, 2011). The exact contribution of astrocyte anion
405 channels to physiological and pathological processes *in vivo* remains largely unknown but
406 the recent molecular identification of several subunits constituting these channels should
407 help resolving this issue (Han et al., 2019; Osei-Owusu, Yang, Vitery, & Qiu, 2018). In the
408 context of epilepsy, additional studies are required to evaluate the relative contribution of
409 astrocytic exocytotic and conductive pathways to a rise in ATP in the extracellular space
410 during episodes of intense neuronal activities. Interestingly, PANX1 channel activation was
411 shown recently to promote seizure generation through ATP signalling involving P2
412 receptors in human cortical brain tissue and in a mouse model of MTLE (intra-hippocampal

413 kainate) (Dossi, Blauwblomme, et al., 2018). However, both neuronal and astroglial
414 PANX1 channels were activated in these conditions and the specific role of PANX1-
415 dependent release of ATP from astrocytes remains to be tested.

416

417 **3. Purinergic signalling in the onset and progression of epilepsy**

418 Despite the long-lasting interest in the study of ATP as a signalling messenger the exact
419 roles of purinergic signalling in pathological conditions is far from being elucidated. Once
420 released into the extracellular space, ATP is rapidly hydrolysed to ADP and adenosine by
421 different ectonucleotidases present in the extracellular environment (Haydon &
422 Carmignoto, 2006). Thereby, the release of ATP generates different purines that can
423 activate different types of receptors, including P2X and P2Y receptors as well as
424 adenosine receptors (Rassendren & Audinat, 2016). The activation of these receptors may
425 have a variety of effects on the onset and progression of epilepsy and this will depend on
426 the extracellular concentrations of the different purines. It is commonly believed that these
427 concentrations increase under pathological conditions, (Franke et al., 2012; Rodrigues et
428 al., 2015) but in the context of epilepsy this has only been convincingly established for
429 adenosine (During & Spencer, 1993; Lopatář, Dale, & Frenguelli, 2011). However, a recent
430 *in vivo* study reported increased ATP concentrations after spontaneous seizures in the
431 chronic phase of the rat pilocarpine model (Doná et al., 2016). New genetically encoded
432 sensors based on the use of mutated P2 receptors should help clarifying this issue for ATP
433 and ADP.

434 Adenosine has a well-established anticonvulsant action (Boison, 2012, 2016; Cieślak,
435 Wojtczak, & Komoszyński, 2017; Masino, Kawamura, & Ruskin, 2014). This primarily
436 relies on the activation of adenosine A1 receptors that decrease excitatory synaptic
437 transmission and, for instance, mice lacking A1 receptors have lower threshold of seizure
438 propagation (Boison, 2012; Fedele, Li, Lan, Fredholm, & Boison, 2006) whereas A2 and
439 A3 receptors have rather convulsive or deleterious effects (Cunha, 2016; Matute &
440 Cavaliere, 2011). The astrocytic enzyme adenosine kinase (ADK) appears to play a key
441 role in regulating the anticonvulsant effect of adenosine. The expression of ADK is
442 upregulated during epilepsy, leading to a deficiency in adenosine concentration and thus
443 of A1 receptor activation (Boison, 2016).

444 In contrast, the role of ATP signalling in epilepsy is less well understood. There is,
445 however, a general agreement that ATP signalling contributes the pathology of epilepsy.
446 Direct application of ATP analogues in the brain evokes neuronal excitation and promotes
447 seizure activity (Rassendren & Audinat, 2016). There is a profound remodelling of the
448 expression of hippocampal P2 receptors following SE in rodents (Avignone, Ulmann,
449 Levavasseur, Rassendren, & Audinat, 2008; Engel et al., 2016; Rassendren & Audinat,
450 2016), suggesting that these receptors contribute to epileptogenesis. Analysis of the role
451 of specific P2 receptors led to contrasting results depending on the identity of these
452 receptors, their cellular expression but also on the type of epilepsy or on the experimental
453 model. Many of these studies were directed toward the analysis of P2X7, P2X4 and
454 P2Y12 receptors expressed by microglia and have been reviewed previously (Engel et al.,
455 2016; Eyo & Wu, 2019; Rassendren & Audinat, 2016). Without entering in too many
456 details, it is nevertheless worth mentioning that P2X4 and P2X7 have rather detrimental
457 effects whereas P2Y12 have more protective effects on cell survival and disease
458 progression in experimental SE or TLE. It should also be remembered that purinergic
459 signalling is an important communication pathway between astrocytes and microglia. For
460 instance, microglial cells sense ATP released by astrocytes (Davalos et al., 2005; Schipke,
461 Boucsein, Ohlemeyer, Kirchhoff, & Kettenmann, 2002) and ATP released by microglia
462 enhances Ca²⁺ signalling and glutamate release by astrocytes (O. Pascual, Ben Achour,
463 Rostaing, Triller, & Bessis, 2012). It is therefore very likely that microglia and astrocyte
464 purinergic signalling influence each other in pathological settings known to favour the
465 release of ATP (see above).

466 The role of metabotropic P2Y1 receptors in epilepsy has been also studied. P2Y1 is
467 expressed mostly in astrocytes, oligodendrocytes and in neurons, in particular
468 hippocampal inhibitory interneurons (Bowser & Khakh, 2004). A recent study based on the
469 analysis of P2Y1 deficient mice but also on the use of selective agonists and antagonists
470 of P2Y1 indicates that these receptors have an anticonvulsant action at the onset of SE,
471 either triggered by systemic pilocarpine or by intra-amygdala kainate, but have
472 proconvulsant effects once SE has been initiated and favour spontaneous seizures during
473 the chronic phase of these models (Alves et al., 2019). Surprisingly, the same study
474 reported an up-regulation of P2Y1 in microglia and in neurons, but no expression of these
475 receptors in astrocytes after SE. Considering the many studies involving a role of P2Y1 in
476 astrocytes, cell type specific knockout of P2Y1 receptors are now needed to help clarifying

477 these complex effects (see also below). Overall these observations support the idea that
478 ATP signalling intervenes in epilepsy, but the exact mechanisms involved and their
479 influence on epileptogenesis and ictogenesis are still unclear. However, several
480 mechanisms involving ATP signalling in astrocytes have been recently updated under
481 pathological conditions and in particular in the context of epilepsy.

482

483 **4. Astrocyte ATP-mediated intercellular signalling in epilepsy**

484 ATP is an essential mediator of communication between astrocytes and between
485 astrocytes and other types brain cells (Butt, 2011)(Fig. 1). Whether astrocyte purine-
486 mediated signalling is impaired in epilepsy is an intriguing question receiving increased
487 attention. Genetic impairment of the vesicular ATP release from astrocytes by expressing
488 a dominant-negative SNARE (see above and (Pascual et al., 2005)) delays the onset of
489 recurrent seizures, decrease epileptiform activity and reduce hippocampal damage after a
490 pilocarpine-induced SE (Clasadonte, Dong, Hines, & Haydon, 2013). These effects
491 probably involve the control of NMDA receptor expression that is regulated by adenosine
492 A1 receptor activation following ATP release from astrocyte (Deng, Terunuma, Fellin,
493 Moss, & Haydon, 2011; Fellin et al., 2009). On the other hand, ATP release through
494 PANX1 appears to contribute to ictal discharges in human epileptic brain tissue and in
495 mouse models of MTL (intra-hippocampal kainate) or SE (systemic kainate) (Dossi,
496 Blauwblomme, et al., 2018; Santiago et al., 2011), although in these two studies the
497 source of ATP was not specifically determined. Once released by astrocytes, ATP could
498 have several effects. Selective activation of hippocampal astrocytes by optogenetics was
499 shown to induce ATP release that led to the excitation of CCK-expressing interneurons,
500 through P2Y1 receptor activation, and to the inhibition of pyramidal cells, through A1
501 receptors (Tan et al., 2017). These effects should rather have anti-convulsive effects but it
502 should be noted that they have been observed and studied in the presence of glutamate
503 and GABA-A receptor antagonists, making it difficult to evaluate their consequences on
504 the whole hippocampal network. Using a similar approach, but in absence of blockers of
505 synaptic transmission, we observed that the initial and dominant effect of optogenetic
506 activation of hippocampal astrocytes was an excitation of pyramidal neurons (Shen,
507 Nikolic, Meunier, Pfrieger, & Audinat, 2017). This excitation was the consequence of an
508 autocrine ATP signalling in astrocytes involving P2Y1 receptors and boosting Ca^{2+}

509 signalling and glutamate release from astrocytes. In CA1, this astrocyte glutamate release
510 led to the activation of post-synaptic NMDA receptors and pre-synaptic mGluR receptors
511 on excitatory terminals. Of note, this initial excitation of pyramidal neurons was followed by
512 an inhibition due to A1 receptor activation (Shen et al., 2017), as observed by Tan et al.
513 (2017). It is difficult to extrapolate from these observations made with optogenetic
514 activation of astrocytes in non-epileptic tissue what would be the net effect of astrocyte
515 ATP signalling in the epileptic hippocampus where concentration of purines and
516 expression of their receptors are modified (see section 3 above). However, enhanced Ca^{2+}
517 signalling in astrocytes, P2Y1 receptors and presynaptic mGluR receptors had been
518 already associated with epilepsy. Ca^{2+} signalling is enhanced in cortical astrocytes during
519 several days after pilocarpine-induced SE and contribute to neuronal excitotoxicity (Ding et
520 al., 2007). Ca^{2+} signals in hippocampal astrocytes facilitate the propagation of epileptiform
521 activity induced by kainate *in vivo* (Heuser et al., 2018) and Ca^{2+} signalling is enhanced in
522 astrocyte endfeet *in vivo* during the latent phase of a mouse model of MTLE (unilateral
523 intra-cortical kainate; (Szokol et al., 2015)). In a rat model (rapid kindling) of MTLE,
524 activation of P2Y1 receptors is responsible for astrocyte Ca^{2+} hyperactivity that induces an
525 increase in glutamate release from astrocytes and a downstream activation of neuronal
526 mGluR5, thereby increasing release probability at the CA3-CA1 synapses (Álvarez-
527 Ferradas et al., 2015). Blocking astrocyte Ca^{2+} signalling or inhibiting P2Y1 or mGluR5
528 receptors relieved this abnormal enhancement of synaptic strength. The same group
529 reported subsequently that astrocyte PANX1 hemichannels are the route through which
530 ATP is released before activating P2Y1 receptors and triggering the increased astroglial
531 Ca^{2+} signalling (Wellmann, Álvarez-Ferradas, Maturana, Sáez, & Bonansco, 2018). These
532 observations are consistent with an enhanced autocrine ATP signalling being responsible
533 for an increased glutamate release from astrocytes promoting neuronal excitation in the
534 epileptic hippocampus. It is not clear yet whether this autocrine signalling pathway is
535 involved in the purine-dependent action of astrocytes favouring ictal discharges in human
536 epileptic brain tissue (see above and (Dossi, Blauwblomme, et al., 2018)). It should be
537 noted, however, that in a recent study using a model of SE induced by systemic injection
538 of kainate, targeted deletion of PANX1 from astrocytes lowers the level of ATP but
539 potentiates seizures during SE (Scemes, Velišek, & Velišková, 2019). This effect was
540 found to be related to the lower level of adenosine consecutive to the increased level of
541 ADK in astrocytes.

