**Abstract**

In the rodent forebrain, the majority of astrocytes are generated during the early postnatal phase. Following differentiation, astrocytes undergo maturation which accompanies the development of the neuronal network. Neonate astrocytes exhibit a distinct morphology and domain size which differs to their mature counterparts. Moreover, many of the plasma membrane proteins prototypical for fully developed astrocytes are only expressed at low levels at neonatal stages. These include connexins and Kir4.1, which define the low membrane resistance and highly negative membrane potential of mature astrocytes. Newborn astrocytes moreover express only low amounts of GLT-1, a glutamate transporter critical later in development. Furthermore, they show specific differences in the properties and spatio-temporal pattern of intracellular calcium signals, resulting from differences in their repertoire of receptors and signalling pathways. Therefore, roles fulfilled by mature astrocytes, including ion and transmitter homeostasis, are underdeveloped in the young brain. Similarly, astrocytic ion signalling in response to neuronal activity, a process central to neuron–glia interaction, differs between the neonate and mature brain. This review describes the unique functional properties of astrocytes in the first weeks after birth and compares them to later stages of development. We conclude that with an immature neuronal network and wider extracellular space, astrocytic support might not be as demanding and critical compared to the mature brain. The delayed differentiation and maturation of astrocytes in the first postnatal weeks might thus reflect a reduced need for active, energy-consuming regulation of the extracellular space and a less tight control of glial feedback onto synaptic transmission.
1 | INTRODUCTION

In the forebrain of rodents, the majority of astrocytes are generated postnatally, at a time point when neuronal differentiation is already largely terminated (Freeman, 2010; Kriegstein & Alvarez-Buylla, 2009). The early postnatal differentiation of astrocytes accompanies the development of the neuronal network, and both processes interact and influence each other. While cells of the astrocytic lineage themselves are key players in the regulation of both neuronal and gliogenesis (Abbott, Ronnback, & Hansson, 2006; Campbell & Götz, 2002; Heins et al., 2002; Kriegstein & Alvarez-Buylla, 2009), astrocytic input in particular has also been shown to be critical for proper synapse and circuit formation in the postnatal brain (Allen & Eroglu, 2017; Clarke & Barres, 2013). The ability of astrocytes to promote neuronal network formation is undoubtedly underpinned by their special properties which change along with their maturation. On the other hand side, astrocytes themselves are too subjected to constant modulation and fine-tuning via a variety of pathways (Freeman, 2010). Not surprisingly then, recent analysis has revealed significant changes in the transcriptome of astrocytes during the first weeks after birth (Cahoy et al., 2008). In addition to this developmental heterogeneity, there is a notable regional heterogeneity. This encompasses morphology, protein expression and signalling pathways, resulting in a substantial diversity of astrocytes that reflects their functional adaptations to the given local neural networks (Zhang & Barres, 2010; Khakh & Sofroniev, 2015). Notably, such heterogeneity is not only observed between different brain regions, such as the hippocampus and cortex, but also within the latter (Seifert & Steinhäuser, 2018; Miller et al., 2019).

In this review, we will give an overview on the properties of astrocytes of the neonate rodent brain, which are indeed very different from mature astrocytes found at later stages of postnatal development. Special focus will be put onto aspects relevant for the specific functions of astrocytes at synapses: the formation of perisynaptic processes and gap junctional coupling, regulation of extracellular K⁺ homeostasis and uptake of neurotransmitters, as well as intracellular calcium signalling. For astrocytes’ involvement in the formation of blood vessels and their functional roles at the blood–brain barrier during postnatal development, we refer the reader to excellent recent reviews covering this subject (e.g. Abbott et al., 2006; Iadecola, 2017).

2 | GENESIS AND MORPHOLOGICAL CHARACTERISTICS OF ASTROCYTES IN THE NEONATE FOREBRAIN

The genesis of astrocytes is a complex multiphase process, which occurs in several waves, and is only completed after birth in the forebrain of rodents (Molofsky & Deneen, 2015). In the developing cortex of vertebrates, astrocytes begin life as neural stem cells in the subventricular zone which undergo the “gliogenic switch” at around E16-18 in order to form astrocyte precursor cells (Ge, Miyawaki, Gage, Jan, & Jan, 2013). These newly formed astrocytes (much like newly formed neurons) reach their final destinations within the cortex by migrating along radial glia. However, migration is not yet complete during early postnatal development, when radial glia are in the process of losing their guiding processes before differentiating themselves (Kriegstein & Alvarez-Buylla, 2009).

While this “second wave” of astrocytes is travelling through the tissue (albeit not reaching quite as far as their “first wave” predecessors), proliferation is also underway throughout the cortex, with astrocytic numbers increasing strongly between birth and the end of the third postnatal week (Kriegstein & Alvarez-Buylla, 2009). During this stage, new astrocytes are formed from differentiating radial glia, subventricular zone progenitors and division of local immature astrocytes and go on to make up around 50% of all astrocytes in the mature cortex (Ge et al., 2013; Molofsky & Deneen, 2015). This time frame, covering the first postnatal weeks in rodents, is also the time period in which the growth of neuronal dendrites and synapses surges and in which mature neuronal communication and networks properties develop (Freeman, 2010; Semple, Blomgren, Gimlin, Ferriero, & Noble-Haeusslein, 2013; Wang & Bordey, 2008).

It is well established that astrocytes—like neurons—are a heterogeneous cell type (Khakh & Sofroniew, 2015; Rusakov, Bard, Stewart, & Henneberger, 2014; Wang & Bordey, 2008; Zhang & Barres, 2010), and several strategies are commonly used for their identification in brain tissue. Besides immunohistochemistry, an approach often employed is the use of transgenic mice, in which a fluorescent protein is expressed under the control of a promoter predominately/specifically active in astrocytes, such as for glial fibrillary acidic protein (GFAP) (Hirrlinger, Scheller, Braun, Hirrlinger, & Kirchhoff, 2006; Nolte et al., 2001; Zhuo et al., 1997),...
glutamate transporters GLAST or GLT-1 (glutamate/aspartate transporter and glutamate transporter-1, respectively) (Mori et al., 2006; Yang et al., 2011) or Aldh1/L1 (Morel et al., 2019; Yang et al., 2011). Alternatively, the vital fluorescent marker sulforhodamine 101 (SR101), which specifically stains astrocytes in the healthy forebrain (Kafitz, Meier, Stephan, & Rose, 2008; Nimmerjahn & Helmchen, 2012; Schnell et al., 2015), may be used in living tissue.

The use of such approaches has revealed that astrocytes undergo substantial changes in their density and cellular morphology during early postnatal development of the forebrain. Studies employing GFAP as a marker have found that the density of astrocytes in the first postnatal week after birth is significantly lower than that in the juvenile and adult brain (Kimoto, Eto, Abe, Kato, & Araki, 2009; Nixdorf-Bergweiler, Albrecht, & Heinemann, 1994; Schreiner et al., 2014) (Figure 1a,b). The increase in astrocyte density is accompanied by an increase in GFAP levels (Kim et al., 2011; Schreiner et al., 2014) (Figure 1a,b). GFAP not only serves as an important structural component of the cytoskeleton, but is also involved in the anchoring and trafficking of different proteins to the membrane (including GLAST and GLT-1 (Middeldorp & Hol, 2011; Sullivan et al., 2007)), thereby promoting the postnatal maturation of astrocytes.

