NEURON–GLIA INTERACTIONS

Astroglial networks: a step further in neuroglial and gliovascular interactions

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Abstract | Dynamic aspects of interactions between astrocytes, neurons and the vasculature have recently been in the neuroscience spotlight. It has emerged that not only neurons but also astrocytes are organized into networks. Whereas neuronal networks exchange information through electrical and chemical synapses, astrocytes are interconnected through gap junction channels that are regulated by extra- and intracellular signals and allow exchange of information. This intercellular communication between glia has implications for neuroglial and gliovascular interactions and hence has added another level of complexity to our understanding of brain function.

Over the past three decades our understanding of intercellular communication between glia has fundamentally changed from the notion that they are organized as a syncytium — a multinucleate mass of cytoplasm resulting from the fusion of cells — to the recognition that they are organized into networks. Indeed, in the 1970s the identification of a large number of gap junctions led to the statement: "Adjacent glial cells, however, including those of mammals, are linked to each other by gap junctions. In this respect they resemble to epithelial and glands cells and heart muscles fibers" (in *From Neuron to Brain* by S.W. Kuffler and J.G. Nicholls[1]). This statement was further supported by ultrastructural data published in an article with the explicit title *Cell junctions of astrocytes, ependyma, and related cells in the mammalian central nervous system, with emphasis on the hypothesis of a generalized syncytium of supporting cells*[2]. Since then, the term glial syncytium has been extensively used in several publications, and even recently a review referred to a pan-glial syncytium[3].

The present Review discusses data suggesting that glia, and in particular astrocytes, are organized as networks and communicate through specialized channels, the so-called gap junctions. We propose that neuroglial and gliovascular interactions should be considered at a network level — that is, beyond a dialogue between single cells. We hope that this working hypothesis will trigger research that will lead to a better understanding of neuroglial network interactions.

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Connexins in astroglial networks

Connexins and gap junctions. Gap junctions (BOX 1) are expressed extensively in the nervous system. One of the first reports of direct cell-to-cell communication presented electrophysiological evidence that gap junctions allowed transmission between neurons at electrical synapses[4]. A few years later it was demonstrated that non-neuronal cells were also extensively coupled by cell-to-cell junctions[5]. In subsequent years, the ultrastructural basis of gap junctions was described and the connexin (Cx) family of proteins were identified as the molecular constituents of gap junction channels (GJCs).

Twenty-one Cxs have been identified so far, and eleven of these have been detected in the vertebrate brain[3,6–8]. Each Cx has its own pattern of expression and typically more than one Cx is expressed in a given cell type[3]. Cxs are organized as hexamers embedded in the plasma membrane that, when associated head-to-head between two neighbouring cells, form a GJC (FIG. 1a). However, recent findings have demonstrated that Cxs can also operate as hemichannels (BOX 2), allowing exchange of molecules between the cytoplasmic and extracellular media[9–12]. Hemichannels can also be composed of pannexins, a distinct family of membrane proteins that are homologous to innexins, the GJC-forming proteins in invertebrates[13].The central pore of hemichannels or GJCs allows the passage of ions (ionic coupling) and small molecules (biochemical or metabolic coupling), with a cut-off selectivity of around 1 to 1.2 kDa. Numerous

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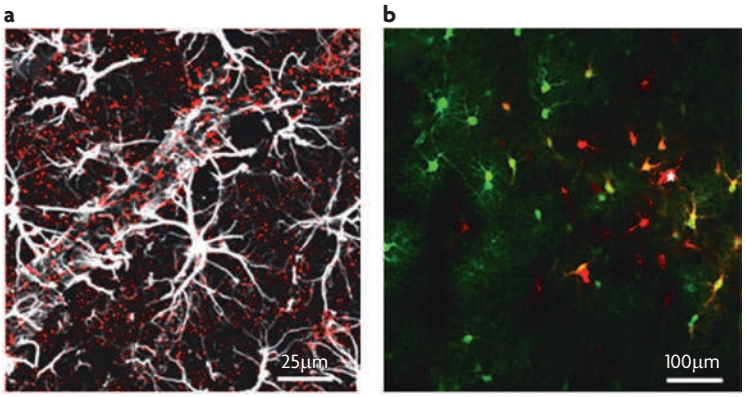
Box 1 | **Gap junctions: what they are and how to study them in astrocytes**



Gap junctions form aqueous channels and are sites of direct intercellular communication. The term was proposed on a morphological basis owing to the 'gap' of ~2 nm that separates the membranes of adjacent cells, as revealed in electron micrographs. Gap junction channels (GJCs) are composed of connexins (Cxs), which can be visualized by immunohistochemical stainings in astrocytes that express glial fibrillary acidic protein (GFAP) (see part **a** of the figure, with GFAP staining in white and Cx30 staining in red) to reveal their expression between and within astrocytic domains and at contacts between endfeet that wrap blood vessels. Connexons are hexameric structures composed of Cx subunits. The connexons of two adjacent cells dock to form a GJC that allows the intercellular diffusion of small molecules of up to 1–1.2 kDa and ~1.5 nm diameter (biochemical or metabolic coupling) and the flow of currents (ionic coupling) when there is a transjunctional voltage difference. A fluorescent dye injected into one cell will diffuse into adjacent cells if they are coupled by GJCs. In the experiment illustrated in part **b** of the figure, biocytin (red) was injected into an astrocyte (white star) in a hippocampal slice from an hGFAP–eGFP mouse (in which the expression of enhanced green fluorescent protein is under the control of the human GFAP promoter). Note that in a single confocal plane, not all eGFP-positive astrocytes are dye coupled and therefore are not part of the same network.