542 Altogether, the majority of the above cited observations point to the conclusion that the
543 actions of astrocyte purinergic signalling determined by the combined effects of ATP,
544 adenosine and glutamate can displace the equilibrium between excitation and inhibition
545 and promote appearance of epileptiform activity. It is important to note that P2Y1
546 regulation of astrocyte Ca^{2+} signalling described in the context of epilepsy has been also
547 reported in a mouse model of Alzheimer's disease (Delekate et al., 2014; Reichenbach et
548 al., 2018) and in a model of traumatic brain injury (Choo et al., 2013). In both cases,
549 blocking this pathway had beneficial consequences on outcome of these models. Impaired
550 astrocyte purinergic signalling could thus be a common signature of different CNS
551 pathologies.

552 Purinergic interactions between microglia and astrocytes are likely to influence how
553 astrocytes regulate neuronal excitability and epilepsy progression. Together with
554 astrogliosis, reactivity of microglia is another typical feature of human and experimental
555 epilepsies and purinergic signalling is enhanced in both cell types (Devinsky et al., 2013;
556 Rassendren & Audinat, 2016). In the hippocampus of naïve animals, activation of
557 microglial TLR4 receptors rapidly induces a release of ATP that activates astrocytic P2Y1
558 receptors and the release of glutamate by astrocytes, which in turn activates mGluR5
559 receptors on presynaptic glutamatergic terminals (Pascual et al., 2012). This cascade
560 eventually leads to an enhancement of excitability and promotes the appearance of
561 epileptiform activities *in vitro* in experimental conditions where GABA receptors are
562 blocked and external Mg^{2+} is low. Since microglial cells also express purinergic receptors,
563 the release of ATP by astrocytes consecutively to the activation of P2Y1 receptors (see
564 above) could initiate a vicious circle by recruiting further microglia. This possibility has not
565 been specifically tested in the context of epilepsy but purine-mediated crosstalk between
566 these glial cells has been reported in the corpus callosum (Davalos et al., 2005; Schipke et
567 al., 2002) and also in the dentate gyrus following pilocarpine induced SE by showing that
568 blocking microglial P2X7 receptors prevents astroglial death (Kim, Kwak, Jo, & Kang,
569 2009). Interestingly, in a model of traumatic brain injury, reactive microglia induces a
570 protective phenotype of astrocytes by down-regulating the expression of astrocytic P2Y1
571 receptors (Shinozaki et al., 2017).

572 Bidirectional interactions between microglia and astrocytes in epilepsy will not only set the
573 level of purinergic signalling but probably also that of microglial release of cytokines. This
574 is particularly important since cytokines are implicated in the control of glutamate release

575 from astrocytes (see next section). Careful examination of purinergic receptors types that
576 contribute to the crosstalk between astrocytes and microglia is required to better
577 characterize paracrine glial signalling in epilepsy. Clearly more targeted genetic
578 approaches involving deletion or activation of specific type of purinergic receptor on
579 astrocytes or microglia could clarify the actual outcome of such purinergic communication
580 on the functional changes in the epileptic brain.

581

582 **5. Cytokine control of astrocyte purinergic signalling in epilepsy**

583 There is strong evidence that high levels of key pro-inflammatory cytokines, such as tumor
584 necrosis factor alpha (TNF α) and IL-1 β , and downstream signalling that these cytokines
585 promote, contribute to the neuronal hyper-excitability and degeneration in epilepsy (van
586 Vliet, Aronica, Vezzani, & Ravizza, 2018). These neuromodulatory actions of cytokines,
587 distinct from their canonical function in the immune system, could induce seizures by
588 acting on astrocytes in multiple ways. For instance, these cytokines released by microglia
589 inhibit gap junctions in astrocytes *in vitro* (Meme et al., 2006) and in MTLE, inhibition of
590 gap junctions by TNF α and IL-1 β leads to the impairment of astrocytic buffering of
591 extracellular K⁺ (Bedner et al., 2015). Furthermore, TNF α could induce seizures through
592 excessive astrocyte-neuron gliotransmitter mediated signalling. Clues supporting this idea
593 can be drawn from studies showing, on the one hand, that cytokines can boost glutamate
594 release from astrocytes (Vesce, Rossi, Brambilla, & Volterra, 2007) and, on the other
595 hand, that astrocytic glutamate promotes abnormal neuronal activity in epilepsy (see
596 sections 1.4 and 4).

597 In the context of purinergic signalling in astrocytes, TNF α is the key cytokine that can
598 amplify astrocytic glutamate release through coupling with P2Y1 receptors (Fig. 1). It was
599 first shown that binding of TNF α to the astrocytic TNFR1 receptors is a powerful positive
600 modulator of glutamate release from astrocytes (Bezzi et al., 2001). Later on, the same
601 group showed that TNF α controls P2Y1R-dependent glutamate release from astrocytes
602 (Domercq et al., 2006) and that gliotransmission driven by this pathway loses its efficacy in
603 modulating glutamatergic synaptic activity in TNF α knockout mice (Santello, Bezzi, &
604 Volterra, 2011). This stimulation of astrocyte glutamate release by a cytokine was
605 demonstrated in the dentate gyrus and leads to increase the release probability at
606 glutamatergic synapses impinging onto granule cells through the activation of pre-synaptic

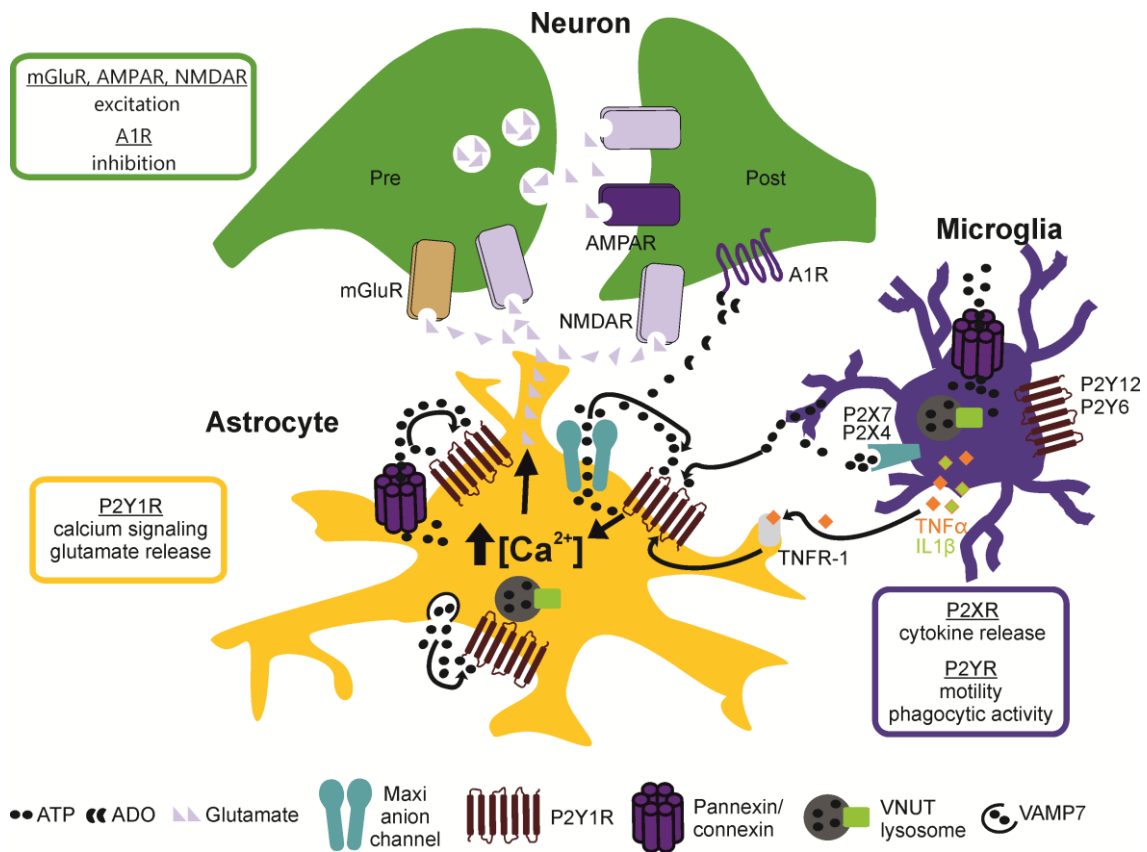
607 NMDA receptors. It is not clear yet whether TNF α also potentiate other outcomes of
608 astrocyte glutamate release in the hippocampus, such as the generation of SICs (Angulo
609 et al., 2004; Fellin et al., 2004) or the mGluR-dependent potentiation of synaptic excitation
610 in CA1 (Gertrudis Perea & Araque, 2007).

611 Although P2Y1R-dependent signalling is one of the main pathways regulating astrocyte-
612 neuron interactions through the release of glutamate, it has not been considered as a
613 specific cytokine target in the epileptic brain. However, TNF α signalling is rapidly
614 upregulated in the hippocampus after SE (Avignone et al., 2008) and astrocytic purinergic
615 P2Y1 signalling is highly active in models of MTLE (see above section 4). Our recent
616 findings indicate that TNF α signalling is one pathway that increases astrocyte purinergic
617 signalling in epilepsy. We indeed confirmed that TNF α triggers a Ca²⁺-dependent
618 glutamate release from astrocytes that boosts excitatory synaptic activity in granule cells of
619 the dentate gyrus through presynaptic NMDA receptors (see above). We further showed
620 that this effect of TNF α can be mimicked by optogenetic activation of astrocyte and
621 demonstrated that this action involves the autocrine activation of astrocyte P2Y1 receptors
622 (Nikolic et al., 2018). In a mouse model of MTLE (unilateral intracortical kainate), this
623 autocrine loop seems permanently activated at the end of the latent phase that precedes
624 the appearance of spontaneous seizures: In the ipsilateral dentate gyrus, the frequency of
625 mEPSCs is higher than in the control conditions, exogenous TNF α does not trigger
626 anymore the increase of excitatory synaptic transmission and blocking P2Y1 receptors,
627 which has no effect in control conditions, reduces excitatory synaptic activity to the level of
628 control conditions (Nikolic et al., 2018). This signalling pathway could therefore be
629 responsible for the lowering of seizure threshold by TNF α (Iori, Frigerio, & Vezzani, 2016)
630 and thus be an important component of the functional remodelling (epileptogenesis) that
631 will drive the hippocampus to spontaneous seizures.

632 Targeting the coupling of TNF α with astrocyte purinergic signalling may thus hold the
633 potential to be a useful therapeutic strategy for reducing astrocytic glutamate release and
634 normalizing synaptic activity in epilepsy. It should be kept in mind, however, that the
635 precise mechanism of the cytokine-P2Y1 interaction in epilepsy is still unclear. A promising
636 candidate to be considered is of course TNFR1 whose expression increased in animal
637 model of seizures and has been reported to mediate the ictogenic effects of TNF α (Iori et
638 al., 2016). Interestingly, TNFR1 has been shown to mediate TNF α effects on astrocyte
639 glutamate signalling in an animal model of multiple sclerosis (Habbas et al., 2015).

640 Inhibiting the function of astrocyte TNFR1, or P2Y1, should prevent the lowering of seizure
641 threshold. Our observation that this TNF α -P2Y1 pathway is activated during the latent
642 phase, *i.e.* before the appearance of the first spontaneous seizures, suggests that
643 targeting this pathway could be a disease-modifying strategy through which the
644 occurrence of spontaneous seizures could be delayed or event prevented.