While growing in number and overall density, astrocytes also rearrange their morphology. During the first two postnatal weeks, astrocytes in grey matter exhibit a less delicate and complex architecture of their fine processes (Figure 1d) (Bushong, Martone, & Ellisman, 2004; Morel, Higashimori, Tolman, & Yang, 2014). Mature astrocytes, in contrast, are characterized by numerous very thin processes, called PAPs (peripheral astrocytic processes) (Derouiche & Frotscher, 2001) that reach out to contact many thousands of synapses (Reichenbach, Derouiche, & Kirchhoff, 2010; Ventura & Harris, 1999).

Furthermore, at early postnatal stages, astrocytes do not display the discrete tiling characteristic of mature brains, in which they occupy non-overlapping individual domains (Bushong, Martone, Jones, & Ellisman, 2002; Halassa, Fellin, Takano, Dong, & Haydon, 2007; Nedergaard, Ransom, & Goldman, 2003). Instead, neonate astrocytes exhibit long processes reaching beyond the borders of their main territory, invading the domains of neighbouring astrocytes (Bushong et al., 2004) (Figure 1d). Notably, in the mature brain, astrocyte tiling may be lost again in response to disease (Oberheim et al., 2008). Formation of discrete astrocytic domains from postnatal day (P) 7 to P26 is accompanied by an approximately fourfold increase in the number of glutamatergic synapses present in the domain of an individual astrocyte (Morel et al., 2014). Silencing glutamatergic synapses during this period results in a reduced domain size, a reduced number of synapses contacted by each astrocyte and a reduced expression of GLT-1 at P26. This growth-promoting effect of glutamatergic transmission on maturing astrocytes has been reported to be related to activation of mGluR5 (metabotropic glutamate receptor 5) and astrocytic calcium signalling (Morel et al., 2014).

3 | CONNEXINS AND ASTROCYTE COUPLING

Astrocytes in the mature mouse hippocampus express connexins (Cx) 30 and 43, which form gap junctions that allow the passage of ions and small molecules between cells (Dermietzel et al., 1989; Giaume, Koulakoff, Roux, Holcman, & Rouach, 2010; Nagy & Rash, 2000; Ransom & Ye, 2005). Moreover, Cx can exist as uncoupled hemichannels serving as gated and selective pores in the cell membrane (Nielson, Hansen, Ransom, Nielsen, & MacAulay, 2017). Recent work has provided evidence that Cx43 hemichannels are involved in the regulation of basal synaptic transmission at glutamatergic synapses (Chever, Lee, & Rouach, 2014).

The fully developed astrocytic gap junction network in the rodent forebrain is extensive, encompassing many dozens of cells (e.g. Rouach, Koulakoff, Abudara, Willecke, & Giaume, 2008; Wallraff, Odermatt, Willecke, & Steinhauser, 2004) (Figure 1c). Importantly, gap junctions also mediate the spread of glucose and its metabolites through the astrocytic network to regions of increased neuronal activity (Clasadonte, Seemes, Wang, Boison, & Haydon, 2017; Rouach et al., 2008), indicating that they play an important role in neuro-metabolic coupling in the mature network (Belanger, Allaman, & Magistretti, 2011; Escartin & Rouach, 2013). Another consequence of gap junctional coupling is that it results in isopotentiality of the astrocytes involved, reducing local depolarizations induced, for example, by elevation of extracellular K⁺ (Kiyoshi et al., 2018; Ma et al., 2016).

While Cx play an important role in embryonic development and development of the cerebral cortex in rodents (Elias & Kriegstein, 2008), astrocytes in the neonate brain show low expression levels of Cx and, therefore, only weak gap junctional coupling as compared to later stages (Houades, Koulakoff, Ezan, Seif, & Giaume, 2008; Schools, Zhou, & Kimelberg, 2006; Yamamoto, Vukelić, Hertzberg, & Nagy, 1992) (Figure 1c). As a consequence, the different physiological functions attributed to a mature astrocytic syncytium, such as an efficient intercellular spread of ions and metabolites, will be less strongly expressed in neonates. This was, for example, shown for the intercellular spread of Na⁺ between astrocytes in the hippocampus, which is considerably reduced at P4 as compared to P16-21 (Langer, Stephan, Theis, & Rose, 2012). The same is likely to hold true for intercellular diffusion of K⁺, which has been implicated to contribute to the regulation of extracellular K⁺ by astrocytes in older animals (Pannasch et al., 2011; Wallraff et al., 2006).
Similarly, one could speculate that the reduced gap junctional coupling of neonate astrocytes reduces metabolite trafficking, thereby resulting in a reduced delivery of metabolites from astrocytes to active synapses compared to more developed brains. Interestingly, it also appears that Cx, namely Cx30, can regulate the spatial proximity of PAPs with glutamatergic synapses and thereby the efficacy of glutamate uptake by astrocytes (Pannasch et al., 2014).

On the other hand, it was shown that neurons themselves control the expression of Cx (Koulakoff, Ezan, & Giaume, 2008). The functional relevance of the morphological arrangement of PAPs at tripartite synapses has been exemplified in the hypothalamus of adult rodents, in which changes in the astrocytic coverage directly modify extracellular ion homeostasis and neuronal synaptic transmission (Theodosis, Poulain, & Oliet, 2008). Astrocyte
morphology, domain formation and gap junctional coupling are thus tightly connected to major physiological roles of astrocytes at glutamatergic synapses, namely the clearance of the synaptic cleft from $K^+$ and glutamate (Pannasch & Rouach, 2013).

4 | $K^+$ CHANNELS, ELECTROPHYSIOLOGICAL PROPERTIES AND EXTRACELLULAR $K^+$ HOMEOSTASIS

The first electrophysiological studies performed on brain tissue slices did not differentiate between bona fide astrocytes and NG2 glia. Originally, the latter cells were instead classified as “complex” cells/astrocytes, “Glur” cells or “outwardly rectifying” glia/astrocytes by the different authors (Bordey & Sontheimer, 2000; Matthias et al., 2003; Zhou, Schools, & Kimelberg, 2006). For the last 20 years, however, NG2 glia have been recognized as a separate type of macroglial cell (Dimou & Gallo, 2015; Nishiyama, 2001).

Classical astrocytes express a variety of different $K^+$ channels, that is inwardly rectifying (Kir), two-pore domain ($K_P$) and voltage-activated ($K_V$) channels (for review see e.g. Verkhratsky & Nedergaard, 2018; Verkhratsky & Steinhauser, 2000). Among those, Kir4.1 is of particular interest as it is the predominant $K^+$ channel in fully developed astrocytes, massively influencing their physiology and behaviour.

