Versatile control of synaptic circuits by astrocytes: where, when and how?

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Abstract | Close structural and functional interactions of astrocytes with synapses play an important role in brain function. The repertoire of ways in which astrocytes can regulate synaptic transmission is complex so that they can both promote and dampen synaptic efficacy. Such contrasting effects raise questions regarding the determinants of these divergent astroglial functions. Recent findings provide insights into where, when and how astroglial regulation of synapses takes place by revealing major molecular and functional intrinsic heterogeneity as well as switches in astrocytes occurring during development or specific patterns of neuronal activity. Astrocytes may therefore be seen as boosters or gatekeepers of synaptic circuits depending on their intrinsic and transformative properties throughout life.

UP and DOWN states
Spontaneous alternation of neuronal membrane potential between two preferential levels corresponding to an active period of depolarization associated with sustained firing (UP state) and a quiescent period with hyperpolarized membrane potential (DOWN state).

Synaptic transmission is no longer viewed as a simple matter of one neuron conveying a signal to another. The factors influencing electrical signals generated at synapses are now known to be multitudinous and originate from both neuronal and non-neuronal sources. Notably, astrocytes closely associate with synapses, with the processes of one astrocyte contacting as many as ~140,000 synapses[1]. Yet, although astrocytes are considered as active partners of neurotransmission, their precise role has been subject to debate. Seminal work assessing the influence of neuroglial interactions on synaptic transmission has mainly been performed in cell cultures and hippocampal slices and has by and large reported that astrocyte stimulation enhances synaptic transmission[2]. However, in the past couple of decades, the technological advances allowing specificity of astrocyte activation, the broadening of brain regions investigated and, importantly, the assessment of neuroglial interactions in physiological contexts (that is, ex vivo or in vivo) at different developmental stages resulted in the realization that astrocyte activation can lead to inhibitory as well as excitatory regulation of neuronal activity. In the present Review, we consider the diversity of these regulatory actions in light of the many aspects of astroglial functions that occur simultaneously, with particular focus on intrinsic astrocyte heterogeneity, developmental changes and patterns or regimes of neuronal activity.

Astroglial functional heterogeneity

Although astrocyte morphological diversity was documented over a century ago (such as the existence of protoplasmic and fibrous astrocytes), their functional and physiological heterogeneity has only recently emerged thanks to the use of novel combinatorial approaches[3]. These have indeed revealed circuit-specialized and even synapse-specialized astrocytes in several brain regions[4–6]. We refer readers to several recent and general reviews on astroglial heterogeneity[3,7,8] and here focus on heterogeneity from a functional and physiological point of view, illustrating the multiplicity and specialization of astrocyte functions within brain circuitry.

Electrophysiological membrane properties

Until recently, astrocytes were thought to be a homogeneous population, displaying typical electrophysiological membrane properties, such as low membrane resistance and resting membrane potential, passive conductances, lack of action potential and extensive gap junction (GJ) coupling. However, numerous studies now indicate that astrocytes show heterogeneity; that is, there are subpopulations of astrocytes with varying electrophysiological properties, within and across brain regions.

In vivo intracellular recordings from anaesthetized rats (4–6 weeks postnatal) revealed that membrane potentials of cortical and hippocampal astrocytes display a Gaussian distribution centred around −85 mV (REF.[9]). These astroglial potentials also slowly fluctuate by ~1 mV according to the level of spontaneous network activity occurring during cortical UP and DOWN states or hippocampal non-theta and theta states. Astrocyte depolarization is thought to reflect inward K^+ currents resulting from activity-dependent increases in extracellular K^+ levels[9]. Although it is challenging to assess specifically the physiological role of these small fluctuations, astroglial uptake of extracellular K^+, in particular through inwardly rectifying K^+ channel Kir4.1, has been shown to regulate neuronal excitability under physiological conditions[10,11]. In addition, alteration in Kir4.1-mediated K^+ uptake has recently been reported to contribute to

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pathological conditions such as major depressive disorder[12] and Huntington disease[13] as a result of neuronal hyperexcitability in the lateral habenula or striatum, respectively. Astrocytes also express a variety of voltage-dependent transporters and channels, including glutamate transporter proteins (GLTs), Na^+/K^+-ATPases or Na^+/HCO_3^--transporters. Astroglial depolarization may thus also favour adaptive functional changes, which could lead to changes in glutamate clearance, rate of glycolysis[14] or intracellular pH homeostasis[15], in turn resulting in altered neuronal activity.

The current profile of astrocytes can also vary, both between different brain regions and within the same brain area[3,16–18]. Indeed, passive astrocytes displaying linear current–voltage relationships or complex astrocytes displaying nonlinear voltage-dependent currents (arising from the expression of a variety of rectifying K^+ conductances) have been reported within various brain areas[17,19]. The nature of the channels underlying the astroglial passive currents remains however uncertain. The present consensus is that astroglial passive conductance reflects intrinsic properties of membrane ion channels from individual astrocytes but not GJ coupling[20–22]. Although K^+ channels are most likely the key players, their precise nature remains elusive. Specifically, the contribution of TWIK1 (also known as KCNK1) and/or TREK1 (also known as KCNK2) K^+ channels, initially thought to mediate astrocyte passive conductance[23,24], is now controversial[25]. Indeed, although pharmacological and short hairpin RNA (shRNA) approaches first suggested that TWIK1/TREK1 were involved, it was recently reported that their genetic deletion using knockout mice has no effect on astrocyte passive conductance. The experimental approaches used in these studies have limitations, including weak selectivity (pharmacology), potential off-target effects (shRNA) or compensatory expression of other genes (knockout mice), which may underlie the discrepancies.

Several studies have also reported electrophysiologically distinct classes of astrocytes displaying different current patterns and K^+ uptake capabilities within the same and across distinct brain regions. Notably, this is the case for hippocampal astrocytes, which are topographically segregated in the CA1 and CA3 areas[17,18]. Such differential capacity for K^+ clearance is likely to translate into distinct astroglial regulation of neuronal excitability. Heterogeneity of astrocytes is also reflected in their activity-dependent currents. For example, glutamate uptake currents, typically found in hippocampal CA1 astrocytes, are hardly detectable in CA3 astrocytes[26,27] and in some hypothalamic astrocytes[28]; this is likely to increase spillover of released glutamate to nearby synapses and could promote synchronization of neuronal populations, a physiological phenomenon well known to occur in these brain regions. In addition, recent data also show that K^+-mediated currents are larger in astrocytes from the hippocampal CA1 area than from the striatum[3]. Such intra-region and inter-region heterogeneity is likely to reflect differential transporter or channel expression levels[3,29] and/or astroglial coverage of synapses[30] and may underlie distinct regulatory powers of astrocytes on neuronal excitability and synaptic transmission.

Another typical electrophysiological feature of astrocytes is their extensive GJ coupling, which initially fuelled the concept of a homogeneous astroglial syncytium. Several studies now indicate, however, that the extent and shape of coupling in astrocytes can widely differ within the same[17] or from different brain areas[31,32], indicating the existence of heterogeneous astroglial networks. Indeed, astroglial networks can be compartmentalized according to the structural and functional organization of local neuronal circuits and display activity-dependent changes in their size. Such plasticity may result from differential connexin (Cx) expression as well as GJ modulation by endogenous factors[33]. As found for the membrane currents, astrocytes from the CA1 and CA3 areas of the hippocampus also differ in their intercellular coupling, with CA1 astrocytes being more coupled than their CA3 counterparts[17]. The functional relevance of this coupling heterogeneity is still unknown. However, because GJ-mediated astroglial networks promote coordination of neuronal populations[34], astroglial control of neuronal synchrony may be greater in the CA1 than in the CA3 area of the hippocampus. Similarly, astrocytes from the barrel cortex[35] or olfactory glomeruli[36] display differential coupling properties according to their location and the neuronal activity within these structures. Indeed, as found for neuronal circuits, astrocyte coupling is confined in individual barrels or olfactory glomeruli, favouring preferential astroglial communication within these structures. Remarkably, such functional compartmentalization is also strengthened in the barrel cortex by the mere GJ-mediated coupling between astrocytes from interbarrel septa. Together, these observations point at the presence of selective and activity-dependent GJ-mediated astroglial networks within specific functional circuits[33].

The reported heterogeneity of the astroglial membrane properties is, however, relatively limited when compared, for example, with the diversity of astrocyte molecular repertoires[3,7]. This is most likely attributable to the approach used to probe astroglial membrane properties (that is, somatic electrophysiology), which is poorly suited to these cells as astrocytes are electrically non-excitable and display low input resistance, high GJ coupling and a complex morphology. Astrocytes display an extensive network of ramified fine processes, termed perisynaptic astroglial processes (PAPs). The putative dynamics and heterogeneous membrane properties of PAPs, which are the relevant nanodomains for integration and processing of local synaptic information, are thus far inaccessible using conventional somatic electrophysiology. The recently developed nanopipette electrodes, used to record intracellular voltage from dendritic spines[37], should be key in advancing our knowledge on the diversity of electrophysiological properties from astroglial membrane subcompartments. These nanopipettes, with tip diameters of 15–30 nm, have recently been used to measure directly currents or voltages from astroglial perisynaptic nanoprocesses, which can be visualized via super-resolution microscopy.