645



647

648 **Figure 1:** Purinergic signalling between astrocytes, neurons and microglial cells in
 649 epilepsy. The mechanisms of ATP release and the involvement of specific purinergic P2X
 650 and P2Y receptors types are depicted for astrocytes and microglia. Activation of astrocytic
 651 P2Y1 receptors leads to the rise in intracellular Ca²⁺ that promotes astrocyte release of
 652 glutamate, which acts on pre- and post- synaptic neuronal glutamate receptors and
 653 increases excitation. ATP released from astrocytes can be converted into adenosine and
 654 promote activation of adenosine A1 receptors which will favour inhibition. The cytokine
 655 TNF α , likely released by microglia, activates the P2Y1R-dependent glutamate release
 656 from astrocytes and thus neuronal excitation.

657

658 **References**

- 659 Abudara, V., Retamal, M. A., Del Rio, R., & Orellana, J. A. (2018). Synaptic Functions of
660 Hemichannels and Pannexons: A Double-Edged Sword. *Frontiers in Molecular*
661 *Neuroscience*, 11. <https://doi.org/10.3389/fnmol.2018.00435>
- 662 Akita, T., Fedorovich, S. V., & Okada, Y. (2011). Ca²⁺ nanodomain-mediated component
663 of swelling-induced volume-sensitive outwardly rectifying anion current triggered by
664 autocrine action of ATP in mouse astrocytes. *Cellular Physiology and Biochemistry :
665 International Journal of Experimental Cellular Physiology, Biochemistry, and
666 Pharmacology*, 28(6), 1181–1190. <https://doi.org/10.1159/000335867>
- 667 Álvarez-Ferradas, C., Morales, J. C., Wellmann, M., Nualart, F., Roncagliolo, M.,
668 Fuenzalida, M., & Bonansco, C. (2015). Enhanced astroglial Ca²⁺ signaling increases
669 excitatory synaptic strength in the epileptic brain. *GLIA*, 63(9), 1507–1521.
670 <https://doi.org/10.1002/glia.22817>
- 671 Alves, M., De Diego Garcia, L., Conte, G., Jimenez-Mateos, E. M., D’Orsi, B., Sanz-
672 Rodriguez, A., ... Engel, T. (2019). Context-Specific Switch from Anti- to Pro-
673 epileptogenic Function of the P2Y₁ Receptor in Experimental Epilepsy. *The Journal of
674 Neuroscience*, 39(27), 5377–5392. <https://doi.org/10.1523/JNEUROSCI.0089-19.2019>
- 675 Alvestad, S., Hammer, J., Hoddevik, E. H., Skare, Ø., Sonnewald, U., Amiry-Moghaddam,
676 M., & Ottersen, O. P. (2013). Mislocalization of AQP4 precedes chronic seizures in
677 the kainate model of temporal lobe epilepsy. *Epilepsy Research*, 105(1–2), 30–41.
678 <https://doi.org/10.1016/j.eplesyres.2013.01.006>
- 679 Anderson, M. A., Ao, Y., & Sofroniew, M. V. (2014). Heterogeneity of reactive astrocytes.
680 *Neuroscience Letters*, 565, 23–29. <https://doi.org/10.1016/j.neulet.2013.12.030>
- 681 Anderson, M. A., Burda, J. E., Ren, Y., Ao, Y., O’Shea, T. M., Kawaguchi, R., ...
682 Sofroniew, M. V. (2016). Astrocyte scar formation aids central nervous system axon
683 regeneration. *Nature*, 532(7598), 195–200. <https://doi.org/10.1038/nature17623>
- 684 Andreone, B. J., Lacoste, B., & Gu, C. (2015). Neuronal and Vascular Interactions. *Annual
685 Review of Neuroscience*, 38(1), 25–46. <https://doi.org/10.1146/annurev-neuro-071714-033835>
- 687 Angulo, M. C., Kozlov, A. S., Charpak, S., & Audinat, E. (2004). Glutamate released from

688 glial cells synchronizes neuronal activity in the hippocampus. *The Journal of*
689 *Neuroscience : The Official Journal of the Society for Neuroscience*, 24(31), 6920–
690 6927.

691 Angulo, M. C., Le Meur, K., Kozlov, A. S., Charpak, S., & Audinat, E. (2008). GABA, a
692 forgotten gliotransmitter. *Progress in Neurobiology*, 86(3), 297–303.
693 <https://doi.org/10.1016/j.pneurobio.2008.08.002>

694 Araque, A., Carmignoto, G., Haydon, P. G., Oliet, S. H., Robitaille, R., & Volterra, A.
695 (2014). Gliotransmitters travel in time and space. *Neuron*, 81(4), 728–739.
696 <https://doi.org/10.1016/j.neuron.2014.02.007>

697 Asadi-Pooya, A. A., Stewart, G. R., Abrams, D. J., & Sharan, A. (2017). Prevalence and
698 Incidence of Drug-Resistant Mesial Temporal Lobe Epilepsy in the United States.
699 *World Neurosurgery*, 99, 662–666. <https://doi.org/10.1016/j.wneu.2016.12.074>

700 Attwell, D., Buchan, A. M., Charpak, S., Lauritzen, M., Macvicar, B. A., & Newman, E. A.
701 (2010). Glial and neuronal control of brain blood flow. *Nature*, 468(7321), 232–243.
702 <https://doi.org/10.1038/nature09613>

703 Auld, D. S., & Robitaille, R. (2003). Glial cells and neurotransmission: an inclusive view of
704 synaptic function. *Neuron*, 40(2), 389–400. [https://doi.org/10.1016/S0896-](https://doi.org/10.1016/S0896-6273(03)00607-X)
705 [6273\(03\)00607-X](https://doi.org/10.1016/S0896-6273(03)00607-X)

706 Avignone, E., Ulmann, L., Levavasseur, F., Rassendren, F., & Audinat, E. (2008). Status
707 epilepticus induces a particular microglial activation state characterized by enhanced
708 purinergic signaling. *The Journal of Neuroscience : The Official Journal of the Society*
709 *for Neuroscience*, 28(37), 9133–9144.

710 Bao, L., Locovei, S., & Dahl, G. (2004). Pannexin membrane channels are
711 mechanosensitive conduits for ATP. *FEBS Letters*, 572(1–3), 65–68.
712 <https://doi.org/10.1016/j.febslet.2004.07.009>

713 Bazargani, N., & Attwell, D. (2016). Astrocyte calcium signaling: the third wave. *Nature*
714 *Neuroscience*, 19(2), 182–189. <https://doi.org/10.1038/nn.4201>

715 Bedner, P., Dupper, A., Huttman, K., Muller, J., Herde, M. K., Dublin, P., ... Steinhauser,
716 C. (2015). Astrocyte uncoupling as a cause of human temporal lobe epilepsy. *Brain*,
717 138(1460-2156 (Electronic)), 1208–1222.

- 718 Bedner, P., Steinhauser, C., & Theis, M. (2012). Functional redundancy and compensation
719 among members of gap junction protein families? *Biochim.Biophys.Acta*, 1818(0006-
720 3002 (Print)), 1971–1984.
- 721 Beghi, E. (2016). Addressing the burden of epilepsy: Many unmet needs. *Pharmacological*
722 *Research*, 107, 79–84. <https://doi.org/10.1016/j.phrs.2016.03.003>
- 723 Bezzi, P., Domercq, M., Brambilla, L., Galli, R., Schols, D., De Clercq, E., ... Volterra, A.
724 (2001). CXCR4-activated astrocyte glutamate release via TNFalpha: amplification by
725 microglia triggers neurotoxicity. *Nat.Neurosci.*, 4(1097–6256), 702–710.
- 726 Binder, D. K., & Steinhäuser, C. (2006). Functional changes in astroglial cells in epilepsy.
727 *Glia*, 54(5), 358–368. <https://doi.org/10.1002/glia.20394>
- 728 Binder, D. K., Yao, X., Zador, Z., Sick, T. J., Verkman, A. S., & Manley, G. T. (2006).
729 Increased seizure duration and slowed potassium kinetics in mice lacking aquaporin-4
730 water channels. *Glia*, 53(6), 631–636. <https://doi.org/10.1002/glia.20318>
- 731 Boison, D. (2012). Adenosine dysfunction in epilepsy. *Glia*, 60(8), 1234–1243.
732 <https://doi.org/10.1002/glia.22285>
- 733 Boison, D. (2016). Adenosinergic signaling in epilepsy. *Neuropharmacology*, 104, 131–
734 139. <https://doi.org/10.1016/j.neuropharm.2015.08.046>
- 735 Bordey, A., & Sontheimer, H. (1998). Properties of human glial cells associated with
736 epileptic seizure foci. *Epilepsy Research*, 32(1–2), 286–303. Retrieved from
737 <http://www.ncbi.nlm.nih.gov/pubmed/9761328>
- 738 Bowser, D. N., & Khakh, B. S. (2004). ATP excites interneurons and astrocytes to increase
739 synaptic inhibition in neuronal networks. *Journal of Neuroscience*, 24(1529-2401
740 (Electronic)), 8606–8620.
- 741 Bowser, D. N., & Khakh, B. S. (2007). Two forms of single-vesicle astrocyte exocytosis
742 imaged with total internal reflection fluorescence microscopy.
743 *Proc.Natl.Acad.Sci.U.S.A*, 104(0027-8424 (Print)), 4212–4217.
- 744 Butt, A. M. (2011). ATP: A ubiquitous gliotransmitter integrating neuron–glial networks.
745 *Seminars in Cell & Developmental Biology*, 22(2), 205–213.
746 <https://doi.org/10.1016/j.semcdb.2011.02.023>

- 747 Cavelier, P., & Attwell, D. (2005). Tonic release of glutamate by a DIDS-sensitive
748 mechanism in rat hippocampal slices. *J.Physiol*, 564(0022–3751), 397–410.
- 749 Cavus, I., Kasoff, W. S., Cassaday, M. P., Jacob, R., Gueorguieva, R., Sherwin, R. S., ...
750 Abi-Saab, W. M. (2005). Extracellular metabolites in the cortex and hippocampus of
751 epileptic patients. *Annals of Neurology*, 57(2), 226–235.
752 <https://doi.org/10.1002/ana.20380>
- 753 Cavus, I., Pan, J. W., Hetherington, H. P., Abi-Saab, W., Zaveri, H. P., Vives, K. P., ...
754 Spencer, D. D. (2008). Decreased hippocampal volume on MRI is associated with
755 increased extracellular glutamate in epilepsy patients. *Epilepsia*, 49(8), 1358–1366.
756 <https://doi.org/10.1111/j.1528-1167.2008.01603.x>
- 757 Chever, O., Djukic, B., McCarthy, K. D., & Amzica, F. (2010). Implication of Kir4.1 channel
758 in excess potassium clearance: an in vivo study on anesthetized glial-conditional
759 Kir4.1 knock-out mice. *The Journal of Neuroscience : The Official Journal of the*
760 *Society for Neuroscience*, 30(47), 15769–15777.
761 <https://doi.org/10.1523/JNEUROSCI.2078-10.2010>
- 762 Choi, D. W. (1988). Glutamate neurotoxicity and diseases of the nervous system. *Neuron*,
763 1(8), 623–634. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/2908446>
- 764 Choo, A. M., Miller, W. J., Chen, Y.-C., Nibley, P., Patel, T. P., Goletiani, C., ... Meaney,
765 D. F. (2013). Antagonism of purinergic signalling improves recovery from traumatic
766 brain injury. *Brain : A Journal of Neurology*, 136(Pt 1), 65–80.
767 <https://doi.org/10.1093/brain/aws286>
- 768 Cieślak, M., Wojtczak, A., & Komoszyński, M. (2017). Role of the purinergic signaling in
769 epilepsy. *Pharmacological Reports : PR*, 69(1), 130–138.
770 <https://doi.org/10.1016/j.pharep.2016.09.018>
- 771 Clasadonte, J., Dong, J., Hines, D. J., & Haydon, P. G. (2013). Astrocyte control of
772 synaptic NMDA receptors contributes to the progressive development of temporal
773 lobe epilepsy. *Proceedings of the National Academy of Sciences of the United States*
774 *of America*, 110(43), 17540–17545. <https://doi.org/10.1073/pnas.1311967110>
- 775 Cope, D. W., Di, G. G., Fyson, S. J., Orban, G., Errington, A. C., Lorincz, M. L., ... Crunelli,
776 V. (2009). Enhanced tonic GABAA inhibition in typical absence epilepsy. *Nat.Med.*,

777 15(1546-170X (Electronic)), 1392–1398.