Neonatal and mature astrocytes show prominent differences in their expression of different $K^+$ channels and can thus be distinguished electrophysiologically. Immature neonatal astrocytes exhibit a non-linear current–voltage relationship due to high expression levels of time- and voltage-dependent $K^+$ currents (Bordey & Sontheimer, 1997; Kafitz et al., 2008) (Figure 2a). In contrast, mature astrocytes display a linear current–voltage relationship due to predominant expression of ohmic currents originating from Kir and $K_P$ channels (Bordey & Sontheimer, 2000; D’Ambrosio, Wenzel, Schwartzkroin, McKhann, & Janigro, 1998; Kafitz et al., 2008; Seifert et al., 2009; Zhou et al., 2006) (Figure 2a). During early postnatal development, there is a shift from non-linear to linear current–voltage relationship (Kafitz et al., 2008; Zhong et al., 2016; Zhou et al., 2006) which is in large part due to a strong upregulation of Kir4.1 during this period (Lunde et al., 2015; Moroni, Inverardi, Regondi, Pennacchio, & Frassoni, 2015; Nwaobi, Lin, Peramsetty, & Olsen, 2014; Olsen et al., 2015; Seifert et al., 2009) (Figure 2b). Moreover, localization of Kir4.1 shifts from a predominantly somatic towards a mainly distal expression in perisynaptic processes and perivascular endfeet (Moroni et al., 2015).

Astrocytes exhibit a highly negative resting membrane potential ($E_M$) independent from the developmental stage (Kafitz et al., 2008; Zhong et al., 2016; Zhou et al., 2006). It is largely defined by the efflux of $K^+$ through Kir4.1 and $K_P$ channels, and therefore, inactivation of Kir4.1 causes strong depolarization of astrocytes (Djukic, Casper, Philpot, Chin, & McCarthy, 2007; Kofuji et al., 2000; Seifert et al., 2009; Stephan et al., 2012). A smaller fraction of the $E_M$ is set by the hyperpolarizing activity of the sodium/potassium ATPase (NKA) (Verkhratsky & Nedergaard, 2018). The negative $E_M$ provides the driving force for electrogenic transport of glutamate, GABA ($\gamma$-aminobutyric acid) and glycine via GLAST/ GLT-1, GAT and GlyT (glycine transporter), respectively. Transport direction and strength of these carriers depend on the electrochemical gradients of the ions and molecules involved. Thus, depolarization reduces or may even reverse transmitter uptake in astrocytes (Barakat & Bordey, 2002; Djukic et al., 2007; Huang, Barakat, Wang, & Bordey, 2004; Stephan et al., 2012) as discussed below.

During neonatal stages, astrocytes exhibit a relatively high membrane resistance ($R_M$). However, there is a developmental decrease in $R_M$ (Bordey & Sontheimer, 1997; Kafitz et al., 2008; Stephan et al., 2012; Zhong et al., 2016; Zhou et al., 2006) due to increased $K^+$ channel expression and consequent increase in $K^+$ permeability (Seifert et al., 2009). The membrane capacitance ($C_M$) of neonatal astrocytes is relatively low (Kafitz et al., 2008; Zhou et al., 2006). $C_M$ is a measure of the membrane surface, and neonatal astrocytes exhibit a low degree of branching (Bushong et al., 2004; Morel et al., 2014). In contrast, mature astrocytes have many finer processes and display much stronger gap junctional coupling as delineated above. Together, the elaborate branching of astrocyte processes and the formation of a large syncytium of electrically coupled cells increase the total surface area, which results in basically immeasurable high $C_M$ (Kafitz et al., 2008; Zhou et al., 2006).

Kir and $K_P$ channels are open under resting conditions causing a high $K^+$ permeability (Stephan et al., 2012; Zhou et al., 2009). In turn, astrocytes are strongly susceptible to changes in $[K^+]_o$ representing near-perfect “$K^+$-electrodes” (Kimelberg, Bowman, Biddlecome, & Bourke, 1979; Ransom & Goldring, 1973; Stephan et al., 2012) (Figure 2c). There is evidence that Kir4.1 mediates temporary local buffering of $K^+$ that is released from neurons upon activation (Bay & Butt, 2012; Chever, Djukic, McCarthy, & Amzica, 2010; D’Ambrosio, Gordon, & Winn, 2002; Larsen et al., 2014; Sibille, Pannasch, & Rouach, 2014). Neonatal astrocytes exhibit a restricted $K^+$ buffering capacity through Kir4.1, which then becomes more effective during development and increased expression of Kir4.1 (Larsen, Stoica, & MacAulay, 2019; Zhong et al., 2016).

Regulation of $[K^+]_o$ by astrocytes is not only mediated through $K^+$ channels. In addition, uptake of $K^+$ into
Astrocytes by the sodium/potassium ATPase (NKA) plays a prominent role (Hertz et al., 2015; Karus, Mondragao, Ziemens, & Rose, 2015; Larsen, Stoica, & MacAulay, 2016). A recent study performed on rat hippocampus revealed that all three isoforms of the α subunit of the NKA are up-regulated during the first three postnatal weeks (Larsen et al., 2019). Similarly, expression levels of the sodium-potassium-2 chloride co-transporter 1 (NKCC1), another transporter likely involved in the regulation of extracellular K+ (MacVicar, Feighan, Brown, & Ransom, 2002; Su, Kintner, & Sun, 2002), increase strongly from P0 to adulthood (Yan, Dempsey, & Sun, 2001).

The capacity for both channels—as well as transporter-mediated astrocytic uptake of K+ from the extracellular space (ECS), is thus relatively weak in the neonate brain. Indeed, the activity-induced extracellular changes in K+ are much larger and longer-lasting in neonate animals and may even exceed the so-called ceiling level of 10–12 mM (Connors, Ransom, Kunis, & Gutnick, 1982; Heinemann & Lux, 1977; Jendelova & Sykova, 1991; Larsen et al., 2019) (Figure 2d). Within the first three postnatal weeks, the number of synapses increases strongly, resulting in a greater activity-related release of K+. At the same time, however, the increase in capacity for astrocytic K+ uptake is even stronger, resulting in smaller and briefer increases in K+ for a given intensity of neuronal activity (Larsen et al., 2019) (Figure 2d). Of note, the first postnatal weeks also experience significant changes in the brain’s ECS, including a decrease in the extracellular volume fraction of about 37% at P4-6 to 24% at P12 to about 15% in adults (Nicholson & Hrabetova, 2017;
Sykova & Nicholson, 2008). Diffusion in the neonate ECS, therefore, plays a much more prominent functional role as compared to later stages of development, where the ECS is narrow and does not enable efficient passive clearance. The apparent weakness of astrocytic K\(^+\) uptake ability in neonate brain seems thus be compensated for by the increased volume fraction of the ECS (Larsen et al., 2019). Vice versa, astrocytes adapt to the requirements of the growing network and neuronal activity by increasing their ability to buffer elevations in [K\(^+\)]\(_o\).

5 | GLUTAMATE TRANSPORTERS

Glutamate uptake by astrocytes is vital to protect the brain from excitotoxic cell damage and related cell death (Maragakis & Rothstein, 2004; Parpura et al., 2012; Schousboe, Scafidi, Bak, Waagepetersen, & McKenna, 2014). By removing synaptically released glutamate from near the synaptic cleft, astrocytes also control its diffusion to peri- and extrasynaptic receptors (Huang & Bergles, 2004; Zheng, Scimemi, & Rusakov, 2008). In the adult neocortex and hippocampus, astrocytes mainly express two isoforms of high-affinity glutamate transporters, namely GLT-1 (glutamate transporter 1; human analogue: EAAT2 (excitatory amino acid transporter 2)) and GLAST (glutamate/aspartate transporter; human analogue: EAAT1 (excitatory amino acid transporter 1)) (Danbolt, 2001). The two transporters share the same stoichiometry, with 3 Na\(^+\) taken up together with 1 H\(^+\) and 1 glutamate in exchange for a K\(^+\), but exhibit differences in their associated anion conductance (Marcaggi & Attwell, 2004; Rose, Ziemens, Untiet, & Fahlke, 2018).