Passive astrocytes
Functional subtype of astrocytes that present a linear current–voltage relationship.

Complex astrocytes
Functional subtype of astrocytes that display voltage-dependent currents.

Perisynaptic astroglial processes
(PAPs). Fine distal processes of astrocytes surrounding synaptic elements, integrating and processing synaptic information.

Calcium signalling

Astrocytes display a wide variety of Ca^{2+} signals that are spatially and temporally distinct, occur either spontaneously or in response to local neuronal activity and are likely to regulate a variety of physiological functions (for review, see REF.[8]). These signals range from spontaneous subcellular transients that are activity-independent[3,38,39] to behaviour-driven global events, which occur in a concerted manner across populations of astrocytes[40,41]. What are the determinants of these multiple signals? Intrinsic molecular diversity of membrane receptors, ionic channels and intracellular signalling machinery, as well as local microcircuits, are likely key drivers. In particular, astrocytes can exhibit various patterns of Ca^{2+} signalling within the same brain region and can discriminate and respond specifically to defined neuronal or synapse subtypes, as now shown in the hippocampus[42], barrel cortex[43] or dorsal striatum[44]. Differences in the pattern of Ca^{2+} activity, such as in spontaneous transients, are also found in astrocytes from the cortex, where in layer 1 asynchronous activity dominates whereas in layer 2/3, more synchronous transients are found[45]. Such diversity is also well illustrated within the hippocampus, where CA1 striatum radiatum astrocytes processes display local Ca^{2+} increase via activation of metabotropic glutamate receptor 5 (mGluR5) in response to Schaffer collateral minimal stimulation[46] (that is, a stimulation that engages only a small number of synaptic inputs and is therefore close to threshold for activating downstream neurons). By contrast, astrocytes from the CA3 stratum lucidum area only respond to strong activity evoked by mossy fibre stimulation. Accordingly, the responses, mediated by activation of mGluR2/mGluR3, occur across all astroglial compartments[47]. Such different activity-dependent responses in stratum lucidum and stratum radiatum astrocytes are likely to reflect the markedly lower astroglial coverage of CA3 compared with CA1 synapses[30] (FIG. 1) as well as the peculiar physiological properties of mossy fibre synapses, characterized by low basal neurotransmitter release probability but pronounced and dynamic facilitation during repetitive stimulation[48]. These data thus suggest that Ca^{2+} activity from stratum radiatum astrocytes can locally regulate neighbouring synapses[46,49], whereas Ca^{2+} activity in the stratum lucidum counterparts may preferentially control coordination of neuronal networks.

What about the patterns of astroglial Ca^{2+} signalling in vivo in behaving mice? Although there have been to date only a few studies recording physiologically relevant and behaviourally induced astroglial Ca^{2+} events within intact brains of awake mice, some common features have emerged. Indeed, both locomotion and startle reflex induce global slow Ca^{2+} events mediated by noradrenaline in cortical astrocytes and cerebellar Bergmann glia from head-fixed mice[40,41,50,51]. Nevertheless, the kinetics of these Ca^{2+} responses varied between these brain regions[51]. Thus, behaviourally driven global Ca^{2+} events in astrocytes may regulate on a slow timescale large neuronal networks involved in the generation of behaviour, as recently shown in *Drosophila* for sensory-driven behaviours[52]. It will now be interesting to investigate whether similar patterns of global and concerted responses also occur in astrocytes of freely behaving mice as well as following other types of behaviour or in other brain areas, in particular related to learning and memory. Furthermore, recording Ca^{2+} signalling in three dimensions, as opposed to the two-dimensional imaging typically performed up to now, would better reflect the genuine complexity of astroglial Ca^{2+} signalling as recently uncovered[53]. Finally, it will be important to explore whether other types of astroglial signal, such as the rapid Na^{+} signalling that responds to neuronal activity[54] and is linked to changes in astrocyte Ca^{2+} dynamics[55], also display heterogeneity and can mediate fast and diverse synaptic modulations.

Synaptogenic effect

There is also functional heterogeneity in the ability of astrocytes to promote neuronal synaptogenesis. Here, again, it has emerged that the determinants of this heterogeneity are the brain region and astrocyte population subtype. Indeed, it is well established that astrocytes play an important role in promoting synaptogenesis, particularly during development. They do this via multiple pathways, including contact-mediated signalling and secretion of molecules (for review, see REFS[56,57]). Expression of several astroglial synaptogenic molecules varies between and within brain regions[58], suggesting specialization of local astrocyte populations within specific synaptic circuits. For example, astrocytes from the cerebellum were recently found to induce more synapse formation than their counterparts from the midbrain, hippocampus or cortex[58], and this occurs via stronger expression of synaptogenic molecules such as hevin and glypican 4. Remarkably, recent data indicate that in several brain regions, the synaptogenic effect of astrocytes actually reflects the predominance of a specific astrocyte population subtype referred to as population 'C'[6]. This subpopulation of astrocytes, isolated from ALDH1L1–eGFP adult mice and identified via the presence of surface markers (CD51, CD63 and CD71), express genes promoting synapse formation and function, in particular associated with glutamate and ion transport, as well as assembly of synapses. In this way, population C augments the frequency, but not amplitude, of cortical excitatory and inhibitory currents[6]. Altogether, these data highlight the functional heterogeneity of the role of astrocytes in synaptic modelling.

In all, little is known about the mechanisms underlying astrocyte molecular and functional diversity. However, recent data suggest that astrocyte specialization is likely to be driven and maintained by both endogenous hardwired programmes in developing astrocytes and extrinsic neuronal cues controlling the local environment[7]. Further elucidation of the signals driving the specialization and functional roles of distinct populations of astrocytes is crucial to gaining deeper insights into astrocyte functional heterogeneity.

Astroglia: from booster to gatekeeper?

The population of astrocytes that has freshly settled in the immature brain sense and respond to neuronal activity in different ways from adult astroglial cells, which results in markedly differential regulation of synaptic functions (FIG. 2).

Mossy fibre synapses
Peculiar synapses with a giant presynaptic bouton possessing, on average, 25 active zones. These synapses occur in the hippocampus between dentate granule cells and CA3 pyramidal neurons as well as in the cerebellum, where inputs from several brain regions synapse onto granule cells.