778 Coulter, D. A., & Eid, T. (2012). Astrocytic regulation of glutamate homeostasis in epilepsy.
779 *Glia*, 60(8), 1215–1226. <https://doi.org/10.1002/glia.22341>

780 Cresto, N., Pillet, L.-E., Billuart, P., & Rouach, N. (2019). Do Astrocytes Play a Role in
781 Intellectual Disabilities? *Trends in Neurosciences*, 42(8), 518–527.
782 <https://doi.org/10.1016/j.tins.2019.05.011>

783 Cunha, R. A. (2016). How does adenosine control neuronal dysfunction and
784 neurodegeneration? *Journal of Neurochemistry*, 139(6), 1019–1055.
785 <https://doi.org/10.1111/jnc.13724>

786 D'Ambrosio, R., Gordon, D. S., & Winn, H. R. (2002). Differential role of KIR channel and
787 Na(+)/K(+)-pump in the regulation of extracellular K(+) in rat hippocampus. *Journal of*
788 *Neurophysiology*, 87(1), 87–102. <https://doi.org/10.1152/jn.00240.2001>

789 Dallérac, G., & Rouach, N. (2016). Astrocytes as new targets to improve cognitive
790 functions. *Progress in Neurobiology*, 144, 48–67.
791 <https://doi.org/10.1016/j.pneurobio.2016.01.003>

792 Dallérac, G., Zapata, J., & Rouach, N. (2018). Versatile control of synaptic circuits by
793 astrocytes: where, when and how? *Nature Reviews Neuroscience*, 19(12), 729–743.
794 <https://doi.org/10.1038/s41583-018-0080-6>

795 Davalos, D., Grutzendler, J., Yang, G., Kim, J. V., Zuo, Y., Jung, S., ... Gan, W. B. (2005).
796 ATP mediates rapid microglial response to local brain injury in vivo. *Nat. Neurosci.*,
797 8(1097-6256 (Print)), 752–758.

798 Delekate, A., Füchtmeier, M., Schumacher, T., Ulbrich, C., Foddiss, M., & Petzold, G. C.
799 (2014). Metabotropic P2Y1 receptor signalling mediates astrocytic hyperactivity in vivo
800 in an Alzheimer's disease mouse model. *Nature Communications*, 5(July 2015), 5422.
801 <https://doi.org/10.1038/ncomms6422>

802 Deng, Q., Terunuma, M., Fellin, T., Moss, S. J., & Haydon, P. G. (2011). Astrocytic
803 activation of A1 receptors regulates the surface expression of NMDA receptors
804 through a Src kinase dependent pathway. *Glia*, 59(7), 1084–1093.
805 <https://doi.org/10.1002/glia.21181>

- 806 Devinsky, O., Vezzani, A., Najjar, S., De Lanerolle, N. C., & Rogawski, M. A. (2013). Glia
807 and epilepsy: excitability and inflammation. *Trends Neurosci.*, 36(1878-108X
808 (Electronic)), 174–184.
- 809 Ding, S., Fellin, T., Zhu, Y., Lee, S. Y., Auberson, Y. P., Meaney, D. F., ... Haydon, P. G.
810 (2007). Enhanced astrocytic Ca²⁺ signals contribute to neuronal excitotoxicity after
811 status epilepticus. *J Neurosci.*, 27(1529-2401 (Electronic)), 10674–10684. Retrieved
812 from <http://0.0.0.12>
- 813 Djukic, B., Casper, K. B., Philpot, B. D., Chin, L.-S., & McCarthy, K. D. (2007). Conditional
814 Knock-Out of Kir4.1 Leads to Glial Membrane Depolarization, Inhibition of Potassium
815 and Glutamate Uptake, and Enhanced Short-Term Synaptic Potentiation. *Journal of
816 Neuroscience*, 27(42), 11354–11365. [https://doi.org/10.1523/JNEUROSCI.0723-
817 07.2007](https://doi.org/10.1523/JNEUROSCI.0723-07.2007)
- 818 Domercq, M., Brambilla, L., Pilati, E., Marchaland, J., Volterra, A., & Bezzi, P. (2006).
819 P2Y1 receptor-evoked glutamate exocytosis from astrocytes: control by tumor
820 necrosis factor-alpha and prostaglandins. *J.Biol.Chem.*, 281(0021-9258 (Print)),
821 30684–30696.
- 822 Doná, F., Conceição, I. M., Ulrich, H., Ribeiro, E. B., Freitas, T. A., Nencioni, A. L. A., & da
823 Silva Fernandes, M. J. (2016). Variations of ATP and its metabolites in the
824 hippocampus of rats subjected to pilocarpine-induced temporal lobe epilepsy.
825 *Purinergic Signalling*, 12(2), 295–302. <https://doi.org/10.1007/s11302-016-9504-9>
- 826 Dossi, E., Blauwblomme, T., Moulard, J., Chever, O., Vasile, F., Guinard, E., ... Rouach,
827 N. (2018). Pannexin-1 channels contribute to seizure generation in human epileptic
828 brain tissue and in a mouse model of epilepsy. *Science Translational Medicine*,
829 10(443), eaar3796. <https://doi.org/10.1126/scitranslmed.aar3796>
- 830 Dossi, E., Vasile, F., & Rouach, N. (2018). Human astrocytes in the diseased brain. *Brain
831 Research Bulletin*, 136, 139–156. <https://doi.org/10.1016/j.brainresbull.2017.02.001>
- 832 During, M. J., & Spencer, D. D. (1993). Extracellular hippocampal glutamate and
833 spontaneous seizure in the conscious human brain. *The Lancet*, 341(8861), 1607–
834 1610. [https://doi.org/10.1016/0140-6736\(93\)90754-5](https://doi.org/10.1016/0140-6736(93)90754-5)
- 835 Eid, T., Ghosh, A., Wang, Y., Beckstrom, H., Zaveri, H. P., Lee, T.-S. W., ... de Lanerolle,

- 836 N. C. (2008). Recurrent seizures and brain pathology after inhibition of glutamine
837 synthetase in the hippocampus in rats. *Brain*, 131(8), 2061–2070.
838 <https://doi.org/10.1093/brain/awn133>
- 839 Eid, T, Thomas, M. J., Spencer, D. D., Rundén-Pran, E., Lai, J. C. K., Malthankar, G. V., ...
840 de Lanerolle, N. C. (2004). Loss of glutamine synthetase in the human epileptogenic
841 hippocampus: possible mechanism for raised extracellular glutamate in mesial
842 temporal lobe epilepsy. *Lancet (London, England)*, 363(9402), 28–37.
843 [https://doi.org/10.1016/s0140-6736\(03\)15166-5](https://doi.org/10.1016/s0140-6736(03)15166-5)
- 844 Eid, Tore, Behar, K., Dhaher, R., Bumanglag, A. V., & Lee, T.-S. W. (2012). Roles of
845 Glutamine Synthetase Inhibition in Epilepsy. *Neurochemical Research*, 37(11), 2339–
846 2350. <https://doi.org/10.1007/s11064-012-0766-5>
- 847 Eid, Tore, Gruenbaum, S. E., Dhaher, R., Lee, T.-S. W., Zhou, Y., & Danbolt, N. C. (2016).
848 The Glutamate–Glutamine Cycle in Epilepsy. In *Advances in neurobiology* (Vol. 13,
849 pp. 351–400). https://doi.org/10.1007/978-3-319-45096-4_14
- 850 Eid, Tore, Lee, T.-S. W., Thomas, M. J., Amiry-Moghaddam, M., Bjørnsen, L. P., Spencer,
851 D. D., ... de Lanerolle, N. C. (2005). Loss of perivascular aquaporin 4 may underlie
852 deficient water and K⁺ homeostasis in the human epileptogenic hippocampus.
853 *Proceedings of the National Academy of Sciences of the United States of America*,
854 102(4), 1193–1198. <https://doi.org/10.1073/pnas.0409308102>
- 855 Engel, T., Alves, M., Sheedy, C., & Henshall, D. C. (2016). ATPergic signalling during
856 seizures and epilepsy. *Neuropharmacology*, 104, 140–153.
857 <https://doi.org/10.1016/j.neuropharm.2015.11.001>
- 858 Etherington, L.-A. V., Patterson, G. E., Meechan, L., Boison, D., Irving, A. J., Dale, N., &
859 Frenguelli, B. G. (2009). Astrocytic adenosine kinase regulates basal synaptic
860 adenosine levels and seizure activity but not activity-dependent adenosine release in
861 the hippocampus. *Neuropharmacology*, 56(2), 429–437.
862 <https://doi.org/10.1016/j.neuropharm.2008.09.016>
- 863 Eyo, U. B., & Wu, L.-J. (2019). Microglia: Lifelong patrolling immune cells of the brain.
864 *Progress in Neurobiology*, 179, 101614.
865 <https://doi.org/10.1016/j.pneurobio.2019.04.003>