The activity of glutamate transporters is critical for adult brains, but also for brain development and maturation. Double knockout mice for GLAST and GLT-1 suffer from perinatal mortality and show multiple brain defects (Matsugami et al., 2006). GLAST knockout mice develop normally, but display moderate motor discoordination and increased vulnerability to excitotoxic damage (Watase et al., 1998). Animals devoid of GLT-1 exhibit a much more severe phenotype, including neurodegeneration and premature death from spontaneous seizures (Rothstein et al., 1997; Tanaka et al., 1997).

Notably, while these results point to an essential role of GLT-1 during embryonic and postnatal development, it is GLAST (and not GLT-1) which is mainly expressed by radial glial cells and by astrocytes of the neonate brain (Regan et al., 2007; Shibata et al., 1997). In the mouse hippocampus, GLAST is well detectable at birth and its expression increases during the first three weeks, after which it remains at a relatively stable level until adulthood (Schreiner et al., 2014; Ullensvang, Lehre, Storm-Mathisen, & Danbolt, 1997) (Figure 3a). Expression of GLT-1 starts to appear only after the first postnatal week and then increases to reach near-adult levels at about three weeks after birth (Cahoy et al., 2008; Schreiner et al., 2014; Ullensvang et al., 1997) (Figure 3a).

In the neocortex, the strong upregulation of GLT-1 happens even later, suggesting that GLAST is indeed the predominant glutamate transporter of immature astrocytes, while GLT-1 is the major isoform in adult brain (Furuta, Rothstein, & Martin, 1997; Hanson et al., 2015). The upregulation of GLT-1 is regulated by neuronal activity (Ghosh, Yang, Rothstein, & Robinson, 2011; Morel et al., 2014; Swanson et al., 1997), but GLT-1 itself is also necessary for the establishment of synapses (Verbich, Prenosil, Chang, Murai, & McKinney, 2012), again illustrating the tight interrelationship of astrocyte maturation and formation of the neuronal network (Benediktsson et al., 2012).

Interestingly, the switch in glutamate transporter expression is not seen in cerebellar Bergmann glial cells and Müller glial cells of the retina, which continue to express high levels of GLAST throughout postnatal development (Rose et al., 2018). The postnatal upregulation of glutamate transporters in Bergmann glial cells was shown to be accompanied by a significant drop in their [Cl\(^-\)]\(_i\) (Untiet et al., 2017), most likely due to the transporter-associated anion conductance of GLAST/GLT-1 (Rose et al., 2018). Based on these results, it was speculated that the high [Cl\(^-\)] of neonatal glial cells might result in a higher ambient GABA concentration in the extracellular space as compared to adulthood (Untiet et al., 2017). In addition, the high astrocytic [Cl\(^-\)] sets the Cl\(^-\)-reversal potential to values more positive than the E\(_M\) of astrocytes, resulting in the efflux of Cl\(^-\) upon the opening of GABA receptors, thereby depolarizing the cells (see below) (Kettenmann, Backus, & Schachner, 1984; Meier, Kafitz, & Rose, 2008).

The relatively low glutamate transporter expression in the first week after birth coincides with a period in which GABA acts as an important depolarizing drive in the rodent forebrain (Ben-Ari, 2001). Despite the low density of functional glutamatergic synapses, however, many neurons express NMDA (N-methyl-D-aspartate receptor) receptors and blocking glutamate uptake results in seizure-like activity in the neuronal network (Demarque et al., 2004). Functional glutamate transport, predominantly through GLAST, is also essential for the maintenance of neuronal Na\(^+\) homeostasis and integrity during recurrent activity in neonates (Karus, Gerkau, & Rose, 2017).

Using the glutamate transporter current in astrocytes as a readout (Bergles & Jahr, 1997), it was shown that the overall increase in transporter expression during the first three postnatal weeks is accompanied by a significant increase in the cellular clearance rate for glutamate (Diamond, 2005) (Figure 3b). While it was first speculated that in young brains, this lower level of active clearance could result in an increased activation of extrasynaptic glutamate receptors, a
A later study provided evidence that the reduced glutamate uptake capacity of astrocytes is compensated for by the larger ECS of neonates (Thomas, Tian, & Diamond, 2011).

Interestingly, electrophysiological analysis during the first postnatal weeks also demonstrated that glutamate transport is substantially slower in the neocortex compared to the hippocampus of rats, most likely due to lower overall transporter expression levels on neocortical astrocytes throughout postnatal development (Hanson et al., 2015) (Figure 3c). The reduced capacity for uptake of glutamate by cortical astrocytes results in a less strict control of extracellular glutamate concentration and a stronger activation of neuronal NMDA receptors compared to the hippocampus (Hanson et al., 2015).

6 | GABA TRANSPORTERS

The three main GABA transporters of the rodent forebrain are (following rat nomenclature) GAT-1, GAT-2 and GAT-3 (Borden, 1996; Conti, Minelli, & Melone, 2004; Ghirardini et al., 2018; Stephan, 2015). Astrocytic expression of the different isoforms seems to be heterogeneous, depending on the subcellular compartment, brain region and developmental state (Scimemi, 2014b). GAT-1 is expressed in the cortex and hippocampus from P1 onwards, but does not appear in the cerebellum until the second postnatal week (Frahm & Draguhn, 2001; Rosina, Morara, & Provini, 1999). While astrocytic GAT-2 is strongly expressed around cortical blood vessels during the first postnatal week, it is downregulated shortly after this and levels in the mature cortex are very low. In contrast, GAT-3 is present at birth and then increases with ongoing development (particularly within the first postnatal week), until adult levels and patterns are reached at P20 (Minelli, Barbaresi, & Conti, 2003; Vitellaro-Zuccarello, Calvaresi, & De Biasi, 2003). GAT-3 is not restricted to astrocyte processes at synapses, but also found at extrasynaptic locations (Minelli, DeBiasi, Brecha, Zuccarello, & Conti, 1996).
Like other transporters, levels of GAT are dynamically regulated, which not only includes changes in gene expression, but also changes in the plasma membrane levels (Scimemi, 2014a). Interestingly, studies in the fruit fly Drosophila indicate that the recruitment of GATs into the astrocytic membrane is upregulated by GABAergic signaling—specifically via the activation of GABA\textsubscript{A} receptors (Muthukumar, Stork, & Freeman, 2014).

GABA uptake requires the co-transport of Na\textsuperscript{+} and Cl\textsuperscript{−} (Barakat & Bordey, 2002; Stephan & Friauf, 2014)—with a stoichiometry of 2 Na\textsuperscript{+}: 1 Cl\textsuperscript{−}: 1 GABA (Eulenburg & Stephan, 2014)—with activity dependent on the electrochemical gradients of the involved ions as well as on GABA concentrations. As these parameters are not fixed and can change with activity, GAT transport capacity and even the transport direction of GATs may change.