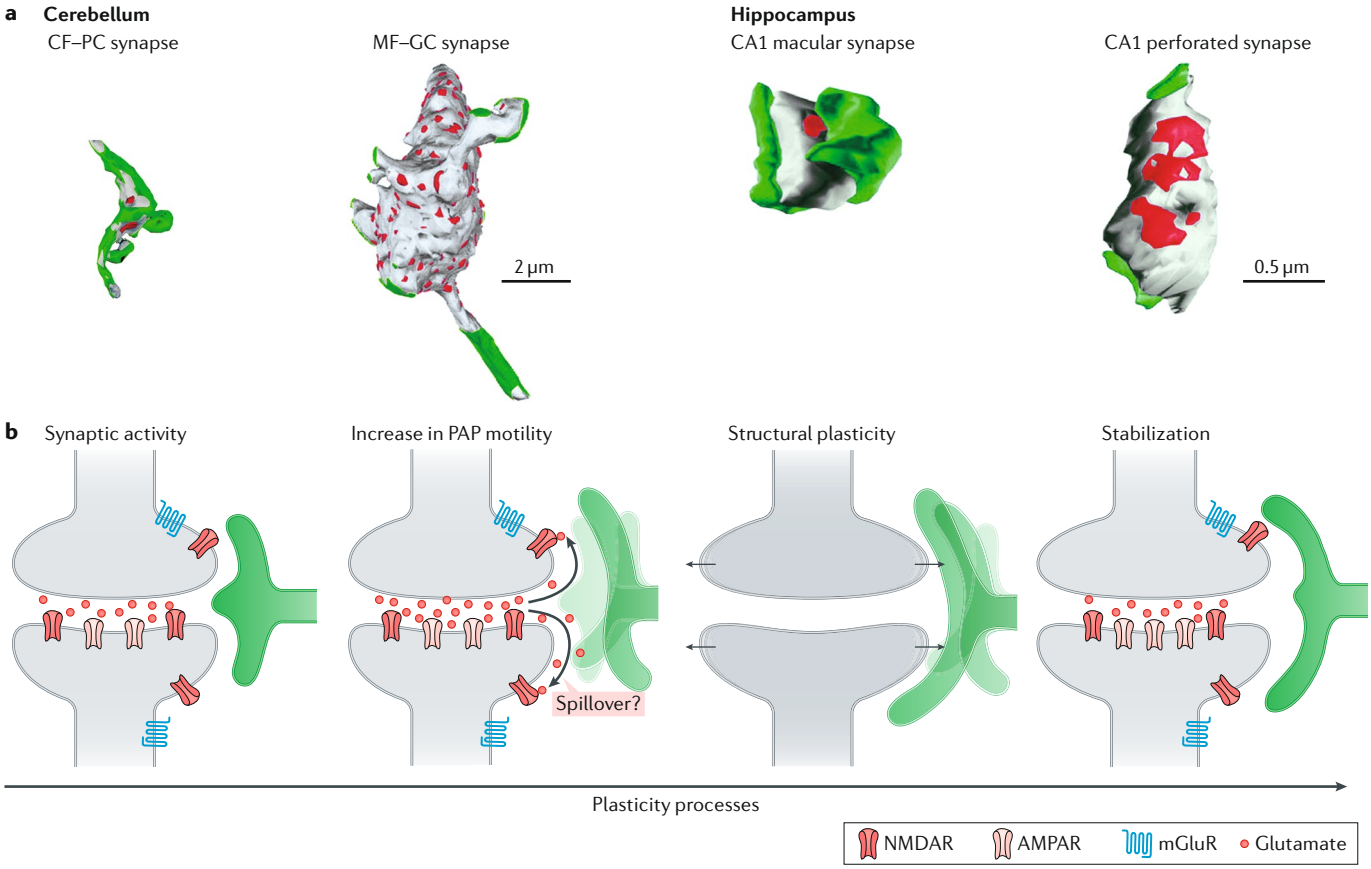

Fig. 1 | **Diversity and plasticity of astroglial coverage of synapses. a** | 3D reconstructions of different types of synapse and their astroglial coverage showing heterogeneity within and between brain regions. In the cerebellum, the climbing fibre–Purkinje cell (CF–PC) synapse (red) has extensive astrocyte coverage (green), whereas the mossy fibre–granule cell (MF–GC) synapse, at which MFs make numerous synaptic contacts with each GC, has relatively little astroglial coverage. In hippocampus, CA1 macular synapses have more astroglial coverage than CA1 perforated synapses. **b** | Possible scenario of changes in synaptic astroglial coverage occurring during plasticity processes. An increase in synaptic activity leads to glutamate release and a temporary increase in perisynaptic astroglial process (PAP) motility[157]. If this results in reduced astrocyte synapse coverage, there is increased potential for spillover of glutamate and structural plasticity of synaptic structures. Stabilization of the enhanced PAP synaptic coverage would terminate these processes. mGluRs, metabotropic glutamate receptors. Part **a** republished with permission of the Society for Neuroscience, from Ultrastructural contributions to desensitization at cerebellar mossy fiber to granule cell synapses, Xu-Friedman et al., *J. Neurosci.*, **23**, 2182–2192, 2003, and from Three-dimensional relationships between hippocampal synapses and astrocytes, Ventura et al., *J. Neurosci.*, **19**, 6897–6906, 1999; permission conveyed through Copyright Clearance Center, Inc. (REFS[144,156]).

Maturation of sensing properties

Electrophysiological membrane properties. The membrane properties of immature astrocytes are different from their mature counterparts: they predominantly display voltage-dependent currents, indicating that most of the immature astrocyte population is composed of so-called 'complex astrocytes'[19,59]. Their ion channel repertoire differs from mature astrocytes, as shown in the hippocampus, where neonatal (that is, immature) astrocytes express a complex combination of voltage-gated K^+ inward and outward conductances but very few or no leak K^+ or Na^+ conductances. The specific set of voltage-dependent conductances present in immature astrocytes at this stage of development appears to determine their ability to sense neuronal activity. In addition, neonatal astrocytes were recently found to exhibit a more negative resting membrane potential (~5 mV hyperpolarization) than mature astrocytes, and this is associated with a poor K^+ uptake capacity in hippocampal slices[19]. Such differences in membrane properties strongly suggest that immature astrocytes poorly regulate neuronal excitability via control of extracellular K^+ homeostasis. Such a feature contrasts with adult astroglial networks, which play a key role in maintaining appropriate K^+ levels during cerebral activation.

Connexins and junctional communication. Astrocytes express high levels of Cxs, which are the proteins composing GJ intercellular channels that enable extensive electrical and metabolic coupling between cells. Importantly, GJ coupling between astrocytes strengthens during development. At embryonic stages, this coupling is mediated exclusively by Cx43. Around postnatal day 15 (P15), Cx30 expression begins and increases until adulthood[60], so that eventually both Cxs similarly contribute to GJ communication in mature astrocytes[61,62].

GJ communication
Gap junction (GJ)-mediated direct electrical and metabolic coupling between adjacent cells allowing cytoplasmic exchanges of small molecules with a molecular mass below 1.5 kDa, including ions, neurotransmitters, second messengers or energy metabolites.