- 866 Fedele, D. E., Li, T., Lan, J. Q., Fredholm, B. B., & Boison, D. (2006). Adenosine A1
867 receptors are crucial in keeping an epileptic focus localized. *Experimental Neurology*,
868 *200*(1), 184–190. <https://doi.org/10.1016/j.expneurol.2006.02.133>
- 869 Fellin, T., Gomez-Gonzalo, M., Gobbo, S., Carmignoto, G., & Haydon, P. G. (2006).
870 Astrocytic glutamate is not necessary for the generation of epileptiform neuronal
871 activity in hippocampal slices. *J Neurosci.*, *26*(1529-2401 (Electronic)), 9312–9322.
872 Retrieved from <http://0.0.0.13>
- 873 Fellin, T., Halassa, M. M., Terunuma, M., Succol, F., Takano, H., Frank, M., ... Haydon, P.
874 G. (2009). Endogenous nonneuronal modulators of synaptic transmission control
875 cortical slow oscillations in vivo. *Proc.Natl.Acad.Sci.U.S.A.*, *106*(1091-6490
876 (Electronic)), 15037–15042.
- 877 Fellin, T., Pascual, O., Gobbo, S., Pozzan, T., Haydon, P. G., & Carmignoto, G. (2004).
878 Neuronal synchrony mediated by astrocytic glutamate through activation of
879 extrasynaptic NMDA receptors. *Neuron*, *43*(0896–6273), 729–743.
- 880 Fiacco, T. A., & McCarthy, K. D. (2018). Multiple Lines of Evidence Indicate That
881 Gliotransmission Does Not Occur under Physiological Conditions. *The Journal of*
882 *Neuroscience*, *38*(1), 3–13. <https://doi.org/10.1523/JNEUROSCI.0016-17.2017>
- 883 Franke, H., Verkhratsky, A., Burnstock, G., & Illes, P. (2012). Pathophysiology of astroglial
884 purinergic signalling. *Purinergic Signalling*. <https://doi.org/10.1007/s11302-012-9300-0>
- 885 Fujii, Y., Maekawa, S., & Morita, M. (2017). Astrocyte calcium waves propagate proximally
886 by gap junction and distally by extracellular diffusion of ATP released from volume-
887 regulated anion channels. *Scientific Reports*, *7*(1), 13115.
888 <https://doi.org/10.1038/s41598-017-13243-0>
- 889 Fujita, T., Chen, M. J., Li, B., Smith, N. A., Peng, W., Sun, W., ... Nedergaard, M. (2014).
890 Neuronal Transgene Expression in Dominant-Negative SNARE Mice. *Journal of*
891 *Neuroscience*, *34*(50), 16594–16604. [https://doi.org/10.1523/JNEUROSCI.2585-](https://doi.org/10.1523/JNEUROSCI.2585-14.2014)
892 [14.2014](https://doi.org/10.1523/JNEUROSCI.2585-14.2014)
- 893 Gomez-Gonzalo, M., Losi, G., Chiavegato, A., Zonta, M., Cammarota, M., Brondi, M., ...
894 Carmignoto, G. (2010). An excitatory loop with astrocytes contributes to drive neurons
895 to seizure threshold. *PLoS Biology*, *8*(4), e1000352.

- 896 <https://doi.org/10.1371/journal.pbio.1000352>
- 897 Gourine, A. V, Kasymov, V., Marina, N., Tang, F., Figueiredo, M. F., Lane, S., ...
898 Kasparov, S. (2010). Astrocytes control breathing through pH-dependent release of
899 ATP. *Science*, 329(1095-9203 (Electronic)), 571–575.
- 900 Habbas, S., Santello, M., Becker, D., Stubbe, H., Zappia, G., Liaudet, N., ... Volterra, A.
901 (2015). Neuroinflammatory TNFalpha Impairs Memory via Astrocyte Signaling. *Cell*,
902 163(7), 1730–1741. <https://doi.org/10.1016/j.cell.2015.11.023>
- 903 Halassa, M. M., Fellin, T., & Haydon, P. G. (2007). The tripartite synapse: roles for
904 gliotransmission in health and disease. *Trends in Molecular Medicine*, 13(2), 54–63.
905 <https://doi.org/10.1016/j.molmed.2006.12.005>
- 906 Halassa, M. M., Florian, C., Fellin, T., Munoz, J. R., Lee, S. Y., Abel, T., ... Frank, M. G.
907 (2009). Astrocytic modulation of sleep homeostasis and cognitive consequences of
908 sleep loss. *Neuron*, 61(2), 213–219. <https://doi.org/10.1016/j.neuron.2008.11.024>
- 909 Hamilton, N. B., & Attwell, D. (2010). Do astrocytes really exocytose neurotransmitters?
910 *Nat.Rev.Neurosci.*, 11(1471-0048 (Electronic)), 227–238.
- 911 Han, Y.-E., Kwon, J., Won, J., An, H., Jang, M. W., Woo, J., ... Lee, C. J. (2019). Tweety-
912 homolog (Ttyh) Family Encodes the Pore-forming Subunits of the Swelling-dependent
913 Volume-regulated Anion Channel (VRAC_{swell}) in the Brain. *Experimental*
914 *Neurobiology*, 28(2), 183. <https://doi.org/10.5607/en.2019.28.2.183>
- 915 Hanstein, R., Negoro, H., Patel, N. K., Charollais, A., Meda, P., Spray, D. C., ... Scemes,
916 E. (2013). Promises and pitfalls of a Pannexin1 transgenic mouse line. *Frontiers in*
917 *Pharmacology*, 4, 61. <https://doi.org/10.3389/fphar.2013.00061>
- 918 Harada, K., Kamiya, T., & Tsuboi, T. (2016). Gliotransmitter Release from Astrocytes:
919 Functional, Developmental, and Pathological Implications in the Brain. *Frontiers in*
920 *Neuroscience*, 9. <https://doi.org/10.3389/fnins.2015.00499>
- 921 Haydon, P. G., & Carmignoto, G. (2006). Astrocyte Control of Synaptic Transmission and
922 Neurovascular Coupling. *Physiological Reviews*, 86(3), 1009–1031.
923 <https://doi.org/10.1152/physrev.00049.2005>
- 924 Heinemann, U, Gabriel, S., Jauch, R., Schulze, K., Kivi, A., Eilers, A., ... Lehmann, T. N.

- 925 (2000). Alterations of glial cell function in temporal lobe epilepsy. *Epilepsia*, 41 Suppl
926 6, S185-9. Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10999542>
- 927 Heinemann, U, Kaufer, D., & Friedman, A. (2012). Blood-brain barrier dysfunction,
928 TGFbeta signaling, and astrocyte dysfunction in epilepsy. *Glia*, 60(1098-1136
929 (Electronic)), 1251–1257.
- 930 Heinemann, Uwe, & Dieter Lux, H. (1977). Ceiling of stimulus induced rises in extracellular
931 potassium concentration in the cerebral cortex of cat. *Brain Research*, 120(2), 231–
932 249. [https://doi.org/10.1016/0006-8993\(77\)90903-9](https://doi.org/10.1016/0006-8993(77)90903-9)
- 933 Hesdorffer, D. C., Logroscino, G., Benn, E. K. T., Katri, N., Cascino, G., & Hauser, W. A.
934 (2011). Estimating risk for developing epilepsy: A population-based study in
935 Rochester, Minnesota. *Neurology*, 76(1), 23–27.
936 <https://doi.org/10.1212/WNL.0b013e318204a36a>
- 937 Heuser, K., Eid, T., Lauritzen, F., Thoren, A. E., Vindedal, G. F., Taubøll, E., ... Lanerolle,
938 N. C. de. (2012). Loss of Perivascular Kir4.1 Potassium Channels in the Sclerotic
939 Hippocampus of Patients With Mesial Temporal Lobe Epilepsy. *Journal of*
940 *Neuropathology & Experimental Neurology*, 71(9), 814–825.
941 <https://doi.org/10.1097/NEN.0b013e318267b5af>
- 942 Heuser, K., Nome, C. G., Pettersen, K. H., Åbjørnsbråten, K. S., Jensen, V., Tang, W., ...
943 Enger, R. (2018). Ca²⁺ Signals in Astrocytes Facilitate Spread of Epileptiform Activity.
944 *Cerebral Cortex*, 28(11), 4036–4048. <https://doi.org/10.1093/cercor/bhy196>
- 945 Hinterkeuser, S., Schröder, W., Hager, G., Seifert, G., Blümcke, I., Elger, C. E., ...
946 Steinhäuser, C. (2000). Astrocytes in the hippocampus of patients with temporal lobe
947 epilepsy display changes in potassium conductances. *The European Journal of*
948 *Neuroscience*, 12(6), 2087–2096. Retrieved from
949 <http://www.ncbi.nlm.nih.gov/pubmed/10886348>
- 950 Illes, P., & Verkhratsky, A. (2016). Purinergic neurone-glia signalling in cognitive-related
951 pathologies. *Neuropharmacology*, 104, 62–75.
952 <https://doi.org/10.1016/j.neuropharm.2015.08.005>
- 953 Iori, V., Frigerio, F., & Vezzani, A. (2016). Modulation of neuronal excitability by immune
954 mediators in epilepsy. *Current Opinion in Pharmacology*, 26, 118–123.

- 955 <https://doi.org/10.1016/j.coph.2015.11.002>
- 956 Jabaudon, D., Shimamoto, K., Yasuda-Kamatani, Y., Scanziani, M., Gahwiler, B. H., &
957 Gerber, U. (1999). Inhibition of uptake unmasks rapid extracellular turnover of
958 glutamate of nonvesicular origin. *Proc.Natl.Acad.Sci.U.S.A*, 96(0027–8424), 8733–
959 8738.
- 960 Jauch, R., Windmüller, O., Lehmann, T.-N., Heinemann, U., & Gabriel, S. (2002). Effects
961 of barium, furosemide, ouabaine and 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid
962 (DIDS) on ionophoretically-induced changes in extracellular potassium concentration
963 in hippocampal slices from rats and from patients with epilepsy. *Brain Research*,
964 925(1), 18–27. [https://doi.org/10.1016/S0006-8993\(01\)03254-1](https://doi.org/10.1016/S0006-8993(01)03254-1)
- 965 Jourdain, P., Bergersen, L. H., Bhaukaurally, K., Bezzi, P., Santello, M., Domercq, M., ...
966 Volterra, A. (2007). Glutamate exocytosis from astrocytes controls synaptic strength.
967 *Nat.Neurosci.*, 10(1097-6256 (Print)), 331–339.
- 968 Kang, N., Xu, J., Xu, Q., Nedergaard, M., & Kang, J. (2005). Astrocytic glutamate release-
969 induced transient depolarization and epileptiform discharges in hippocampal CA1
970 pyramidal neurons. *J.Neurophysiol.*, 94(0022-3077 (Print)), 4121–4130.
- 971 Kasymov, V., Larina, O., Castaldo, C., Marina, N., Patrushev, M., Kasparov, S., & Gourine,
972 A. V. (2013). Differential Sensitivity of Brainstem versus Cortical Astrocytes to
973 Changes in pH Reveals Functional Regional Specialization of Astroglia. *Journal of*
974 *Neuroscience*, 33(2), 435–441. <https://doi.org/10.1523/JNEUROSCI.2813-12.2013>
- 975 Khakh, B. S., & Sofroniew, M. V. (2015). Diversity of astrocyte functions and phenotypes in
976 neural circuits. *Nature Neuroscience*, 18(7), 942–952. <https://doi.org/10.1038/nn.4043>
- 977 Kim, J.-E., Kwak, S.-E., Jo, S.-M., & Kang, T.-C. (2009). Blockade of P2X receptor
978 prevents astroglial death in the dentate gyrus following pilocarpine-induced status
979 epilepticus. *Neurological Research*, 31(9), 982–988.
980 <https://doi.org/10.1179/174313209X389811>
- 981 Kimelberg, H. K., MacVicar, B. A., & Sontheimer, H. (2006). Anion channels in astrocytes:
982 biophysics, pharmacology, and function. *Glia*, 54(0894-1491 (Print)), 747–757.
- 983 Kivi, A., Lehmann, T. N., Kovacs, R., Eilers, A., Jauch, R., Meencke, H. J., ... Gabriel, S.
984 (2000). Effects of barium on stimulus-induced rises of [K⁺]_o in human epileptic non-