The lack of specific pharmacological tools[111,123,124] requires an alternative, genetic approach, such as Cx-knockout mice to study the role of astroglial GJCs[111]. This strategy has already been used in brain slices[21,22] and *in vivo*[112]. However, as there are two main Cxs in astrocytes, namely Cx30 and Cx43, double-knockout mice have to be used. Moreover, as Cx43 is widely expressed in different organs, an astrocyte-targeted Cx43 knockout was designed using an hGFAP promoter-driven *cre* transgene. As expected, dye coupling in astrocytes from double-knockout mice was not observed, and hence these mice allow us to study the function of astroglial networks, for instance in pathological models of epilepsy[21] or hypoglycaemia[22]. However, such an approach also has its limits as Cx genes, in particular the gene encoding Cx43, may be important for regulating brain gene expression[125]. Alternatively, small interfering RNAs or engineered lentiviral vectors targeting astrocytes[126] could provide other strategies to silence astroglial Cxs in brain slices or *in vivo* models.

studies have shown that the selective compatibility of different Cxs for the assembly of Cx channels, their gating properties, selectivity and regulation depend on the nature of their molecular constituents, which has led to the concept of a Cx 'language'[14]. Although GJCs have been extensively studied, less information is available for hemichannels (BOX 2).

Connexins in astrocytes. In the adult brain, Cx43 (also known as GJA1) and Cx30 (also known as GJB6) are the main Cxs in astrocytes[15–18]. Their relative levels vary according to the developmental stage and brain region[19]. Cx43 is expressed from early in development, around the twelfth day of gestation in rat radial glial processes. As development proceeds, Cx43 expression increases and is detectable throughout the brain as immunoreactive puncta. Cx30 is expressed in astrocytes in juvenile rodents during the third postnatal week, with a punctate staining pattern[17,19] (BOX 1). Cx30 and Cx43 are clearly co-expressed at junctional plaques of mature grey matter astrocytes. However, there are regional differences in the expression of these two connexins, the major difference being that there is no Cx30 expression in astrocytes of white matter tracts[18]. Although single-cell reverse transcription PCR performed in hippocampal astrocytes has detected the presence of other Cx mRNAs[20], no intercellular communication — as assessed by dye coupling experiments — was observed between astrocytes of Cx43;Cx30 double-knockout mice[21,22], strengthening the notion that Cx43 and Cx30 are the major astroglial Cxs. Interestingly, as well as their role in GJCs and hemichannels, Cxs are involved in other glial functions, such as adhesion[23] and modulation of purinergic receptors[24].

When comparing neurons and astrocytes, one has to keep in mind that whereas neurons usually have overlapping dendritic fields in addition to axonal projections, protoplasmic astrocytes of the grey matter occupy very restricted and independent spatial territories. Indeed, based on glial fibrillary acidic protein (GFAP) immunostaining, astrocytes were initially described as stellate cells with large interdigitations between the processes of adjacent cells[25]. Recently, morphological analysis of dye-filled astrocytes in the hippocampus and the cortex indicated that they are more 'spongiform' than star-shaped and occupy separate anatomical domains[26–28]. Consequently, to coordinate information transfer in a reliable and efficient manner, astrocytes need a strong modality of intercellular communication: Cx-mediated pathways can certainly fulfil this function. Indeed, injections of intercellular tracers into one astrocyte revealed that hundreds of cells can contribute to astroglial

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networks in the hippocampus[20,22,29,30], the cerebral cortex[31], the inferior colliculus[32] and the striatum[33]. Finally, although gap junctions are observed at the interface between two neighbouring astrocytes and at contacts between glial endfeet that enwrap blood vessels[18,22], immunohistochemical stainings for Cx43 and Cx30 revealed that their expression is not restricted to these locations (BOX 1). This suggests that, in addition to their role in intercellular communication, Cxs could operate between processes originating from a single astrocyte (FIG. 1a). Such 'reflexive' gap junctions, already described at the ultrastructural level[25], could be part of astrocytic microdomains[34] and contribute to their integrative responses.