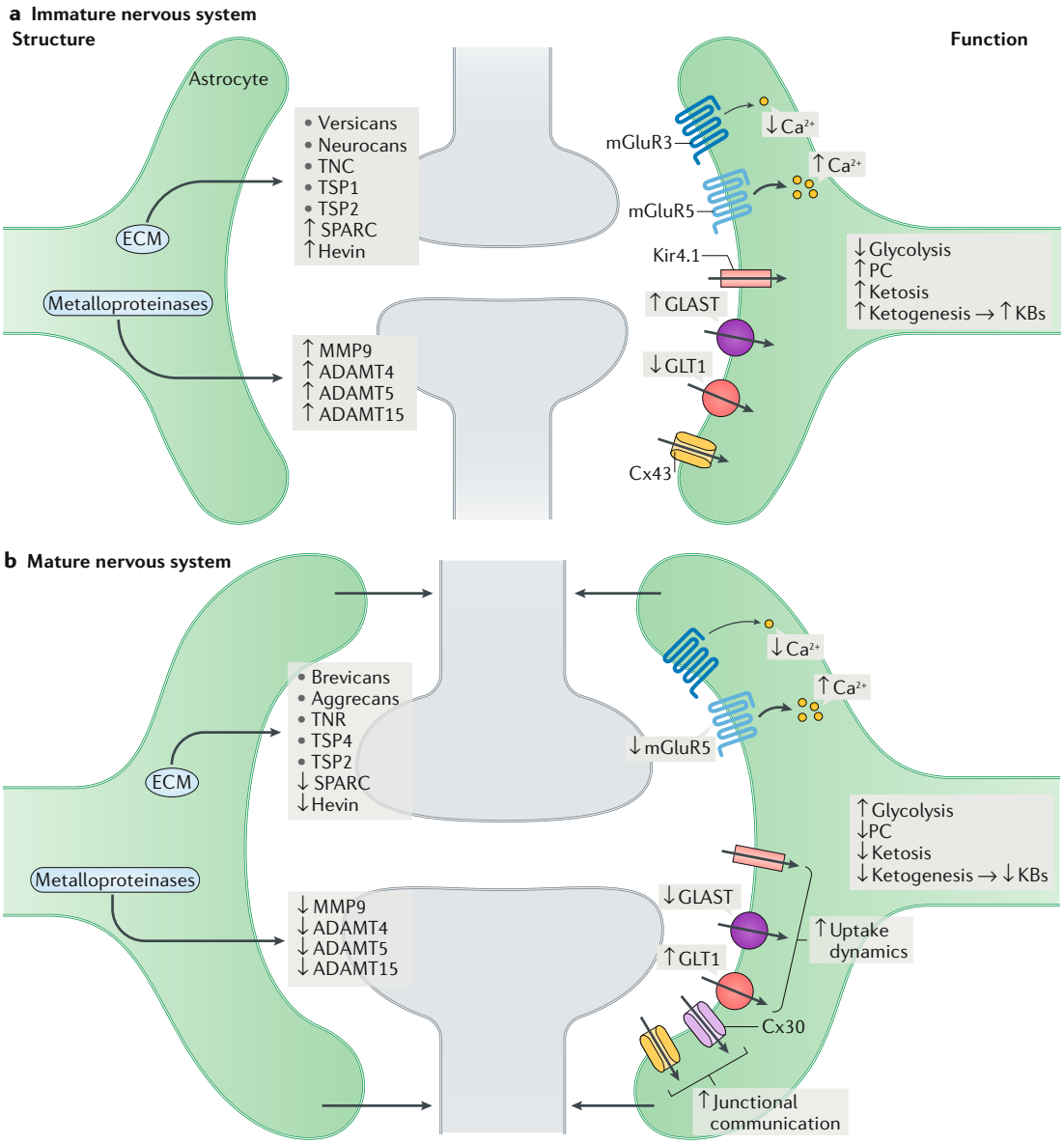

Fig. 2 | **Structural and functional changes in astroglial perisynaptic processes during postnatal development.** Schematic drawing illustrating the structure and function of astroglial perisynaptic processes in the immature and mature nervous system. Perisynaptic astroglial processes are presented farther from the synapse in the young than in the adult brain as synaptic coverage is likely to increase during development (BOX 2). **a** | Left (structure): astrocyte-derived molecules composing the perisynaptic extracellular matrix (ECM) in the immature nervous system mainly include the chondroitin sulfate proteoglycans (CSPGs) versicans and neurocans, tenascin C (TNC), the matricellular proteins thrombospondin 1 and 2 (TSP1 and TSP2, respectively), secreted protein acidic and cysteine-rich (SPARC) and hevin. Expression of metalloproteinases secreted by young astrocytes mainly includes metalloproteinase 9 (MMP9) and a disintegrin and metalloproteinase with thrombospondin motifs 4, 5 and 15 (ADAMT4, ADAMT5 and ADAMT15, respectively). Right (function): Immature astrocytes express the metabotropic glutamate receptors (mGluRs) of both mGluR3 and mGluR5 types. At this stage, the main connexin (Cx) expressed by astrocytes is Cx43. With regard to metabolism, young astrocytes mainly produce energy through ketogenesis (that is, production of ketone bodies (KBs)). The glucose used is mainly taken up for production of glutamate and GABA through the transformation of pyruvate into oxaloacetate with pyruvate carboxylase (PC), an enzyme expressed only in astrocytes. **b** | Left (structure): as the brain matures, CSPGs' contribution to the ECM changes to the benefit of brevicans and aggrecans, TNR replaces TNC, TSP4 becomes prominent and SPARC expression decreases. Astroglial production of metalloproteinases MMP9, ADAMT4, ADAMT5 and ADAMT15 decreases in the adult. Right (function): during postnatal development, mGluR5 is downregulated, leading to a change in glutamate-induced Ca^{2+} signalling. Expression of the inwardly rectifying K^+ channel Kir4.1 increases during development, as does the expression of glutamate transporter 1 (GLT1), occurring in parallel with a decrease in glutamate-aspartate transporter (GLAST). Together, this leads to an increase in the ability of perisynaptic astroglial processes to efficiently take up the K^+ and glutamate released during synaptic transmission. Astroglial expression of Cx30 arises in the mature brain, thus increasing astroglial junctional communication. The production of energy in adult astrocytes is primarily carried out through glycolysis.

Polarity
Reorientation of CA1 striatum radiatum astrocyte stem processes polarizing perpendicularly to the pyramidal cell layer during postnatal maturation.

We and others have shown that, through their role in intercellular communication, Cx43 and Cx30 together tune neurotransmission by locally fuelling active synapses with metabolic substrates[61] and by preventing excessive neuronal activity through efficient uptake of synaptically released glutamate and K^+ (REFS[22,34,63]). Furthermore, we very recently showed that Cx30 controls the spatial properties of GJ-mediated networks by regulating the morphological polarity of astrocytes established during postnatal development[64] and may thereby shape specific distal synaptic circuits (BOX 1). Taken together, these findings suggest that postnatal maturation of astroglial GJ communication, by enabling long-range extracellular homeostasis, restricts temporally and spatially the transfer of synaptic information and thus acts as a 'gatekeeper' at the synapse.

Glutamate transporters. Astroglial glutamate transporters are major sensors of excitatory synaptic activity and play a crucial role in synaptic transmission by rapidly taking up glutamate and therefore limiting glutamate spillover from the synaptic cleft. Astrocytes can take up glutamate through the glutamate-aspartate transporter (GLAST; also known as SLC1A3) and glutamate transporter 1 (GLT1; also known as SLC1A2). Following astrogliogenesis (that is, in the immature postnatal brain), GLAST appears before GLT1 (REF.[65]); GLT1 expression rapidly increases during the first 3 postnatal weeks and, by and large, eventually overcomes GLAST expression. Both transporters preferentially localize at perisynaptic astroglial membranes[66]. Interestingly, although GLT1 and GLAST show no significant difference in turnover rate and affinity for glutamate[67], their functional regulation appears to differ, possibly leading to non-equivalent functional properties. Recruitment of GLAST at the astroglial cell surface is upregulated by glutamate, involves translocation of a reserve pool of transporters to the membrane and occurs within minutes through a mechanism involving the actin cytoskeleton[68]. GLT1 expression at astrocyte membranes is also upregulated by glutamate, but via a more dynamic process involving fast diffusion that results in the spatial recruitment of GLT1 near the active zone where it can shape synaptic transmission[69]. Thus, the higher GLT1 density in the membrane and its more dynamic spatial distribution compared with GLAST suggests that the change in astroglial glutamate transporter type during development will result in more contained synaptic transmission through a tight control of glutamate diffusion.

Calcium signalling. Little is known about how astroglial Ca^{2+} signalling changes during postnatal development. Yet, recent data indicate that developmental changes occur in both the pattern of individual Ca^{2+} signals and their spatial distribution within astrocytes[70]. Astrocytes in late juvenile mice (P30) indeed show larger subcellular Ca^{2+} signals than their immature counterparts (P7) as well as a higher proportion of somatic transients. Mechanistically, astroglial mGluR3 and mGluR5 enable these cells to sense extracellular glutamate and trigger astroglial Ca^{2+} signalling, which in turn influences synaptic activity[71]. Interestingly, mGluR5, the main glutamate receptor triggering Ca^{2+} signalling in astrocytes and supporting synaptic transmission, markedly decreases after the first 3 weeks of postnatal development[72,73]. However, despite this low expression, astroglial mGluR5 appears to remain at play as mGluR5-dependent astroglial activation has been reported in adult mice in vivo[74]. In contrast to mGluR5, mGluR3 is negatively linked to the release of Ca^{2+} from intracellular stores and shows stable expression in astrocytes during brain development[73,75]. Thus, although the functional consequences of the changes in astroglial mGluR expression remain to be further characterized, the available data suggest that the ability of glutamate to activate astroglial Ca^{2+} signalling is enhanced in juveniles compared with mature animals. As Ca^{2+} signalling is lower in immature compared with mature astrocytes, this suggests that glutamate is not the only player in astroglial excitability during adulthood.

Interestingly, GABA has also been reported to induce Ca^{2+} signalling in hippocampal astrocytes via activation of $GABA_A$ and $GABA_B$ receptors[76]. Whereas $GABA_A$ receptors rapidly induce Ca^{2+} transients in hippocampal astrocytes throughout development, $GABA_B$ receptors evoke delayed transients, which are prominent in young animals (P11–15) but are minor in young pups (P3) and late juvenile mice (P32–34)[76]. GABA is of particular interest as it is a neurotransmitter with a dual action during postnatal development[77]. During the first postnatal week, GABA has an excitatory effect on neurons and thereafter exerts its well-known inhibitory action. Thus, $GABA_B$-mediated Ca^{2+} signalling in astrocytes may have a functional role in the establishment

Box 1 | **A role for astroglial polarity in functional maturation of synaptic circuits?**

Astroglial processes are sites of privileged interactions with synapses and constitute relevant nanodomains for integration and processing of synaptic information[135]. During development, hippocampal astrocytes become polarized, their orientation in the striatum radiatum being almost perpendicular to the striatum pyramidale[136,137]. Around the third postnatal week (that is, at the time of synaptogenesis), astrocytes from the hippocampal CA1 area indeed reorient their processes along the axis of pyramidal cell dendrites, where synapses are located. Such spatial reorganization confers polarized secretory and uptake competence to mature astrocytes, which likely favours strong local functional interactions with synapses and regulatory power. Radial astrocyte reorientation also shapes the spatial properties of gap junction (GJ) coupling in striatum radiatum astroglial networks[137]. This shaping of GJ spatial properties results in preferential anisotropic coupling at postnatal stages, which promotes intercellular diffusion of molecules perpendicular to the pyramidal cell layer[64,137]. Given the role of GJ-mediated astroglial networks in long-range extracellular homeostasis and metabolic support controlling synaptic transmission and neuronal coordination[22,34,61,63], this rearrangement may contribute to the recruitment of distal synaptic circuits and the emergence of synchronous activity such as sharp wave ripples, which are typically observed in the hippocampus after the second postnatal week[138].

Although polarity of hippocampal astroglial processes persists into adulthood, it is a plastic feature that is dynamically regulated, either negatively during physiological conditions such as enriched environment or physical exercise[139,140] or positively in pathological contexts such as post-traumatic stress disorder[140]. This suggests that polarity is a signature of astrocyte maturity in the hippocampus and that enhanced physiological activity, by inhibiting polarity, may revert astrocytes to an immature state and favour synaptic plasticity. It is noteworthy that astrocyte polarization also occurs in other brain areas such as the striatum or sensorimotor cortex during pathological conditions such as stroke[141] and might also serve in the formation of glial scars for brain repair and neuroregeneration purposes. Thus, astrocyte polarization likely contributes to the structural maturation of local astroglial circuits within neuronal functional units.

of hippocampal networks that occurs during a specific period of postnatal development.

Taken together, recent findings suggest that glutamate and GABA exert a more prominent effect on Ca^{2+} signalling of astrocytes in juveniles compared with older animals. During adulthood, other pathways mediating stronger and more global events are likely at play, as recently suggested by in vivo imaging studies showing the involvement of neuromodulators such as noradrenaline in astroglial responsiveness to neuronal activity[40,41,50,51].