Dynamic control of extracellular GABA through GATs in fact seems to play a critical role during development. In the early postnatal brain, local increases in synaptic GABA concentrations during periods of high activity drive inward transport through subsynaptic GATs (Savtchenko, Megalogeni, Rusakov, Walker, & Pavlov, 2015), reducing the activation of GABA\textsubscript{A} receptors driving giant depolarizing potentials (GDPs) (Sipila, Huttu, Voipio, & Kaila, 2004). The latter arise as a consequence of the high intracellular Cl\textsuperscript{−} concentrations in neonate neurons which set their Cl\textsuperscript{−}-reversal potential to values more positive than their $E_{M}$, resulting in Cl\textsuperscript{−} efflux upon the opening of GABA\textsubscript{A} receptors (Ben-Ari, 2001).

At the same time, the limited uptake capability of GATs expressed at extrasynaptic sites effectively clamps ambient GABA at a level high enough to tonically activate both GABA\textsubscript{A} and extrasynaptic GABA\textsubscript{A} receptors. These GABA\textsubscript{A} receptors have different subunit compositions to their subsynaptic counterparts, bestowing them with a higher sensitivity and reduced level of desensitization (Belelli et al., 2009). Their tonic activation has been implicated in processes such as the modulation of neuronal migration and differentiation. While they are persistently activated by ambient GABA, they are not saturated at resting levels and transient increases in extracellular GABA concentrations can therefore induce additional phasic depolarization (Luhmann, Fukuda, & Kilb, 2015; Song et al., 2013).

Activation of GABA uptake in neonate astrocytes may have functional consequences for astrocyte signalling itself. In the developing olfactory bulb, increases in Na\textsuperscript{+} caused by astrocytic inward GAT activity can be sufficient to decrease the activity of the sodium/calcium exchanger (NCX), thereby increasing intracellular Ca\textsuperscript{2+} levels enough to trigger IP\textsubscript{3}-mediated Ca\textsuperscript{2+} release from intracellular stores (Doengi et al., 2009). GAT-induced rise in intracellular Ca\textsuperscript{2+} signalling has also been shown to induce release of ATP (adenosine triphosphate) from astrocytes in juvenile animals, which heterosynaptically activates pre- and postsynaptic A1 receptors on hippocampal pyramidal neurons and exerts an inhibitory effect on the network (Boddum et al., 2016; Matos et al., 2018).

In addition to being dependent on GABA, transport activity through GATs is dependent on Na\textsuperscript{+} and Cl\textsuperscript{−} concentrations which bring the reversal potential close to the resting membrane potential (Richerson & Wu, 2003). Increases in the intracellular Na\textsuperscript{+} concentration occurring after glutamate uptake, depolarization via extracellular K\textsuperscript{+} or an increase in intracellular GABA, are therefore all sufficient to reverse astrocytic GABA transport in a dose-dependent manner—thereby increasing extracellular GABA (Heja et al., 2012; Wu, Wang, Diez-Sampedro, & Richerson, 2007).

As mentioned above, astrocytes display a highly negative $E_{M}$ already from birth on. Moreover, astrocyte Na\textsuperscript{+} concentrations are apparently stable in this period (Felix, Ziemens, Seifert, & Rose, 2020), amounting to about 12-15 mM (Rose, Ziemens, & Verkhratsky, 2019). The situation is, however, less clear for Cl\textsuperscript{−}. Reported values for astrocytic Cl\textsuperscript{−} concentration have (independent from age) varied from 20 to 50 mM depending on the technique and preparation used (Wilson & Mongin, 2019). A recent study employing fluorescence lifetime microscopy has now shown neonate Bergmann glia to have a [$\text{Cl}^{-}$], of around 52 mM, a value significantly higher than their mature counterparts, which sit at roughly 35 mM (Untiet et al., 2017). It should be noted that the high Cl\textsuperscript{−} content in young astrocytes is most likely not primarily due to influx via the NKCC1 (as is the case in neurons), as astrocytic expression of the transporter remains low during early development and only reaches maximal values after the third postnatal week (Yan et al., 2001).

The developmental change in glial Cl\textsuperscript{−} is of particular importance, as the higher [$\text{Cl}^{-}$], in neonates as compared to the mature brain shift the reversal potential of GATs. Indeed, Unichenko, Dvorzhak, and Kirischuk (2013) showed that in the neonatal neocortex, Na\textsuperscript{+} influx caused by glutamate uptake is sufficient to keep GATs functioning primarily in the outward mode, resulting in a higher ambient level of extracellular GABA (around 250 nM as opposed to 125 nM in older animals, Figure 3d) (Dvorzhak, Myakhar, Unichenko, Kirmse, & Kirischuk, 2010; Kirmse & Kirischuk, 2006; Untiet et al., 2017).

### 7 | Activity Patterns and Calcium Signalling in the Neonate Brain

Patterns of synchronized, universal neuronal activity emerge at birth and characterize the neonatal stage. This early activity shapes local circuit creation in the developing
astrocytes, with ~70% showing Ca\(^{2+}\) oscillations and ~40% changes in both of these ions appear in a subset of measured Na\(^{+}\) fluctuations in the hippocampus. Unlike Ca\(^{2+}\) waves bursts of synchronized Ca\(^{2+}\) elevations, moving in slow so-called early network oscillations (ENOs), which are due to the well-documented “chloride switch”—the result of the excitatory action of GABA during early development is Hanse, & Konnerth, 1998; Mohajerani & Cherubini, 2006). The excitatory action of GABA during early development is not clear, developing astrocytes in the neonatal forebrain (Ben-Ari, 2001; Griguoli & Cherubini, 2017; Hangau-Opitz, 2010; Khazipov & Luhmann, 2006; Kilb, Kirischuk, & Luhmann, 2011; Yang, Hangau-Opitz, Sun, & Luhmann, 2009). These patterns include GDPs of the hippocampus (Ben-Ari, Cherubini, Corradetti, & Gaiarsa, 1989), which have been largely attributed to GABAergic excitation of neonate neurons (Ben-Ari, 2001; Canepari, Mammano, Kachalsky, Rahamimoff, & Cherubini, 2000; Garaschuk, Hanse, & Konnerth, 1998; Mohajerani & Cherubini, 2006). Another feature of the developing postnatal brain is the so-called early network oscillations (ENOs), which are bursts of synchronized Ca\(^{2+}\) elevations, moving in slow oscillatory waves throughout entire populations of neurons in the hippocampus and cortex (Garaschuk, Linn, Eilers, & Konnerth, 2000; Nakayama, Sasaki, Tanaka, & Ikegaya, 2016). Within the cortex, they appear to be generated by glutamatergic signals and are suspended by the end of the first postnatal week (Allene et al., 2008; Corlew, Bosma, & Moody, 2004; Garaschuk et al., 2000). In contrast, hippocampal ENOs are present to the end of the second postnatal week and are the products of GABA\(_A\) activation and glutamatergic innervation (Barger, Easton, Neuzil, & Moody, 2016; Bolea, Sanchez-Andres, Huang, & Wu, 2006; Canepari et al., 2000; Garaschuk et al., 1998).

