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Role of astrocyte purinergic signalling in epilepsy

Running title: Astrocyte purinergic signalling in epilepsy

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

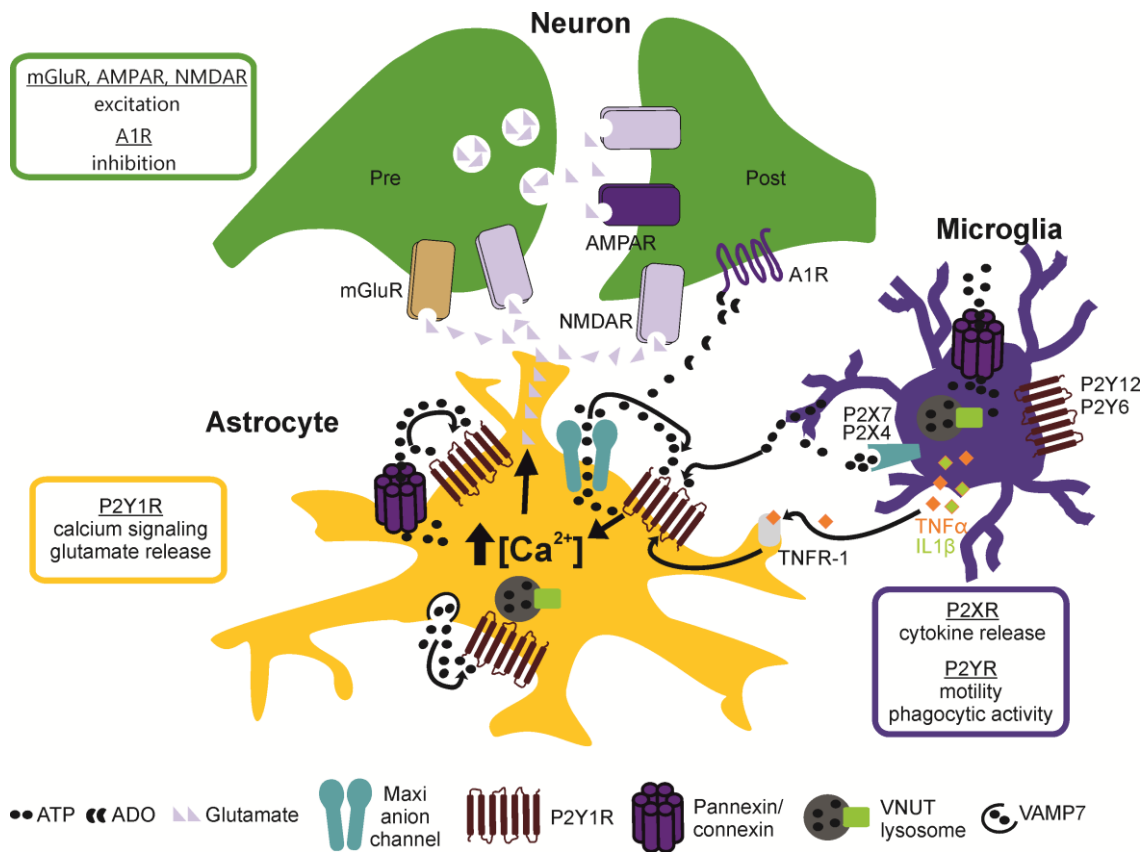
607 NMDA receptors. It is not clear yet whether TNF α also potentiate other outcomes of
608 astrocyte glutamate release in the hippocampus, such as the generation of SICs (Angulo
609 et al., 2004; Fellin et al., 2004) or the mGluR-dependent potentiation of synaptic excitation
610 in CA1 (Gertrudis Perea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

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