Evolving control of synaptic environment

Perisynaptic extracellular matrix. Astrocytes are key players in settling the extracellular matrix (ECM) in which synapses are embedded. They secrete ECM molecules that not only take part in the perineuronal nets surrounding specific neurons but also operate at a more discrete level in the perisynaptic extracellular space. Important ECM protagonists expressed by astrocytes are hyaluronans (HAs), chondroitin sulfate proteoglycans (CSPGs) and tenascins (TNs). HA is a large, unsulfated glycosaminoglycan that is not attached to a protein core. HA is thought to serve as a backbone onto which CSPGs attach whereas TNs stabilize neighbouring HA–CSPG complexes by linking CSPGs to each other. Although the HA backbone is produced throughout postnatal development, there are marked changes in the types of CSPG and TN composing the ECM.

In the juvenile brain, the main CSPGs expressed are versicans and neurocans[78,79]. This composition confers to perisynaptic ECM a high diffusivity that allows permissive properties regarding synaptic structural changes and synaptogenesis, which contributes to the high plasticity characteristic of 'critical periods' of postnatal development[80–82].

As the brain matures, the ECM changes composition and becomes denser and less permissive. Adult brain CSPGs are mostly aggrecans and brevicans[78,79], which are also mainly produced by astrocytes. Although they restrict spine motility[80], such rigidity is important for synaptic plasticity as brevican-deficient mice display an impaired maintenance of long-term potentiation (LTP)[83], suggesting that inappropriate spine motility prevents the consolidation of synaptic changes. Endogenous enzymes produced by astrocytes that degrade brevicans and aggrecans (for example, metalloproteinases such as a disintegrin and metalloproteinase with thrombospondin motifs 4 (ADAMTS4) and metalloproteinase 9 (MMP9)) have also been suggested to play an important role in the structural plasticity that accompanies changes in synaptic strength[84,85]. Indeed, mice deficient in the MMP9 enzyme likewise show an impaired maintenance of LTP[86]. Therefore, it appears that, in the mature brain, perisynaptic ECM is necessary for maintaining synaptic changes but that digestion of this same ECM is also required for prolonged plasticity. One possible explanation for this apparent contradiction is that astrocytes regulate synaptic plasticity in a timely manner by first secreting metalloproteinases that weaken the HA-based perisynaptic ECM when synaptic remodelling is necessary, and by subsequently secreting CSPGs when the morphological adjustments that have occurred must be consolidated. Insofar as mature perisynaptic CSPGs are known to prevent lateral (extrasynaptic) diffusion of synaptic receptors[87], another or complementary explanation for these findings is that ECM enzymatic digestion would temporarily enable extrasynaptic receptor activation through spillover, after which astroglial synthesis of CSPGs would terminate this signalling.

In addition to these physical considerations, the ECM molecule TNC, which is expressed early in development, has been found to promote the activation of L-type voltage-dependent Ca^{2+} channels (VDCCs). Indeed, TNC-deficient mice exhibit impairment in a form of synaptic plasticity that depends on VDCC activation in the CA1 area of the hippocampus[88].

Another major ECM change that occurs during maturation is the marked downregulation of TNC production by astrocytes, which is replaced by TNR produced by both neurons and astrocytes. Similar to TNC, TNR also influences synaptic transmission but acts on voltage-gated Na^{+} channels rather than Ca^{2+} channels[89], and thus differently influences plasticity mechanisms[90]. Thus, changes in ECM constituents secreted by astrocytes throughout postnatal development enable a progressively increasing control of synaptic motility and plasticity.

Matricellular proteins and synapses. Although secreted into the ECM, matricellular proteins are not structural components of the matrix. They instead modulate membrane adhesion and synaptic functions by interacting with cell-surface receptors as well as structural matrix proteins[91]. Astrocyte-derived matricellular proteins that highly influence synaptic functions are the thrombospondins (TSPs) and the secreted protein acidic and cysteine-rich (SPARC) family proteins SPARC and hevin.

In the first weeks of postnatal development, which corresponds to a period of massive synaptogenesis in most brain areas, TSP1 and TSP2 are highly expressed and have been found to initiate formation of synapses[92]. This process occurs through binding of several membrane proteins, including the postsynaptic cell adhesion molecule neuroligin 1 (REF.[93]) and the Ca^{2+} channel subunit α2δ1 (REF.[94]). Once the period of early postnatal synaptogenesis is over, TSP1 and TSP2 are downregulated[92] and, concomitantly, TSP4 astroglial secretion increases[95]. The latter has recently been shown, in the spinal cord, to also regulate synaptogenesis as well as presynaptic function by binding the α2δ1 subunit[96,97]. TSP4 is, however, a shorter thrombospondin than TSP1 and TSP2 as it lacks the procollagen and properdin-like (type I) repeats that enable interactions with glycosaminoglycans, integrin-associated proteins and activation of TGFβ[98]. Thus, whereas TSP1-deficient and TSP2-deficient mice display functional defects attributable to decreased synaptic inputs[99], TSP4-deficient mice show increased synaptic activity due to a lack of regulation of presynaptic Ca^{2+} channels[96]. Hence, the change in TSP type provided by astrocytes at the perisynaptic level during development results in differential structural and functional regulation of synapses.

Akin to TSPs, SPARC and hevin matricellular proteins are highly expressed in the first weeks of postnatal development. Whereas hevin has been found to promote synapse formation[100], SPARC by contrast triggers

Matricellular proteins
Proteins that are secreted to the extracellular environment but do not play a structural role in this location and instead modulate cell functions by interacting with cell-surface receptors and structural elements of the matrix.

a cell-autonomous programme of synapse elimination[101]. It has thus been hypothesized that the competition between the two proteins regulates the development of synaptic circuits. In the adult brain, both proteins are downregulated, which could underpin the reduction in synapse motility and remodelling that occurs at this stage of development, as these matricellular proteins interact with pathways involved in synapse plasticity such as fibroblast growth factor 2 (FGF2), TGFβ and integrins. Signalling from the SPARC family is therefore markedly different in adult compared with juvenile brains.

It is noteworthy that these developmental changes in TSPs and SPARC signalling are consistent with the synaptogenic effects of immature astrocytes recently proposed to reflect the predominance at early postnatal stages of the aforementioned astrocyte subpopulation C, in which the expression of synapse-related genes is enriched[6].

Although it is well established that immature astrocytes promote massive synapse formation during early development by secreting factors in the ECM, several studies suggest that during adulthood, mature astrocytes hinder synapse formation and contribute to synapse elimination in different brain regions. In the supraoptic nucleus of the hypothalamus, the retraction of astrocytes from synapses that occurs in the adult during physiological situations such as lactation indeed results in an increased number of synaptic contacts[102]. Similarly, Bergmann glia retraction from Purkinje cell synapses enhances innervation of Purkinje cells by climbing fibres[103,104]. PAPs in adult animals may thus inhibit further synaptogenesis by physically competing with neurons, thereby preventing them from forming additional contacts with the postsynaptic element, and/or by inducing synaptic pruning, as recently shown in the somatosensory cortex[105].

In summary, through the secretion of ECM constituents and matricellular proteins, astrocytes set environmental conditions that evolve over time such that they switch from synapse boosters in early postnatal life to gatekeepers in the mature brain.

Metabolic support through life

Energy supply. Glucose is well known for being the main metabolite used by the brain as its energy supply. However, the requirement and consumption of glucose in the brain change throughout the lifespan, and this is paralleled by evolving astroglial metabolic properties. Fuel sources in the neonatal brain comprise not only glucose but also, for a large part, ketone bodies (KBs), which are energetic metabolites produced by the degradation of fatty acids. It has been estimated that the use of glucose in the newborn brain can be as little as 12% of adult consumption, in accordance with a lower expression of glucose transporters at this stage[106]. As a result, glycolysis is also down in the neonatal brain, meaning that the activity of glycolytic enzymes such as lactate dehydrogenase (LDH) is low[107]. This observation is important as LDH is an essential component of the astrocyte–neuron lactate shuttle by which astrocytes transform glucose into lactate and transport it to neurons[108]. Strikingly, LDH activity has been found to be directly linked to neuronal activity. Indeed, a recent study indicates that inhibiting LDH in astrocytes or neurons results in the hyperpolarization of neuronal membranes through the modulation of K_{ATP} channels, thus influencing neuronal activity and synaptic transmission[109]. Astrocytes are also considered as a source of KBs for neurons, which, as mentioned above, are an important energy source in the young brain[110]. This is reflected by the high expression level of the monocarboxylate transporter MCT1 (also known as SLC16A1), which transports KBs and plays a role in synaptic transmission by inhibiting glutamate vesicular transporters[111]. Later on in development, expression of MCT1 is markedly reduced in the adult brain[112]. Thus, although both young and old brain astrocytes are key energy providers for neurons, the relative proportion of energetic molecules providing optimal brain activity is drastically different. At the synaptic level, this has important implications as it keeps neuronal activity and synaptic transmission low early on in development, consistent with the slow metabolic rate reported in neonatal versus adult brains[113]. Therefore, changes in astroglial metabolic activity take part in controlling the maturation of neuronal activities and shaping synaptic circuits.