Strong hippocampal and cortical ENOs can also involve a delayed recruitment of astrocytic networks (Figure 4a) (Barger et al., 2016). While the functional consequences astrocytic contributions to ENOs have not been investigated in detail, local and global Ca\(^{2+}\) elevations and can lead to release of glio-transmitters feeding back onto neurons as formalized by the term “tripartite synapse” (Araque, Purpura, Sanzgiri, & Haydon, 1999). However, it is worth noting that recent studies have called the concept back into question (for a review of both arguments, see reviews from (Fiacco & McCarthy, 2018) and (Savtchouk & Volterra, 2018)). Although most evidence for glio-transmission has been gained in the juvenile and mature rodent brain, it cannot be excluded from also playing an important role in neonates.

While the exact contribution of astrocytes to GDPs and ENOs is not clear, developing astrocytes in the neonatal neocortex, hippocampus, ventral thalamus and several other brain regions have also been shown to have synchronous, seemingly spontaneous oscillations in somatic Ca\(^{2+}\) concentrations (Figure 4b) (Aguado, Espinosa-Parrilla, Carmona, & Soriano, 2002), and more recently, asynchronous slow fluctuations in somatic Na\(^{+}\) (Felix et al., 2020). Spontaneous changes in both of these ions appear in a subset of measured astrocytes, with ~70% showing Ca\(^{2+}\) oscillations and ~40% Na\(^{+}\) fluctuations in the hippocampus. Unlike Ca\(^{2+}\) waves seen in mature astrocytes, this early activity apparently occurs independently of neuronal transmission, as it was not reduced after inhibition of GABAergic, glutamatergic and purinergic receptors (Aguado et al., 2002; Felix et al., 2020; Parri, Gould, & Crunelli, 2001). In fact, it appears that astrocytic Ca\(^{2+}\) oscillations may trigger Ca\(^{2+}\) transients in neighbouring neurons, via the stimulation of NMDA receptors (Parri et al., 2001). The resulting transients can be eradicated in neurons by application of TTX (tetrodotoxin), but remain present in astrocytes—although the lack of neuronal input appears to reduce their network synchronicity (Aguado et al., 2002). Additionally, similar—purinergic transmission independent—waves have been shown to travel throughout neonatal populations of astrocyte-like “B cells” via gap junction coupling (Lacar, Young, Platel, & Bordley, 2011). The temporal regulation of such intrinsically triggered Ca\(^{2+}\) oscillations are heterogeneous, with neocortical astrocytes showing a reduction but not complete ablation with developmental progression, and hippocampal astrocytes displaying oscillating behaviour to a similar level in mature cells (Aguado et al., 2002; Nett, Oloff, & McCarthy, 2002).

While the preponderance of former studies have concentrated on detecting somatic Ca\(^{2+}\) oscillations in neonatal astrocyte networks, astrocytes also show subcellular Ca\(^{2+}\) elevations already during early development. The latter tend to be small in amplitude but grow as the cells reach morphological maturation (Nakayama et al., 2016). This temporal correlation may be related to the increase in the surface-to-volume ratio produced by the increasing arborization of processes, as morphology has been shown to influence the form and frequency of calcium transients (Wu et al., 2019). While the amplitude of subcellular Ca\(^{2+}\) events increases with age, the mechanism (which remains to be identified) could remain constant—as events in both P7 and P30 animals continue at a similar level after applications of antagonists for purinergic receptors and TRPA1 (transient receptor potential) channels (although mGluRs inhibitors have showed mixed results). Additionally, spontaneous subcellular events in neither young nor mature astrocytes appear to be connected to neuronal activity (Nakayama et al., 2016; Rungta et al., 2016; Zur Nieden & Deitmer, 2006). These subcellular signals constitute the majority of spontaneous Ca\(^{2+}\) signals in adult astrocytes and have been shown to be confined to and synchronized within individual cellular compartments (Bindocci et al., 2017; Rungta et al., 2016; Stobart et al., 2018).

In addition to spontaneous subcellular activity, both developing and mature astrocytes respond to and integrate a range of sensory signals with changes in their Ca\(^{2+}\) concentration (Slezak et al., 2019). In the last 10 years, a broad range of highly sensitive and selective tools has been developed to study these changes. These include the creation of astrocyte-specific transgenic mouse lines, the improvement of imaging to allow multiphoton 3D depiction of entire cells, and resolutions high enough to show interactions at individual
FIGURE 4  
Ca²⁺ activity and transmitter receptors on astrocytes. (a) Propagation of a Ca²⁺ wave through the neocortex, with neurons reacting first, followed by astrocytes. Scale bar: 2 s, 10% ΔF/F. (b) (Left) Wide field microscope image of the stratum radiatum of the hippocampal CA1 region (P6) labelled with Fura-2. (Right) Example traces from individual astrocytes in the hippocampus (top) and cortex (bottom), showing spontaneous oscillations in intracellular Ca²⁺. (c) GABA-induced Ca²⁺ signals in cortical astrocytes (P15-20) and effect of blockers for GABA A (PTX: picrotoxin) and GABA B (CGP52432) receptors. (d) Top: Wide field images of neonatal cortex (left) and hippocampus (right), co-stained with SBFI-AM and SR101 as indicated. Bottom: Astrocyte Na⁺ transients after puff applications of glutamate in both regions, both with and without the presence of the NMDA channel blocker APV. (e) Ca²⁺ signals measured in hippocampal astrocytes (P10-13) after Schaffer collateral stimulation and effect of the mGluR5 inhibitor MCPG (1 mM). Data taken from: (a) Barger et al. (2016), modified; (b) Aguado et al. (2002), modified, Copyright [2002] Society for Neuroscience; (c) Mariotti et al. (2016); (d) Ziemens et al. (2019); (e) Porter & McCarthy (1996) Copyright [1996] Society for Neuroscience.
synapses, models which integrate the complex morphology of mature cells and fluorescence lifetime imaging to reveal absolute Ca\(^{2+}\) concentrations within cell subcompartments (Bindocci et al., 2017; Reynolds, Zheng, & Rusakov, 2019; Savtchenko et al., 2018; Srinivasan et al., 2016; Zheng & Rusakov, 2015). While these technical advances have until now been primarily used to investigate adult signalling, they will undoubtedly also further our understanding of the described “spontaneous” astrocytic Ca\(^{2+}\) signals in the neonatal brain in future studies.

8 | GABA RECEPTORS ON ASTROCYTES

As outlined above, GABA plays an important role in early postnatal development (Ben-Ari, 2001). In addition to binding and taking up GABA through GATs, astrocytes are capable of reacting to released GABA directly via both GABA\(_A\) and GABA\(_B\) receptors (Losi, Mariotti, & Carmignoto, 2014; Schousboe, 2000). These interactions have been shown to play a critical role in astrocytic maturation.

For example, in culture, the introduction of GABAergic neurons induced a shift in astrocyte morphology and produced a more complex shape in cells with neuronal contact (Matsutani & Yamamoto, 1997). This was confirmed in situ, wherein astrocytes from areas with higher levels of GABAergic terminals also display the highest degree of stimulation. Furthermore, increasing GABA or activating GABA\(_A\) receptors in vivo increases GFAP content and astrocytic branching across the forebrain (Runquist & Alonso, 2003). These results may be linked to steroid-induced differentiation, as astrocytic GABA\(_A\) receptor activation has been shown to be a modulating factor here (Mong, Nunez, & McCarthy, 2002). In addition to branching, GABA\(_A\) receptor activation appears to play a role in cell migration, as low concentration applications (levels which would stimulate tonic, but not phasic GABA\(_A\) receptor activation) reduce migration distances, an effect which can be rescued by application of bicuculline (Bolteus & Bordey, 2004). The exact mechanisms of these effects are not yet fully understood; however, the two direct effects of GABA on astrocytes are depolarization, and Ca\(^{2+}\) elevations, the details of which will be discussed below.