- 985 sclerotic and sclerotic hippocampal area CA1. *Eur.J Neurosci.*, 12(0953-816X (Print)),
986 2039–2048.
- 987 Kofuji, P., & Newman, E. A. (2004). Potassium buffering in the central nervous system.
988 *Neuroscience*, 129(4), 1043–1054.
989 <https://doi.org/10.1016/j.neuroscience.2004.06.008>
- 990 Lado, F. A., & Moshé, S. L. (2008). How do seizures stop? *Epilepsia*, 49(10), 1651–1664.
991 <https://doi.org/10.1111/j.1528-1167.2008.01669.x>
- 992 Lalo, U., Palygin, O., Rasooli-Nejad, S., Andrew, J., Haydon, P. G., & Pankratov, Y.
993 (2014). Exocytosis of ATP From Astrocytes Modulates Phasic and Tonic Inhibition in
994 the Neocortex. *PLoS Biology*, 12(1). <https://doi.org/10.1371/journal.pbio.1001747>
- 995 Lazarowski, E. R. (2012). Vesicular and conductive mechanisms of nucleotide release.
996 *Purinergic Signalling*, 8(3), 359–373. <https://doi.org/10.1007/s11302-012-9304-9>
- 997 Le Meur, K., Galante, M., Angulo, M. C. M. C., & Audinat, E. (2007). Tonic activation of
998 NMDA receptors by ambient glutamate of non-synaptic origin in the rat hippocampus.
999 *The Journal of Physiology*, 580(Pt. 2), 373–383.
1000 <https://doi.org/10.1113/jphysiol.2006.123570>
- 1001 Lee, T.-S., Bjørnsen, L. P., Paz, C., Kim, J. H., Spencer, S. S., Spencer, D. D., ... de
1002 Lanerolle, N. C. (2006). GAT1 and GAT3 expression are differently localized in the
1003 human epileptogenic hippocampus. *Acta Neuropathologica*, 111(4), 351–363.
1004 <https://doi.org/10.1007/s00401-005-0017-9>
- 1005 Lian, H., Yang, L., Cole, A., Sun, L., Chiang, A. C.-A., Fowler, S. W., ... Zheng, H. (2015).
1006 NFκB-activated astroglial release of complement C3 compromises neuronal
1007 morphology and function associated with Alzheimer’s disease. *Neuron*, 85(1), 101–
1008 115. <https://doi.org/10.1016/j.neuron.2014.11.018>
- 1009 Liddelw, S. A., & Barres, B. A. (2017). Reactive Astrocytes: Production, Function, and
1010 Therapeutic Potential. *Immunity*, 46(6), 957–967.
1011 <https://doi.org/10.1016/j.immuni.2017.06.006>
- 1012 Liddelw, S. A., Guttenplan, K. A., Clarke, L. E., Bennett, F. C., Bohlen, C. J., Schirmer, L.,
1013 ... Barres, B. A. (2017). Neurotoxic reactive astrocytes are induced by activated
1014 microglia. *Nature*, 541(7638), 481–487. <https://doi.org/10.1038/nature21029>

- 1015 Lopatář, J., Dale, N., & Frenguelli, B. G. (2011). Minor contribution of ATP P2 receptors to
1016 electrically-evoked electrographic seizure activity in hippocampal slices: Evidence
1017 from purine biosensors and P2 receptor agonists and antagonists.
1018 *Neuropharmacology*, 61(1–2), 25–34.
1019 <https://doi.org/10.1016/j.neuropharm.2011.02.011>
- 1020 MacDonald, A. J., Robb, J. L., Morrissey, N. A., Beall, C., & Ellacott, K. L. J. (2019).
1021 Astrocytes in neuroendocrine systems: An overview. *Journal of Neuroendocrinology*,
1022 31(5), e12726. <https://doi.org/10.1111/jne.12726>
- 1023 Malarkey, E. B., & Parpura, V. (2008). Mechanisms of glutamate release from astrocytes.
1024 *Neurochemistry International*, 52(1–2), 142–154.
1025 <https://doi.org/10.1016/j.neuint.2007.06.005>
- 1026 Marchi, N., Granata, T., & Janigro, D. (2014). Inflammatory pathways of seizure disorders.
1027 *Trends in Neurosciences*, 37(1878-108X (Electronic)), 55–65.
1028 <https://doi.org/10.1016/j.tins.2013.11.002>
- 1029 Mariotti, L., Losi, G., Lia, A., Melone, M., Chiavegato, A., Gómez-Gonzalo, M., ...
1030 Carmignoto, G. (2018). Interneuron-specific signaling evokes distinctive somatostatin-
1031 mediated responses in adult cortical astrocytes. *Nature Communications*, 9(1), 82.
1032 <https://doi.org/10.1038/s41467-017-02642-6>
- 1033 Mariotti, L., Losi, G., Sessolo, M., Marcon, I., & Carmignoto, G. (2016). The inhibitory
1034 neurotransmitter GABA evokes long-lasting Ca²⁺ oscillations in cortical astrocytes.
1035 *Glia*, 64(3), 363–373. <https://doi.org/10.1002/glia.22933>
- 1036 Maroso, M., Balosso, S., Ravizza, T., Liu, J., Aronica, E., Iyer, A. M., ... Vezzani, A.
1037 (2010). Toll-like receptor 4 and high-mobility group box-1 are involved in ictogenesis
1038 and can be targeted to reduce seizures. *Nat.Med.*, 16(1546-170X (Electronic)), 413–
1039 419.
- 1040 Masino, S. A., Kawamura, M., & Ruskin, D. N. (2014). Adenosine Receptors and Epilepsy.
1041 In *International review of neurobiology* (Vol. 119, pp. 233–255).
1042 <https://doi.org/10.1016/B978-0-12-801022-8.00011-8>
- 1043 Matos, M., Bosson, A., Riebe, I., Reynell, C., Vallée, J., Laplante, I., ... Lacaille, J.-C.
1044 (2018). Astrocytes detect and upregulate transmission at inhibitory synapses of

- 1045 somatostatin interneurons onto pyramidal cells. *Nature Communications*, 9(1), 4254.
1046 <https://doi.org/10.1038/s41467-018-06731-y>
- 1047 Matute, C., & Cavaliere, F. (2011). Neuroglial interactions mediated by purinergic
1048 signalling in the pathophysiology of CNS disorders. *Seminars in Cell & Developmental*
1049 *Biology*, 22(2), 252–259. <https://doi.org/10.1016/j.semcdb.2011.02.011>
- 1050 Mederos, S., Hernández-Vivanco, A., Ramírez-Franco, J., Martín-Fernández, M.,
1051 Navarrete, M., Yang, A., ... Perea, G. (2019). Melanopsin for precise optogenetic
1052 activation of astrocyte-neuron networks. *Glia*, 67(5), 915–934.
1053 <https://doi.org/10.1002/glia.23580>
- 1054 Mederos, S., & Perea, G. (2019). GABAergic-astrocyte signaling: A refinement of inhibitory
1055 brain networks. *Glia*, glia.23644. <https://doi.org/10.1002/glia.23644>
- 1056 Medina, A., Watson, S. J., Bunney, W., Myers, R. M., Schatzberg, A., Barchas, J., ...
1057 Thompson, R. C. (2016). Evidence for alterations of the glial syncytial function in
1058 major depressive disorder. *Journal of Psychiatric Research*, 72, 15–21.
1059 <https://doi.org/10.1016/j.jpsychires.2015.10.010>
- 1060 Meldrum, B. S. (1994). The role of glutamate in epilepsy and other CNS disorders.
1061 *Neurology*, 44(11 Suppl 8), S14-23. Retrieved from
1062 <http://www.ncbi.nlm.nih.gov/pubmed/7970002>
- 1063 Meme, W., Calvo, C.-F. F., Froger, N., Ezan, P., Amigou, E., Koulakoff, A., ... Giaume, C.
1064 (2006). Proinflammatory cytokines released from microglia inhibit gap junctions in
1065 astrocytes: potentiation by beta-amyloid. *FASEB Journal*, 20(1530-6860 (Electronic)),
1066 494–496. <https://doi.org/10.1096/fj.05-4297fje>
- 1067 Messing, A., Brenner, M., Feany, M. B., Nedergaard, M., & Goldman, J. E. (2012).
1068 Alexander Disease. *Journal of Neuroscience*, 32(15), 5017–5023.
1069 <https://doi.org/10.1523/JNEUROSCI.5384-11.2012>
- 1070 Nagy, J. I., & Rash, J. E. (2000). Connexins and gap junctions of astrocytes and
1071 oligodendrocytes in the CNS. *Brain Research. Brain Research Reviews*, 32(1), 29–44.
1072 Retrieved from <http://www.ncbi.nlm.nih.gov/pubmed/10751655>
- 1073 Nikolic, L., Shen, W., Nobili, P., Virenque, A., Ulmann, L., & Audinat, E. (2018). Blocking
1074 TNF α -driven astrocyte purinergic signaling restores normal synaptic activity during

1075 epileptogenesis. *GLIA*, 66(12), 2673–2683. <https://doi.org/10.1002/glia.23519>

1076 Noè, F., Cattalini, A., Vila Verde, D., Alessi, C., Colciaghi, F., Figini, M., ... Curtis, M.
1077 (2019). Epileptiform activity contralateral to unilateral hippocampal sclerosis does not
1078 cause the expression of brain damage markers. *Epilepsia*, 60(6), epi.15611.
1079 <https://doi.org/10.1111/epi.15611>

1080 Orkand, R. K., Nicholls, J. G., & Kuffler, S. W. (1966). Effect of nerve impulses on the
1081 membrane potential of glial cells in the central nervous system of amphibia. *Journal of*
1082 *Neurophysiology*, 29(4), 788–806. <https://doi.org/10.1152/jn.1966.29.4.788>

1083 Ortinski, P. I., Dong, J., Mungenast, A., Yue, C., Takano, H., Watson, D. J., ... Coulter, D.
1084 A. (2010). Selective induction of astrocytic gliosis generates deficits in neuronal
1085 inhibition. *Nature Neuroscience*, 13(5), 584–591. <https://doi.org/10.1038/nn.2535>

1086 Osei-Owusu, J., Yang, J., Vitery, M. D. C., & Qiu, Z. (2018). Molecular Biology and
1087 Physiology of Volume-Regulated Anion Channel (VRAC). *Current Topics in*
1088 *Membranes*, 81, 177–203. <https://doi.org/10.1016/bs.ctm.2018.07.005>

1089 Oya, M., Kitaguchi, T., Yanagihara, Y., Numano, R., Kakeyama, M., Ikematsu, K., &
1090 Tsuboi, T. (2013). Vesicular nucleotide transporter is involved in ATP storage of
1091 secretory lysosomes in astrocytes. *Biochemical and Biophysical Research*
1092 *Communications*, 438(1), 145–151. <https://doi.org/10.1016/j.bbrc.2013.07.043>

1093 Panatier, A., & Robitaille, R. (2016). Astrocytic mGluR5 and the tripartite synapse.
1094 *Neuroscience*. <https://doi.org/10.1016/j.neuroscience.2015.03.063>

1095 Pannasch, U., Vargova, L., Reingruber, J., Ezan, P., Holcman, D., Giaume, C., ... Rouach,
1096 N. (2011). Astroglial networks scale synaptic activity and plasticity.
1097 *Proc.Natl.Acad.Sci.U.S.A*, 108(1091-6490 (Electronic)), 8467–8472.