Glutamine–glutamate cycle. Refilling of the presynaptic pools of glutamate in excitatory synapses is undertaken by the glutamine–glutamate cycle, a process by which glutamate is taken up by astrocytes via GLT1, where it is transformed into glutamine by glutamine synthase and transported back to neurons, ready to be converted again into glutamate by glutaminases. In accordance with the high proportion of glucose used for pyruvate-carboxylase-dependent synthesis of glutamate, the rate at which glutamine is transferred to neurons is higher in the first postnatal weeks than in adult tissues[114]. In sharp contrast, the transport of glutamate from neurons to astrocytes is negligible, consistent with the low levels of neuronal activity occurring in the neonatal brain[114] and the gradually rising expression of GLT1 during postnatal development[65]. Thus, in the early phases of postnatal development, the glutamine–glutamate cycle is imbalanced, whereas in adult brains, the glutamate–glutamine cycle is highly efficient for fast clearance at synaptic clefts, which ensures isolation of neighbouring synapses and prompts resetting of presynaptic boutons. The poor glutamate transport from neurons to astrocytes taking place at early stages likely promotes activation of extrasynaptic receptors through spillover of glutamate, proposed to participate in the formation of new synapses during development[115,116]. It therefore appears that juvenile neuroglial interactions are mostly involved in providing the fuel, molecular protagonists and structural changes necessary for the emergence and shaping of new synaptic networks. By contrast, in the adult nervous system, astrocytes are more inclined to control the synaptic efficacy and specificity of already-formed circuits.

Activity-dependent control of synapses

Astrocytes modulate synaptic transmission by various mechanisms involving typical functional properties such as the release of neuroactive molecules, Ca^{2+} signalling, interconnectivity through GJs or synaptic coverage (BOX 2). Remarkably, each of these astroglial properties

Glutamine–glutamate cycle
Metabolic pathway consisting of the recycling of glutamate released during synaptic transmission. Glutamate is taken up and metabolized into glutamine by astrocytes and then transported back to neurons as a precursor.

Box 2 | **Versatile astroglial synapse coverage throughout life?**

Synaptic coverage represents an important aspect of the astroglial control of synaptic functions. Although the majority of synapses are contacted by perisynaptic astroglial processes (PAPs), with an average of ~60%, there is an important variability according to brain regions[142–145] (FIG. 1). For example, astrocytes contact 62% of hippocampal synapses[143], only 15% of cerebellar mossy fibre to granule cell synapses[144] and the majority (87%) of cerebellar climbing fibre inputs[145]. Whether such coverage is developmentally regulated remains to be investigated as all these studies are performed on adult tissues. Interestingly, astroglial synapse coverage is preferentially associated with large postsynaptic densities[143], which are more abundant in adults[146,147]. This observation suggests that astroglial coverage gradually increases during postnatal development.

PAP dynamics are regulated through Ca^{2+}-dependent mechanisms eventually controlling synapse coverage. Whisker stimulation in the somatosensory cortex[148,149] and synaptic potentiation in the hippocampus[148,150,151] increase the motility of astroglial processes as well as the coverage of excitatory synapses. The functional relevance of such an observation remains however unclear. One possibility is that sustained synaptic activation triggers an increase in PAP motility, thus providing an adequate time window for glutamate spillover and structural remodelling of the dendritic spine and/or synaptic bouton (FIG. 1). PAP stabilization with an enhanced coverage would terminate this process and seize up the level of synaptic efficacy. However, further modifications of astroglial synapse coverage may occur at later phases of plasticity, as suggested by PAP retraction from synapses of the amygdala during fear memory consolidation[152]. Interestingly, such PAP retraction also occurs in the supraoptic nucleus of the hypothalamus[4,65] and in the frontal cortex[153], where neuronal synchronized activity takes place during lactation or during sleep, respectively. As a result, PAP retraction, by favouring spillover of glutamate, would promote coordination of synaptic circuits[153–155]. Together, these studies indicate that astroglial coverage may contribute to activity-dependent plasticity processes.

can lead to opposite effects on synaptic transmission according to the pattern of activity at play.

Gliotransmitter release

Astrocytes modulate synaptic transmission by various mechanisms such as Ca^{2+}-dependent release of neuroactive substances called gliotransmitters. These molecules have recently been found to control integrated physiological processes such as vigilance states, as recently shown for D-serine (a co-agonist of NMDARs). Release of D-serine from astrocytes fluctuates with the activity of septal cholinergic neurons that vary between sleep and awake states[117]. D-Serine regulation of synaptic transmission is straightforward as it solely acts on NMDARs; comparatively, the effects of other gliotransmitters, which act at multiple neuronal targets, are more complex. Among them, ATP and its metabolite, adenosine, regulate synaptic efficacy by targeting disparate presynaptic purinergic receptor subtypes that can be differentially activated depending on whether neuronal activity is basal or sustained (FIG. 3). Indeed, minimal stimulation of CA3–CA1 projections elicits local increases in astroglial Ca^{2+}, which enhances synaptic efficacy via activation of presynaptic adenosine A_{2A} receptors[46]. By contrast, astroglial release of ATP following tetanic stimulation of the same presynaptic fibres primarily targets presynaptic adenosine A_1 receptor activation and thereby mediates the activity-dependent heterosynaptic depression of adjacent unstimulated synapses[118,119]. It is noteworthy that the versatility of the astroglial purinergic regulations of synaptic efficacy also goes beyond the operating regime of activity. Indeed, astrocytes exposed to an identical regime of neural activity have recently been evidenced to differentially regulate specific neuronal circuits. Indeed, single astrocytes can either release ATP, which acts simultaneously on different receptors expressed at distinct synapses, or sequentially release distinct gliotransmitters, such as glutamate and ATP, which differentially and temporally regulate neurotransmission at the same synapse. This finding has been demonstrated in the central amygdala, where activation of astrocytes, either by endocannabinoids or chemogenetic stimulation, simultaneously depressed excitatory synapses from the basolateral amygdala by acting on adenosine A_1 receptors and enhanced inhibitory synapses from the lateral subdivision of the central amygdala by activation of adenosine A_{2A} receptors[4]. By contrast, in the hippocampus, biphasic and opposite regulation of the same CA3–CA1 synapse is mediated by distinct gliotransmitters released by a single astrocyte, where potentiation is mediated by glutamate and is followed by an ATP-mediated depression[120].

Akin to ATP, glutamate is also released by astrocytes and can differentially modulate adjacent neurons according to the level of activity. For instance, under conditions of basal activity, glutamate release from astrocytes in the dentate gyrus upregulates the frequency of spontaneous excitatory postsynaptic currents from granule cells via activation of presynaptic NMDARs[121]. Similarly, during minimal stimulation of single CA3–CA1 synapses, astroglial glutamate enhances release probability, however via activation of group I mGluRs[49]. Yet, during stronger regimes of activity, glutamate release from astrocytes induced by Ca^{2+} uncaging decreases evoked postsynaptic currents in hippocampal neurons by acting on the presynaptic mGluR2/mGluR3 present on either glutamatergic[122] or GABAergic terminals[123]. Together, these data suggest that, depending on the regime of activity and brain area, astroglial gliotransmitters target distinct neuronal receptor subtypes, leading to specific regulations of synaptic transmission.

Ca^{2+} signalling

Although astroglial Ca^{2+} signalling shares common grounds with the gliotransmitter regulation of synaptic transmission discussed above, its role in synaptic physiology is still matter of debate. As previously mentioned, Ca^{2+} transients can arise spontaneously as well as in response to neuronal activity and occur in both the soma and fine processes. Do these different types of Ca^{2+} elevation encode for a low versus high range of neuronal activities? Ca^{2+} responses in astroglial processes following minimal synaptic activation have been reported[46,124], but the complexity of astroglial morphology and limitations of 2D imaging have hindered verification of this hypothesis. Only recently, advances in fast genetically encoded Ca^{2+} indicators (GECIs) and fast-scanning imaging have allowed the identification of distinct spatiotemporal patterns of Ca^{2+} transients. By recording from the entire 3D structure of hippocampal astrocytes in situ, one study elegantly demonstrated that astrocytes sense and respond to minimal and selective synaptic activation in a small portion of nearby microdomains[53]. Similarly, a comparison of Ca^{2+} imaging in neurons and astrocytes following whisker stimulation revealed fast Ca^{2+} responses in astroglial

Astroglial synapse coverage
Ensheathment of synaptic structures by astroglial membranes that can vary with cell types, activity and physiological state.

Gliotransmitters
Neuroactive substances released by astrocytes such as glutamate, ATP or lactate.