In contrast to neurons, astrocytes retain their NKCC1 expression into adulthood and do not switch to KCC2 (potassium chloride co-transporter 2) (Losi et al., 2014). Taken together with an extracellular Cl\(^{-}\) concentration of around 120 mM, this produces a [Cl\(^{-}\)]\(_e\) of roughly −25 to −30 mV (at intracellular [Cl\(^{-}\)] of 40–50 mM, 37°C), resulting in GABA\(_A\) receptor activation being depolarizing for astrocytes (Fraser, Mudrick-Donnon, & MacVicar, 1994). It has been suggested that GABA-induced Cl\(^{-}\) efflux from astrocytes could help to maintain high extracellular Cl\(^{-}\) content during high frequency interneuron firing (Bormann & Kettenmann, 1988; MacVicar, Tse, Crichton, & Kettenmann, 1989).

GABA\(_A\) receptor-induced depolarization in astrocytes can be sufficient to open L-type voltage-gated Ca\(^{2+}\) channels, creating Ca\(^{2+}\) signals (Figure 4c) (Fraser et al., 1995; Nilsson, Eriksson, Ronnback, & Hansson, 1993). In the mouse hippocampus, GABA\(_A\) receptor-mediated Ca\(^{2+}\) signals such as these are present from the first postnatal week and remain consistent throughout development (Meier et al., 2008). GABA\(_B\) receptor activation too has been linked to intracellular oscillations in Ca\(^{2+}\), which are mainly attributed to IP\(_3\)-related release from intracellular stores (Figure 4c) (Losi et al., 2014; Mariotti, Losi, Sessolo, Marcon, & Carmignoto, 2016; Meier et al., 2008). In hippocampal slices, this reaction was demonstrated using application of the GABA\(_B\) agonist baclofen—and was shown to be greatest during the second postnatal week—wherein around 60% of cells respond, in contrast to around 10% in earlier and later stages (Meier et al., 2008). In contrast to this, cortical astrocytes display GABA\(_B\) receptor-evoked, extended Ca\(^{2+}\) signals in both developing and mature mice (Mariotti et al., 2016). The functional importance of GABA\(_B\) receptors on neonate as well as mature astrocytes is still not entirely clear. However, it has been shown that GABAergic stimulation of astrocytes increases their Ca\(^{2+}\) and can trigger release of other transmitters (such as glutamate or ATP) and thereby modulate activity of neighbouring cells (Kang et al., 2008; Mariotti et al., 2016; Serrano, Haddjeri, Lacaille, & Robitaille, 2006).

9 | GLUTAMATE RECEPTORS ON ASTROCYTES

Astrocytic glutamate receptors are heterogeneously expressed across the brain. One of the best described examples of this is the spatial patterning of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors, which are present in cerebellar Bergmann glia, have low expression levels in the neocortex and are apparently largely absent in the hippocampus (Matthias et al., 2003; Molders, Koch, Menke, & Klocke, 2018; Verkhratsky & Nedergaard, 2018; Zhou & Kimelberg, 2001). Additionally, these receptors are temporally regulated, with expression in astrocytes sorted from whole brains highest in first postnatal week (Molders et al., 2018). Their presence in the cerebellum appears to be involved in the formation of Purkinje cell synapses, as deletion leads to a retraction of astrocytic processes from neuronal contacts and therefore a reduction in glutamate uptake and delayed synaptogenesis (Iino et al., 2001; Saab et al., 2012).

NMDA receptors have been shown to be expressed in astrocytes within the cortex throughout development (Verkhratsky & Kirchhoff, 2007). They account for around 50% of the
astrocytic Na⁺ response to glutamate both in neonates (P2) and in adult mice (P90) (Ziemens, Ochsmann, Germkau, & Rose, 2019) (Figure 4d). This is in contrast to hippocampus, where astrocytic responses to NMDA are either very small or absent (Figure 4d) (Serrano, Robitaille, & Lacaillle, 2008; Ziemens et al., 2019). NMDA receptors on astrocytes differ in properties to their neuronal counterparts, appearing to lack the Mg²⁺ block and displaying a lower permeability to Ca⁴⁺ (Palygin, Lalo, Verkhratsky, & Pankratov, 2010). Inhibition of NMDA receptors has developmental consequences and induced a prolonged proliferative phase, delaying neuronal maturation in the neocortex (Hirasawa, Wada, Kohsaka, & Uchino, 2003) and disrupting migration (Reiprich, Kilb, & Luhmann, 2005).

One of the most thoroughly studied mechanisms for astrocytic glutamate response is the developmental regulation of metabotropic glutamate receptors (mGluR). The majority of hippocampal astrocytes (82%) were shown to respond to the application of glutamate in the first postnatal week via mGluR5 (Figure 4e) (Cai, Schools, & Kimelberg, 2000; Porter & McCarthy, 1996). mGluR5 mRNA levels are gradually downregulated over the first three postnatal weeks, at the end of which it is almost completely replaced with mGluR3 (Sun et al., 2013). It is important to note that although mRNA levels have been heavily reduced in adult animals, mGluR5 receptors may continue to be functional in subdomains, with expression being limited to PAPs at specific synapses (Panatier & Robitaille, 2016). The two receptor subtypes have contrasting consequences, with mGluR5 triggering Ca²⁺ increases through IP₃-mediated release from internal stores (Panatier & Robitaille, 2016) and mGluR3 decreasing cAMP (cyclic adenosine monophosphate) levels and thereby also transmitter release probabilities (Grosche & Reichenbach, 2013).

The mGluR5-induced Ca²⁺ elevations have been implicated in glio-transmission, such as the local release of purines by astrocytes after glutamatergic innervation, which can upregulate the activity of a single presynaptic terminal by binding A₂A receptors. However, if mGluR5 activation is sustained over a longer period, an extrasynaptic release of glutamate is triggered, acting to synchronize a wider network of neurons (Panatier & Robitaille, 2016). This flexibility in response makes mGluR5 ideally suited to striking balances in the dynamic postnatal brain environment, and it has been shown to be a key player in several specifically developmental processes such as astrocytic domain growth, GLT-1 induction and synaptic ensheathing (Morel et al., 2014). Additionally, there is evidence that mGluR5 mediates the spontaneous Ca²⁺ oscillations seen in hippocampal astrocytes (see Figure 4b) (Zur Nieden & Deitmer, 2006).

Of note, the importance of IP₃ receptors for astrocytic Ca²⁺ signals is a topic of debate. Several studies have shown that knocking out IP₃ has little effect on Ca²⁺ signalling within astrocytic distal processes, the location for the majority of Ca²⁺ events (Rungta et al., 2016; Srinivasan et al., 2016; Stobart et al., 2018). However, a recent study utilizing high resolution Ca²⁺ imaging has suggested that in these animals, IP₃ independent mechanisms may create Ca²⁺ “nanodomains” which could compensate the loss and prevent an effect from being seen (Okubo et al., 2019). This and other questions surrounding the receptor may be clarified, for example, using the recently developed photo-switchable mGluRs which can be expressed and measured in vivo (Acosta-Ruiz et al., 2020) and/or using advanced imaging techniques in astrocytic processes as outlined above (Bindocci et al., 2017; Reynolds et al., 2019; Savchenko et al., 2018; Srinivasan et al., 2016; Zheng & Rusakov, 2015).