1098 Papadopoulos, M. C., & Verkman, A. S. (2013). Aquaporin water channels in the nervous
1099 system. *Nature Reviews. Neuroscience*, 14(4), 265–277.
1100 <https://doi.org/10.1038/nrn3468>

1101 Papouin, T., Dunphy, J. M., Tolman, M., Dineley, K. T., & Haydon, P. G. (2017). Septal
1102 Cholinergic Neuromodulation Tunes the Astrocyte-Dependent Gating of Hippocampal
1103 NMDA Receptors to Wakefulness. *Neuron*, 94(4), 840-854.e7.
1104 <https://doi.org/10.1016/j.neuron.2017.04.021>

- 1105 Papouin, T., Dunphy, J., Tolman, M., Foley, J. C., & Haydon, P. G. (2017). Astrocytic
1106 control of synaptic function. *Philosophical Transactions of the Royal Society B:
1107 Biological Sciences*, 372(1715), 20160154. <https://doi.org/10.1098/rstb.2016.0154>
- 1108 Parri, H. R., Gould, T. M., & Crunelli, V. (2001). Spontaneous astrocytic Ca²⁺ oscillations
1109 in situ drive NMDAR-mediated neuronal excitation. *Nat. Neurosci.*, 4(1097–6256),
1110 803–812.
- 1111 Pascual, O., Ben Achour, S., Rostaing, P., Triller, A., & Bessis, A. (2012). Microglia
1112 activation triggers astrocyte-mediated modulation of excitatory neurotransmission.
1113 *Proceedings of the National Academy of Sciences*, 109(4), E197–E205.
1114 <https://doi.org/10.1073/pnas.1111098109>
- 1115 Pascual, Olivier, Casper, K. B., Kubera, C., Zhang, J., Revilla-Sanchez, R., Sul, J.-Y. Y.,
1116 ... Haydon, P. G. (2005). Astrocytic Purinergic Signaling Coordinates Synaptic
1117 Networks. *Science*, 310(5745), 113–116. <https://doi.org/10.1126/science.1116916>
- 1118 Patel, D. C., Tewari, B. P., Chaunsali, L., & Sontheimer, H. (2019). Neuron–glia
1119 interactions in the pathophysiology of epilepsy. *Nature Reviews Neuroscience*, 20(5),
1120 282–297. <https://doi.org/10.1038/s41583-019-0126-4>
- 1121 Pelegrin, P., & Surprenant, A. (2006). Pannexin-1 mediates large pore formation and
1122 interleukin-1beta release by the ATP-gated P2X7 receptor. *EMBO J.*, 25(0261-4189
1123 (Print)), 5071–5082.
- 1124 Pellerin, L., Bouzier-Sore, A.-K., Aubert, A., Serres, S., Merle, M., Costalat, R., &
1125 Magistretti, P. J. (2007). Activity-dependent regulation of energy metabolism by
1126 astrocytes: An update. *Glia*, 55(12), 1251–1262. <https://doi.org/10.1002/glia.20528>
- 1127 Perea, G, & Araque, A. (2005). Properties of synaptically evoked astrocyte calcium signal
1128 reveal synaptic information processing by astrocytes. *Journal of Neuroscience*,
1129 25(1529-2401 (Electronic)), 2192–2203.
- 1130 Perea, G, Navarrete, M., & Araque, A. (2009). Tripartite synapses: astrocytes process and
1131 control synaptic information. *Trends Neurosci.*, 32(1878-108X (Electronic)), 421–431.
- 1132 Perea, Gertrudis, & Araque, A. (2007). Astrocytes potentiate transmitter release at single
1133 hippocampal synapses. *Science*, 317(5841), 1083–1086.
1134 <https://doi.org/10.1126/science.1144640>

- 1135 Petrelli, F., & Bezzi, P. (2016). Novel insights into gliotransmitters. *Current Opinion in*
1136 *Pharmacology*, 26, 138–145. <https://doi.org/10.1016/j.coph.2015.11.010>
- 1137 Pirttimaki, T., Parri, H. R., & Crunelli, V. (2013). Astrocytic GABA transporter GAT-1
1138 dysfunction in experimental absence seizures. *The Journal of Physiology*, 591(4),
1139 823–833. <https://doi.org/10.1113/jphysiol.2012.242016>
- 1140 Ransom, C. B., Ransom, B. R., & Sontheimer, H. (2000). Activity-dependent extracellular
1141 K⁺ accumulation in rat optic nerve: the role of glial and axonal Na⁺ pumps. *The*
1142 *Journal of Physiology*, 522 Pt 3(3), 427–442. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-7793.2000.00427.x)
1143 [7793.2000.00427.x](https://doi.org/10.1111/j.1469-7793.2000.00427.x)
- 1144 Rasooli-Nejad, S., Palygin, O., Lalo, U., & Pankratov, Y. (2014). Cannabinoid receptors
1145 contribute to astroglial Ca²⁺ -signalling and control of synaptic plasticity in the
1146 neocortex. *Philosophical Transactions of the Royal Society B: Biological Sciences*,
1147 369(1654), 20140077. <https://doi.org/10.1098/rstb.2014.0077>
- 1148 Rassendren, F., & Audinat, E. (2016). Purinergic signaling in epilepsy. *Journal of*
1149 *Neuroscience Research*, 94(9), 781–793. <https://doi.org/10.1002/jnr.23770>
- 1150 Reichenbach, N., Delekate, A., Breithausen, B., Keppler, K., Poll, S., Schulte, T., ...
1151 Petzold, G. C. (2018). P2Y1 receptor blockade normalizes network dysfunction and
1152 cognition in an Alzheimer's disease model. *The Journal of Experimental Medicine*,
1153 215(6), 1649–1663. <https://doi.org/10.1084/jem.20171487>
- 1154 Robel, S., Buckingham, S. C., Boni, J. L., Campbell, S. L., Danbolt, N. C., Riedemann, T.,
1155 ... Sontheimer, H. (2015). Reactive Astroglialosis Causes the Development of
1156 Spontaneous Seizures. *Journal of Neuroscience*, 35(8), 3330–3345.
1157 <https://doi.org/10.1523/JNEUROSCI.1574-14.2015>
- 1158 Rodrigues, R. J., Tome, A. R., & Cunha, R. A. (2015). ATP as a multi-target danger signal
1159 in the brain. *Front Neurosci.*, 9(1662-4548 (Print)), 148.
- 1160 Rossi, D., & Volterra, A. (2009). Astrocytic dysfunction: Insights on the role in
1161 neurodegeneration. *Brain Research Bulletin*, 80(4–5), 224–232.
1162 <https://doi.org/10.1016/j.brainresbull.2009.07.012>
- 1163 Rossini, L., Garbelli, R., Gnatkovsky, V., Didato, G., Villani, F., Spreafico, R., ... de Curtis,
1164 M. (2017). Seizure activity per se does not induce tissue damage markers in human

- 1165 neocortical focal epilepsy. *Annals of Neurology*. <https://doi.org/10.1002/ana.25005>
- 1166 Rusakov, D. A., Bard, L., Stewart, M. G., & Henneberger, C. (2014). Diversity of astroglial
1167 functions alludes to subcellular specialisation. *Trends in Neurosciences*, 37(4), 228–
1168 242. <https://doi.org/10.1016/j.tins.2014.02.008>
- 1169 Santello, M, Bezzi, P., & Volterra, A. (2011). TNFalpha controls glutamatergic
1170 gliotransmission in the hippocampal dentate gyrus. *Neuron*, 69(1097-4199
1171 (Electronic)), 988–1001.
- 1172 Santello, Mirko, Toni, N., & Volterra, A. (2019). Astrocyte function from information
1173 processing to cognition and cognitive impairment. *Nature Neuroscience*, 22(2), 154–
1174 166. <https://doi.org/10.1038/s41593-018-0325-8>
- 1175 Santiago, M. F., Veliskova, J., Patel, N. K., Lutz, S. E., Caille, D., Charollais, A., ...
1176 Scemes, E. (2011). Targeting Pannexin1 Improves Seizure Outcome. *PLoS ONE*,
1177 6(9), e25178. <https://doi.org/10.1371/journal.pone.0025178>
- 1178 Savtchouk, I., & Volterra, A. (2018). Gliotransmission: Beyond Black-and-White. *The*
1179 *Journal of Neuroscience*, 38(1), 14–25. [https://doi.org/10.1523/JNEUROSCI.0017-
1180 17.2017](https://doi.org/10.1523/JNEUROSCI.0017-17.2017)
- 1181 Sawada, K., Echigo, N., Juge, N., Miyaji, T., Otsuka, M., Omote, H., ... Moriyama, Y.
1182 (2008). Identification of a vesicular nucleotide transporter. *Proceedings of the National*
1183 *Academy of Sciences*, 105(15), 5683–5686. <https://doi.org/10.1073/pnas.0800141105>
- 1184 Scemes, E., Velišek, L., & Velišková, J. (2019). Astrocyte and Neuronal Pannexin1
1185 Contribute Distinctly to Seizures. *ASN Neuro*, 11, 1759091419833502.
1186 <https://doi.org/10.1177/1759091419833502>
- 1187 Scheffer, I. E., Berkovic, S., Capovilla, G., Connolly, M. B., French, J., Guilhoto, L., ...
1188 Zuberi, S. M. (2017). ILAE classification of the epilepsies: Position paper of the ILAE
1189 Commission for Classification and Terminology. *Epilepsia*, 58(4), 512–521.
1190 <https://doi.org/10.1111/epi.13709>
- 1191 Schipke, C. G., Boucsein, C., Ohlemeyer, C., Kirchhoff, F., & Kettenmann, H. (2002).
1192 Astrocyte Ca²⁺ waves trigger responses in microglial cells in brain slices. *Faseb J.*,
1193 16(2), 255–257. <https://doi.org/10.1096/fj.01-0514fje>

- 1194 Schröder, W., Hinterkeuser, S., Seifert, G., Schramm, J., Jabs, R., Wilkin, G. P., ...
1195 Steinhauser, C. (2000). Functional and molecular properties of human astrocytes in
1196 acute hippocampal slices obtained from patients with temporal lobe epilepsy.
1197 *Epilepsia*, 41 Suppl 6(0013-9580 (Print)), S181–S184. Retrieved from
1198 <http://www.ncbi.nlm.nih.gov/pubmed/10999541>
- 1199 Schwartzkroin, P. A., Baraban, S. C., & Hochman, D. W. (1998). Osmolarity, ionic flux, and
1200 changes in brain excitability. *Epilepsy Research*, 32(1–2), 275–285. Retrieved from
1201 <http://www.ncbi.nlm.nih.gov/pubmed/9761327>
- 1202 Seifert, G., Hüttmann, K., Binder, D. K., Hartmann, C., Wyczynski, A., Neusch, C., &
1203 Steinhäuser, C. (2009). Analysis of astroglial K⁺ channel expression in the developing
1204 hippocampus reveals a predominant role of the Kir4.1 subunit. *The Journal of*
1205 *Neuroscience : The Official Journal of the Society for Neuroscience*, 29(23), 7474–
1206 7488. <https://doi.org/10.1523/JNEUROSCI.3790-08.2009>
- 1207 Shen, W., Nikolic, L., Meunier, C., Pfrieger, F., & Audinat, E. (2017). An autocrine
1208 purinergic signaling controls astrocyte-induced neuronal excitation. *Scientific Reports*,
1209 7(1), 11280. <https://doi.org/10.1038/s41598-017-11793-x>
- 1210 Shinozaki, Y., Shibata, K., Yoshida, K., Shigetomi, E., Gachet, C., Ikenaka, K., ... Koizumi,
1211 S. (2017). Transformation of Astrocytes to a Neuroprotective Phenotype by Microglia
1212 via P2Y₁ Receptor Downregulation. *Cell Reports*, 19(6), 1151–1164.
1213 <https://doi.org/10.1016/j.celrep.2017.04.047>
- 1214 Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar
1215 formation. *Trends in Neurosciences*, 32(12), 638–647.
1216 <https://doi.org/10.1016/j.tins.2009.08.002>
- 1217 Steinhauser, C., & Boison, D. (2012). Special Issue: Astrocytes and Epilepsy. *Glia*, 60(8).
- 1218 Steinhäuser, C., Grunnet, M., & Carmignoto, G. (2016). Crucial role of astrocytes in
1219 temporal lobe epilepsy. *Neuroscience*, 323, 157–169.
1220 <https://doi.org/10.1016/j.neuroscience.2014.12.047>
- 1221 Strohschein, S., Hüttmann, K., Gabriel, S., Binder, D. K., Heinemann, U., & Steinhäuser,
1222 C. (2011). Impact of aquaporin-4 channels on K⁺ buffering and gap junction coupling
1223 in the hippocampus. *Glia*, 59(6), 973–980. <https://doi.org/10.1002/glia.21169>