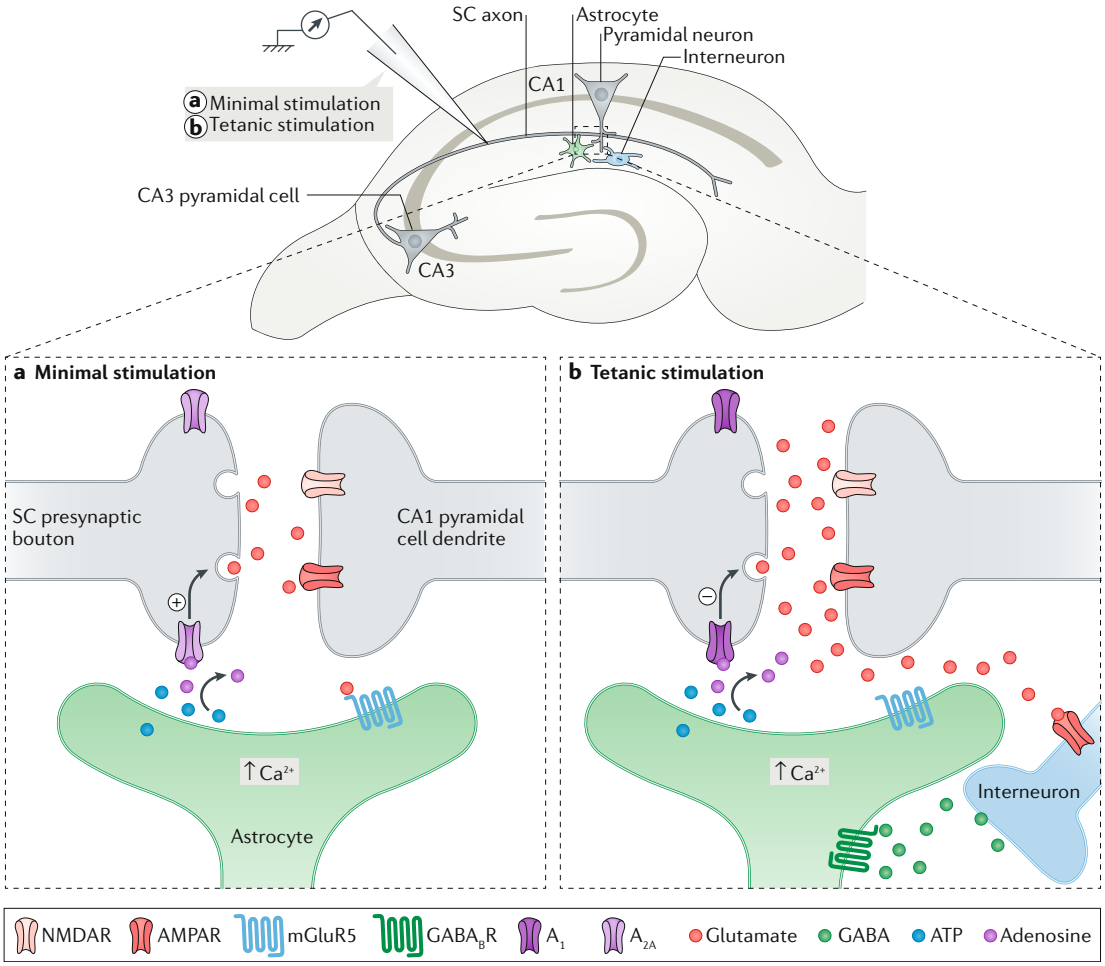

Fig. 3 | **Activity-dependent synaptic regulation by purinergic gliotransmission.** Following minimal stimulation (panel **a**), glutamate released by Schaffer collateral (SC) synapses present on SC axons activates astroglial metabotropic glutamate receptor 5 (mGluR5) on CA1 pyramidal cell dendrites, leading to Ca^{2+}-dependent exocytosis of ATP. The released ATP is then metabolized into adenosine, which activates presynaptic adenosine A_{2A} receptors, increasing the probability of glutamate synaptic release[46]. Under stronger synaptic activation such as that occurring during tetanic stimulation (100 Hz, 1 s; panel **b**), the greater amount of glutamate released by pyramidal cells activates NMDARs expressed on nearby interneurons, which release GABA, leading to activation of astroglial $GABA_B$ receptors ($GABA_BR$) and Ca^{2+}-dependent exocytosis of ATP. The released ATP is then metabolized into adenosine, which activates adenosine A_1 presynaptic receptors, decreasing the probability of synaptic glutamate release[118,119].

microdomains on similar timescales to neuronal activity. Interestingly, these fast microdomain Ca^{2+} dynamics were independent of the inositol triphosphate type 2 receptor (IP3R2), one of the main sources of somatic Ca^{2+} elevations[125]. These IP3R2-dependent somatic Ca^{2+} events are not only slower and less frequent but have also been found to be triggered by more sustained neuronal activity[50,53]. Thus, recent findings reveal multitudinous Ca^{2+} astroglial signals in response to different patterns of neuronal activities. The precision and complexity of astroglial Ca^{2+} signalling suggest that distinct Ca^{2+} signals within the same astrocyte induce different responses according to the surrounding pattern of neuronal and synaptic activity. This notion is supported by recent investigations in which astroglial signalling has been disrupted. These studies show that stimulating astrocytes with optogenetic tools, uncaging or DREADDs (designer receptors exclusively activated by designer drugs) mostly supports synaptic efficacy[49,117,126–128] and plasticity[49,127] (TABLE 1). Indeed, such astroglial stimulation is likely to mimic the high Ca^{2+} elevations that occur in response to sustained neuronal activity. Interestingly, analysing the consequences of hindering endogenous astroglial activation gives a more complex picture as both increases[129,130] and decreases[46,131] in synaptic efficacy have been reported. Thus, more research is needed to understand the relevance of discrete astroglial Ca^{2+} activation on synaptic function. Still, the difference in results obtained by strong activation of astrocytes and inhibition of endogenous Ca^{2+} signals hints that the distinct Ca^{2+} activation in response to low compared with high activities translates into differential regulation of neurotransmission. One of the mechanisms underlying this issue might rely on a threshold of astroglial Ca^{2+} concentration being reached, which triggers distinct downstream signalling, leading to an opposite effect on synaptic transmission, in a way reminiscent of the sliding threshold model of

Table 1 | **Effect of astroglial Ca^{2+} signalling on neuronal activity depending on the type of intervention**

Astroglial Ca^{2+}	Synaptic transmission and/or neuronal activity	Methods	Structure	Refs
Prevent endogenous astrocyte				
↓	↓ EPSP (block t-LTP)	BAPTA-AM	Hippocampus	159
↓	↓ fEPSP (block LTP)	Ca^{2+} clamp	Hippocampus	131
↓	↓ fEPSP (decrease LTP)	TRP channels	Hippocampus	160
↓	↓ fEPSP (block LTP)	Heparin	Hippocampus	161
↓	↓ Synaptic efficacy	BAPTA	Hippocampus	46
↓	↑ REM sleep and theta power	VIPP	Hippocampus	158
↓	↑ Spontaneous EPSC	BAPTA	Barrel cortex	129
↓	↑ Evoked EPSP	BAPTA	Hippocampus	130
↓	↑ fEPSP (block PSD)	BAPTA	Hippocampus	162
↓	↑ fEPSP (block h-LTD)	BAPTA	Hippocampus	163
↓	↑ fEPSP (block h-LTD)	BAPTA	Hippocampus	119
↓	↑ EPSP (block t-LTD)	Ca^{2+} clamp	Barrel cortex	164
↓	↑ NMDA-EPSC	IP3 sponge	Hippocampus	165
↓	→← (no change)	*IP3R2*$^{-/-}$	Hippocampus	166
Mimic astrocyte activation by sustained neuronal activity				
↑	↑ Pr	NP-EGTA	Hippocampus	49
↑	↑ Pr	NP-EGTA	Hippocampus	127
↑	↑ Cortical UP state	Arch	Cortex	167
↑	↑ Neuronal synchronization	Arch	Visual cortex	168
↑	↑ sEPSC and/or sIPSC	ChR2	Visual cortex	128
↑	↑ NMDA fEPSP	ChR2	Hippocampus	117
↑	↑ Neuronal spiking	FMFR and/or MrgA1	Cerebellum	169
↑	↑ EPSC (↑LTP)	NP-EGTA	Hippocampus	49
↑	↑ EPSC (↑LTP)	NP-EGTA	Hippocampus	127
↑	↑ Signal-to-noise ratio	FMFR and/or MrgA1	Hippocampus	126
↑	↓ fEPSP	ChR2	Hippocampus	163
↑	→← (no change)	FMFR and/or MrgA1	Hippocampus	166
↑	→← (no change)	FMFR and/or MrgA1	Hippocampus	170
↑	→← (no change)	NP-EGTA	Hippocampus	162
↑	→← (no change)	FMFR and/or MrgA1	Hippocampus	166

ChR2, channelrhodopsin 2; EPSC, excitatory postsynaptic current; EPSP, excitatory postsynaptic potential; fEPSP, field EPSP; h-LTD, heterosynaptic long term depression; IP3, inositol 1,4,5-triphosphate; LTD, long-term depression; LTP, long-term potentiation; Pr, probability of release; PSD, postsynaptic density; REM, rapid eye movement; sEPSC, spontaneous EPSC; sIPSC, spontaneous inhibitory postsynaptic current; t-LTD, transient LTD; t-LTP, transient LTP.

synaptic plasticity, in which different levels of Ca^{2+} rise lead to induction of LTP or long-term depression[132].