10 | PURINERGIC RECEPTORS ON ASTROCYTES

An established form of astrocytic communication is the triggering of Ca²⁺ waves by ATP (Kuga, Sasaki, Takahara, Matsuki, & Ikegaya, 2011). While these waves persist in adult tissue, they are more prominent in the developing brain, where their propagation through radial glia has been shown to modulate proliferation and migration of neurons and astrocytes, as well as DNA production and cellular differentiation (Weissman, Riquelme, Ivic, Flint, & Kriegstein, 2004). Transcriptional and immunohistochemical studies have shown that mammalian astrocytes express both ionic P2X (Palygin et al., 2010; Verkhratsky, Pankratov, Lalo, & Nedergaard, 2012) and metabotropic P2Y receptors (Cheung, Ryten, & Burnstock, 2003) from before birth and throughout development. However, the largest proportion of hippocampal astrocytes reacts to ATP application in the first postnatal week (Cai et al., 2000).

P2X receptors expressed in vitro appear to be functional—opening upon binding to allow an influx of Na⁺ and Ca²⁺ and efflux of K⁺ (Verkhratsky et al., 2012). However, their ion permeability, as well as their sensitivity and rate of desensitization, is determined by their subunit composition, which varies by region in situ. In slice preparations, ATP might undergo rapid extracellular break down into adenosine (Centemeri et al., 1997). Acutely isolated astrocytes from somatosensory cortex have P2X receptors with a higher sensitivity. While these showed a consistent Ca²⁺ response to ATP (via application and neuronal afferent stimulation) in adult astrocytes, the response in young brains was significantly smaller (Palygin et al., 2010).

Being metabotropic and therefore G protein-coupled, P2Y receptors are generally considered to have long-lasting and trophic actions. They are expressed by around 10% of freshly isolated rat cortical astrocytes from the first postnatal week and by 20%–40% of hippocampal astrocytes (Zhu &
et al., 2018). Like GABA, the Cl- switch renders the action of
nile hippocampus were shown to express GlyT1 (Ghirardini
& Valente, 2011). In line with this, astrocytes in the juve -

cision in astrocytes, these findings point to a more prominent
11 | OTHER RECEPTORS

Despite the fact that the majority of developmental astrocyte
research has focused on the three transmitters detailed above,
immature astrocytes are able to respond to a much broader
range of signals. Here, we will briefly explore some of these
mechanisms and their place in the context of development.

Glycine receptors have long been reported to be expressed
across spinal cord astrocytes, where they are involved in in -
terneuron differentiation and synaptogenesis (Pastor, Chvatal,
Sykoca, & Kettenmann, 1995). More recently, however, the
receptors have been shown to modulate ATP-induced Ca2+
signals in cultured cortical astrocytes (Morais, Coelho, Vaz,
Sebastiao, & Valente, 2017). Furthermore, mRNA and pro -
tein analysis from the whole hippocampus show peak expres-
sion of GlyRs at P7, suggesting that the transmitter may also
of adult astrocytes (Malarkey & Parpura, 2008). These have
been reported within the first two postnatal weeks (Cai
et al., 2000).

Aside from transmitter receptors and transporters, astrocytic ion signalling can also be instigated by a variety of
membrane proteins—most notably, mechanosensitive chan-
nels. As astrocytes mature, they stretch out new processes
and begin arborization, the progression of which would
continuously activate mechanosensitive channels. While studies looking at their
functions and roles are still limited, there is evidence that they are upregulated during development and that they play a role in cell fate determination (Sugimoto et al., 2017) and vascular development (Ranade et al., 2014).

Another class of mechanosensitive channel important for
astroglial function is TRP channels. TRPV4, the channel re-
ponsible for sensing vasodilation/constriction at astrocytic
endfeet in mature tissue, is downregulated during early de-
velopment (Dunn, Hill-Eubanks, Liedtke, & Nelson, 2013).
In contrast, TRPC1 channels are already present on around
50% of astrocytes freshly isolated from P1 animals and 100%
of adult astrocytes (Malarkey & Parpura, 2008). These have
been identified as the primary pathway for Ca2+ release from
stores in astrocytes. In addition, they are permeable for Na+, and studies have shown the regulation of both ion fluxes to be
dynamic. This along with their co-localization with the NCX
makes them ideal coordinators for Ca2+- and Na+ signalling
(Reyes, Verkhratsky, & Parpura, 2013; Verkhratsky, Trebak,
Perocchi, Khananshvili, & Sekler, 2018).

12 | SYNOPSIS

The first postnatal weeks of the rodent brain constitute a
period in which cellular networks undergo morphological
and functional maturation. This not only relates to neuronal
maturation, involving a surge in dendritic outgrowth and syn-
apse formation, but also comprises the terminal differentia-
tion and maturation of astrocytes and the astrocytic network
(Figure 5). Generally, the neonate brain has a significantly
lower functional expression of many plasma membrane
proteins than the adult brain. These proteins form the basis of established astrocyte functions at active synapses, such as regulation of extracellular K⁺ or uptake of transmitters (Figure 5). Astrocytes in the neonatal brain, therefore, appear to play a different role, with a reduced focus on the support of active neurons in terms of controlling the ionic composition of the ECS. On the other hand side, the clearance of substances released by active neurons in the neonate might not be as demanding as compared to the mature brain. First of all, the much lower density of synapses will result in a lower overall release of K⁺ and transmitters. Moreover, the increased size of the ECS enables for a more efficient and rapid diffusion, counteracting a local accumulation of both. The delayed differentiation and functional maturation of astrocytes, compared to neurons in the first postnatal weeks, might thus reflect the reduced need for active (and energy-consuming) regulation of the ECS.

This is by no means to say that astrocytes within young brains are silent bystanders. In fact, multiple lines of evidence point to them as playing a formative role. They do this both by taking up and/or responding to ions and molecules released by other cells (Figure 5) and by actively releasing signalling compounds themselves. This is a tightly regulated process, which has to shift over time to suit the brain’s needs as it matures. Dynamic control of astrocyte activity is governed primarily by Ca²⁺ signalling, which offers astrocytes an alternative to classical electrical excitability. Many studies have shown the broad patterns of activity that move across populations of both neurons and astrocytes during development, and the signalling systems involved have largely been described. Yet, the fine differences that distinguish one outcome from another in the wake of a Ca²⁺ influx have yet to be elucidated. The last decade has greatly expanded the toolbox available for Ca²⁺ studies and has undoubtedly furthered our understanding of astrocytic Ca²⁺ signals in the juvenile and mature brain. Implementing these techniques in the neonatal brain will provide valuable insight into the mechanisms fine-tuning Ca²⁺ signals in maturing astrocytes and determining their consequences. In turn, we may be able to better understand and treat the plethora of developmental disorders which impair the growth and development of the CNS.

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