- 1224 Studer, F. E., Fedele, D. E., Marowsky, A., Schwerdel, C., Wernli, K., Vogt, K., ... Boison,
1225 D. (2006). Shift of adenosine kinase expression from neurons to astrocytes during
1226 postnatal development suggests dual functionality of the enzyme. *Neuroscience*,
1227 *142*(1), 125–137. <https://doi.org/10.1016/j.neuroscience.2006.06.016>
- 1228 Szokol, K., Heuser, K., Tang, W., Jensen, V., Enger, R., Bedner, P., ... Nagelhus, E. A.
1229 (2015). Augmentation of Ca(2+) signaling in astrocytic endfeet in the latent phase of
1230 temporal lobe epilepsy. *Front Cell Neurosci.*, *9*(1662-5102 (Electronic)), 49.
- 1231 Tan, Z., Liu, Y., Xi, W., Lou, H., Zhu, L., Guo, Z., ... Duan, S. (2017). Glia-derived ATP
1232 inversely regulates excitability of pyramidal and CCK-positive neurons. *Nature*
1233 *Communications*, *8*, 13772. <https://doi.org/10.1038/ncomms13772>
- 1234 Tanaka, K., Watase, K., Manabe, T., Yamada, K., Watanabe, M., Takahashi, K., ... Wada,
1235 K. (1997). Epilepsy and Exacerbation of Brain Injury in Mice Lacking the Glutamate
1236 Transporter GLT-1. *Science*, *276*(5319), 1699–1702.
1237 <https://doi.org/10.1126/science.276.5319.1699>
- 1238 Theodosis, D. T., Poulain, D. A., & Oliet, S. H. R. (2008). Activity-Dependent Structural
1239 and Functional Plasticity of Astrocyte-Neuron Interactions. *Physiological Reviews*,
1240 *88*(3), 983–1008. <https://doi.org/10.1152/physrev.00036.2007>
- 1241 Thom, M. (2014). Review: Hippocampal sclerosis in epilepsy: a neuropathology review.
1242 *Neuropathology and Applied Neurobiology*, *40*(5), 520–543.
1243 <https://doi.org/10.1111/nan.12150>
- 1244 Tian, G. F., Azmi, H., Takano, T., Xu, Q., Peng, W., Lin, J., ... Nedergaard, M. (2005). An
1245 astrocytic basis of epilepsy. *Nat.Med.*, *11*(1078-8956 (Print)), 973–981.
- 1246 Tong, X., Ao, Y., Faas, G. C., Nwaobi, S. E., Xu, J., Haustein, M. D., ... Khakh, B. S.
1247 (2014). Astrocyte Kir4.1 ion channel deficits contribute to neuronal dysfunction in
1248 Huntington's disease model mice. *Nature Neuroscience*, *17*(5), 694–703.
1249 <https://doi.org/10.1038/nn.3691>
- 1250 Trevelyan, A. J., Muldoon, S. F., Merricks, E. M., Racca, C., & Staley, K. J. (2015). The
1251 Role of Inhibition in Epileptic Networks. *Journal of Clinical Neurophysiology*, *32*(3),
1252 227–234. <https://doi.org/10.1097/WNP.0000000000000160>
- 1253 van der Hel, W. S., Notenboom, R. G. E., Bos, I. W. M., van Rijen, P. C., van Veelen, C.

- 1254 W. M., & de Graan, P. N. E. (2005). Reduced glutamine synthetase in hippocampal
1255 areas with neuron loss in temporal lobe epilepsy. *Neurology*, *64*(2), 326–333.
1256 <https://doi.org/10.1212/01.WNL.0000149636.44660.99>
- 1257 van Vliet, E. A., Aronica, E., & Gorter, J. A. (2015). Blood–brain barrier dysfunction,
1258 seizures and epilepsy. *Seminars in Cell & Developmental Biology*, *38*, 26–34.
1259 <https://doi.org/10.1016/j.semcdb.2014.10.003>
- 1260 van Vliet, E. A., Aronica, E., Vezzani, A., & Ravizza, T. (2018). Review: Neuroinflammatory
1261 pathways as treatment targets and biomarker candidates in epilepsy: emerging
1262 evidence from preclinical and clinical studies. *Neuropathology and Applied
1263 Neurobiology*, *44*(1), 91–111. <https://doi.org/10.1111/nan.12444>
- 1264 Verderio, C., Cagnoli, C., Bergami, M., Francolini, M., Schenk, U., Colombo, A., ...
1265 Matteoli, M. (2012). TI-VAMP/VAMP7 is the SNARE of secretory lysosomes
1266 contributing to ATP secretion from astrocytes. *Biology of the Cell*, *104*(4), 213–228.
1267 <https://doi.org/10.1111/boc.201100070>
- 1268 Verkhatsky, A., Matteoli, M., Parpura, V., Mothet, J.-P., & Zorec, R. (2016). Astrocytes as
1269 secretory cells of the central nervous system: idiosyncrasies of vesicular secretion.
1270 *The EMBO Journal*, *35*(3), 239–257. <https://doi.org/10.15252/embj.201592705>
- 1271 Verkhatsky, Alexei, & Parpura, V. (2016). Astroglipathology in neurological,
1272 neurodevelopmental and psychiatric disorders. *Neurobiology of Disease*, *85*, 254–
1273 261. <https://doi.org/10.1016/j.nbd.2015.03.025>
- 1274 Vesce, S., Rossi, D., Brambilla, L., & Volterra, A. (2007). Glutamate release from
1275 astrocytes in physiological conditions and in neurodegenerative disorders
1276 characterized by neuroinflammation. *Int.Rev.Neurobiol.*, *82*(07), 57–71.
1277 [https://doi.org/10.1016/S0074-7742\(07\)82003-4](https://doi.org/10.1016/S0074-7742(07)82003-4)
- 1278 Vezzani, A., French, J., Bartfai, T., & Baram, T. Z. (2011). The role of inflammation in
1279 epilepsy. *Nat.Rev.Neurol.*, *7*(1759-4766 (Electronic)), 31–40.
1280 <https://doi.org/10.1038/nrneurol.2010.178>
- 1281 Volterra, A., Liaudet, N., & Savtchouk, I. (2014). Astrocyte Ca²⁺ signalling: an unexpected
1282 complexity. *Nature Reviews Neuroscience*, *15*(5), 327–335.
1283 <https://doi.org/10.1038/nrn3725>

- 1284 Wallraff, A., Kohling, R., Heinemann, U., Theis, M., Willecke, K., & Steinhauser, C. (2006).
1285 The impact of astrocytic gap junctional coupling on potassium buffering in the
1286 hippocampus. *J Neurosci.*, 26(1529-2401 (Electronic)), 5438–5447.
- 1287 Walz, W. (2000). Role of astrocytes in the clearance of excess extracellular potassium.
1288 *Neurochemistry International*, 36(4–5), 291–300. Retrieved from
1289 <http://www.ncbi.nlm.nih.gov/pubmed/10732996>
- 1290 Wang, Y., Zaveri, H. P., Lee, T.-S. W., & Eid, T. (2009). The development of recurrent
1291 seizures after continuous intrahippocampal infusion of methionine sulfoximine in rats:
1292 a video-intracranial electroencephalographic study. *Experimental Neurology*, 220(2),
1293 293–302. <https://doi.org/10.1016/j.expneurol.2009.08.034>
- 1294 Wellmann, M., Álvarez-Ferradas, C., Maturana, C. J., Sáez, J. C., & Bonansco, C. (2018).
1295 Astroglial Ca²⁺-Dependent Hyperexcitability Requires P2Y1 Purinergic Receptors
1296 and Pannexin-1 Channel Activation in a Chronic Model of Epilepsy. *Frontiers in*
1297 *Cellular Neuroscience*, 12, 446. <https://doi.org/10.3389/fncel.2018.00446>
- 1298 Wilcock, D. M., Vitek, M. P., & Colton, C. A. (2009). Vascular amyloid alters astrocytic
1299 water and potassium channels in mouse models and humans with Alzheimer's
1300 disease. *Neuroscience*, 159(3), 1055–1069.
1301 <https://doi.org/10.1016/j.neuroscience.2009.01.023>
- 1302 Xiong, Y., Sun, S., Teng, S., Jin, M., & Zhou, Z. (2018). Ca²⁺-Dependent and Ca²⁺-
1303 Independent ATP Release in Astrocytes. *Frontiers in Molecular Neuroscience*, 11,
1304 224. <https://doi.org/10.3389/fnmol.2018.00224>
- 1305 Zamanian, J. L., Xu, L., Foo, L. C., Nouri, N., Zhou, L., Giffard, R. G., & Barres, B. A.
1306 (2012). Genomic Analysis of Reactive Astrogliosis. *Journal of Neuroscience*, 32(18),
1307 6391–6410. <https://doi.org/10.1523/JNEUROSCI.6221-11.2012>
- 1308 Zhang, Z., Chen, G., Zhou, W., Song, A., Xu, T., Luo, Q., ... Duan, S. (2007). Regulated
1309 ATP release from astrocytes through lysosome exocytosis. *Nature Cell Biology*, 9(8),
1310 945–953. <https://doi.org/10.1038/ncb1620>
- 1311 Zhao, B., Gu, L., Liu, K., Zhang, M., & Liu, H. (2017). Maxi-anion channels play a key role
1312 in glutamate-induced ATP release from mouse astrocytes in primary culture.
1313 *NeuroReport*, 28(7), 380–385. <https://doi.org/10.1097/WNR.0000000000000759>

1314 Zhao, Z., Nelson, A. R., Betsholtz, C., & Zlokovic, B. V. (2015). Establishment and
1315 Dysfunction of the Blood-Brain Barrier. *Cell*, 163(5), 1064–1078.
1316 <https://doi.org/10.1016/j.cell.2015.10.067>

1317 Zimmermann, H. (1994). Signalling via ATP in the nervous system. *Trends in*
1318 *Neurosciences*, 17(10), 420–426. Retrieved from
1319 <http://www.ncbi.nlm.nih.gov/pubmed/7530880>

1320

1321