Gap junctions

A dual role for GJ-mediated astroglial networks in regulating neurotransmission according to the level of activity also currently emerges (FIG. 4). During basal transmission, astroglial GJs play a dampening role, as mice deficient for both astroglial Cx30 and Cx43 (*Cx30*$^{-/-}$*Cx43*$^{-/-}$) display enhanced hippocampal excitatory synaptic transmission[22]. Such an effect reflects alteration in the astroglial control of extracellular homeostasis, which modifies both presynaptic and postsynaptic functions. Indeed, disconnection of astrocytes impairs the astroglial clearance rate of synaptically released K^+ and glutamate that results from the inability of astrocytes to redistribute these molecules through the GJ-mediated astroglial network. This in turn results in enhanced extracellular levels of K^+ and glutamate during basal synaptic activity, thus increasing neuronal excitability, release probability and postsynaptic activity.

By contrast, during stronger neuronal activation, astroglial GJs promote ex vivo sustained coordinated neuronal bursts and, in vivo, paroxysmal events as well as convulsive behaviour. In effect, mice deficient for astroglial Cx43 and Cx30 exhibit reduced neuronal synchronization as well as decreased severity of evoked seizures and associated convulsions[34] (FIG. 4). This effect

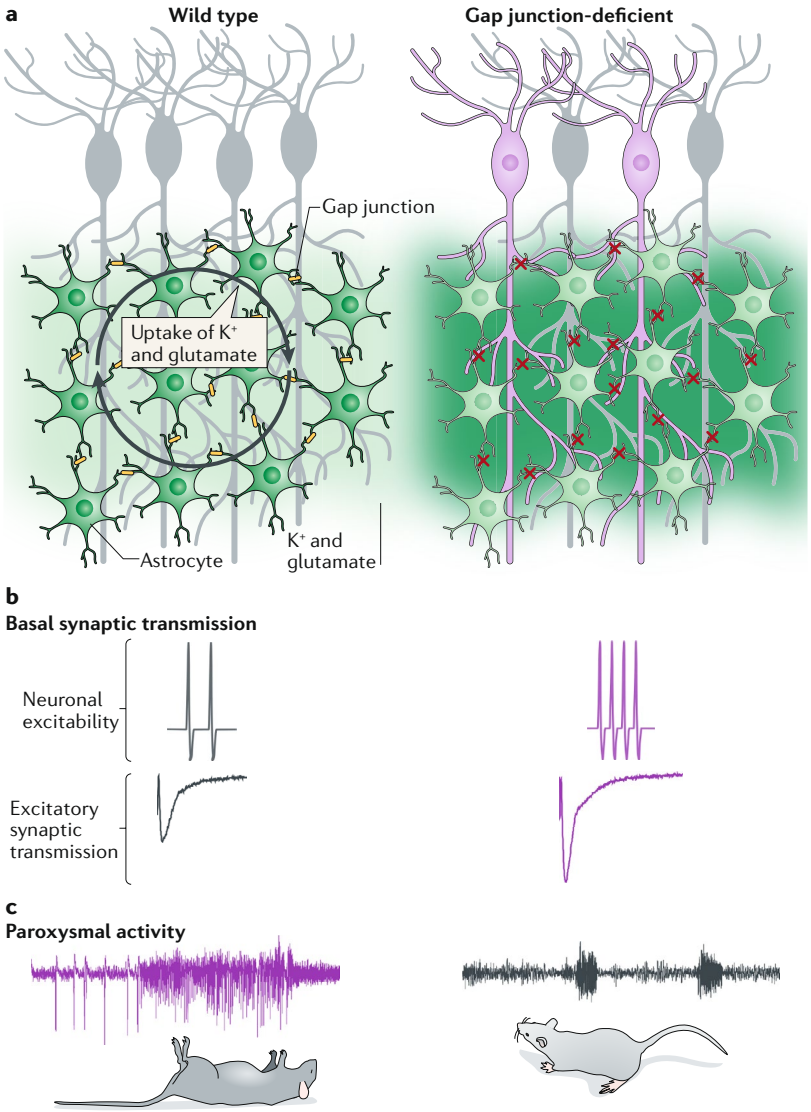

Fig. 4 | **Dual role of gap junction-mediated astroglial networks in basal and network activity. a** | Schematic model showing the altered K^+ and glutamate extracellular homeostasis resulting in enhanced neuronal activity but decreased neuronal synchrony in gap junction (GJ)-deficient mice. In wild-type mice (left panel), individual astrocytes take up extracellular K^+ and glutamate, which are released by active synapses, and redistribute them into astroglial networks via GJ channels (black arrows)[22]. Following synaptic activity, K^+ and glutamate are thereby removed extracellularly (light green) and accumulate in astrocytes (dark green), enabling termination of synchronous neuronal activation and return to the basal state (light grey). In GJ-deficient mice (right panel), individual astrocytes can still take up the synaptically released K^+ and glutamate, but they cannot redistribute them into astroglial networks. This inability leads to prolonged accumulation of K^+ and glutamate in the extracellular space and may thereby extend neuronal activation (purple) in a non-synchronous manner. **b** | Under basal conditions (basal synaptic transmission, left side of figure), GJs restrict neurotransmission (grey). Mice deficient for both astroglial connexins (Cxs) (GJ-deficient, right side of figure) display enhanced hippocampal excitability and excitatory synaptic transmission (purple). This effect results from increased extracellular levels of synaptically released K^+ and glutamate[22] owing to the absence of GJ channels that normally mediate ion and neurotransmitter redistribution throughout the astroglial network. **c** | Under conditions of stronger activation, GJs instead promote in vivo paroxysmal events and convulsive behaviour (purple), as mice deficient for astroglial Cxs display a decreased severity of evoked paroxysmal events and associated convulsions (grey). The effect of GJs on paroxysmal events and convulsive behaviour results from strong neuronal synchronization enabled by adequate neuronal firing and increased synaptic release probability[34], most likely ensured by efficient GJ-mediated extracellular K^+ homeostasis.

results from decreased neuronal recruitment during sustained network activity and is likely to be mediated by impaired GJ-mediated extracellular K^+ homeostasis. Indeed, astroglial GJ disruption increases extracellular K^+ level, which enhances background spontaneous synaptic activity and causes neuronal depolarization. The latter is associated with increased neuronal excitability but decreased synaptic release probability. In fact, the rise in basal spontaneous activity may reduce the size of the readily releasable pool of synaptic vesicles, which is essential for the recruitment of neurons during network activity[133,134]. Such a mechanism could thereby limit synaptic efficacy and neuronal synchrony. Remarkably, the decreased release probability found during sustained neuronal activation is in contrast to the enhanced release probability reported under basal conditions in the same mice with disconnected astrocytes[22].

Overall, these data suggest that by setting basal active states via regulation of extracellular ions and neurotransmitters, astroglial networks exert opposite modulations of neuronal functions depending on the regime of activity.

Conclusion and future directions

Over the past decade, it has become increasingly evident that, depending on a vast amount of factors, astrocytes can provide multidirectional regulations of the synaptic environment, structure and efficacy. Such observations highlight the versatile nature of astrocytes. Although there are a tremendous number of variables at play in determining how neuroglial interactions shape synaptic transmission, they can be grouped into three main themes: the intrinsic molecular and functional heterogeneity of astrocytes within or from different brain regions, the developmental stage under consideration and the regime of surrounding neuronal activity. Combinations of these variables generate diversity in astroglial regulation of synapses throughout life and can translate into apparently opposite, yet physiologically relevant, control of synaptic circuits via selective engagement of distinct mechanisms ranging from homeostatic control of the synaptic environment to active signalling and release of neuroactive molecules.

Determining the signals driving the generation of astroglial population subtypes and their plasticity during postnatal development or various physiological contexts will be crucial to expand further our understanding of astrocyte functional heterogeneity. Greater insights into the diversity of structural and functional interactions between astrocytes and synapses in vivo and across development using innovative approaches such as 3D large-scale imaging (as opposed to the 2D imaging typically performed up to now) would better reflect the genuine complexity of astroglial activations as recently uncovered[53]. Another interesting advance would be to explore whether other types of astroglial signal, such as rapid activity-dependent Na^+ signalling[54], also participate in fast and/or diverse synaptic modulations. In addition, super-resolution technology coupled to nanopipettes is also likely to be crucial to fully understand the roles of astrocytes in synaptic wiring, transmission and plasticity. Finally, deciphering the molecular mechanisms driving the functional diversity of perisynaptic astrocytes

according to brain area, developmental stage and regime of activity should not only advance our comprehension of cerebral development and plasticity, but also provide a novel framework for identifying dysfunctions underlying neurological or psychiatric disorders as well as revealing alternative therapeutic targets.

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