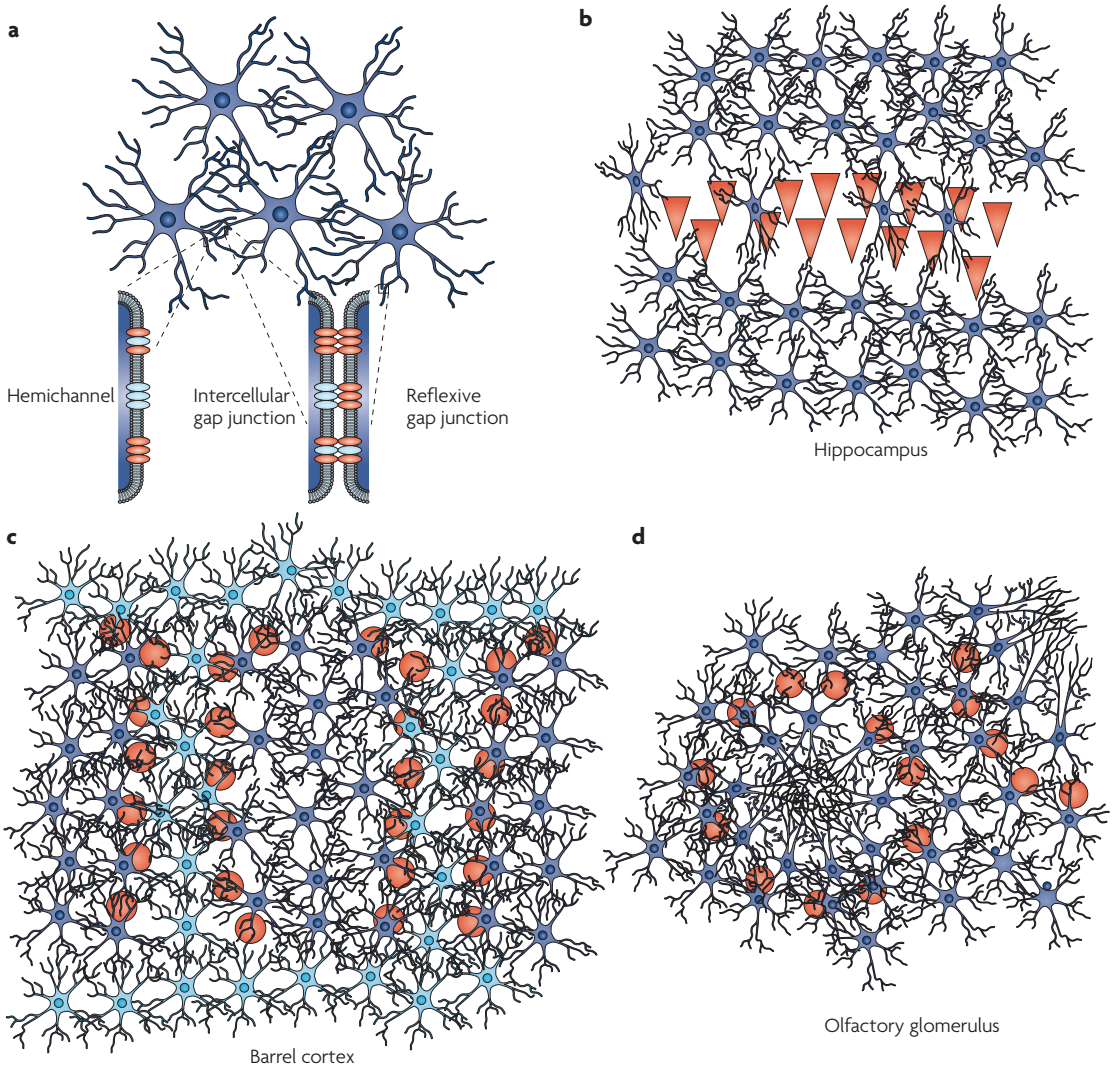

Figure 1 | **Astroglial connexins and communicating networks. a** | Localization and structure of connexin (Cx) channels in astrocytes. Domains that are occupied by neighbouring astrocytes do not overlap, and intercellular gap junction channels (GJCs) are located at contact points between neighbouring astrocytes. In an individual astrocyte, one process can contact another process and form 'reflexive' gap junctions. Alternatively, in defined situations (BOX 2), Cxs can also operate as hemichannels at the surface of the cell membrane to exchange information with the extracellular space. As astrocytes express mainly two Cxs (BOX 1), several combinations of Cx43 (blue) and Cx30 (red) can be encountered: both GJCs and hemichannels can be made of only one Cx (homomeric channels) or of a combination of two Cxs (heteromeric channels); GJCs can also be heterotypic — that is, composed of two different homomeric or heteromeric hemichannels. Parts **b**–**d** illustrate the organization of different astroglial networks. **b** | In the hippocampus the layer of pyramidal neurons (red) limits the number of astrocytes in this compartment and affects their morphology[22,43]. **c** | In the somatosensory cortex the distribution of astrocytes is homogeneous but Cx30 and Cx43 are differentially expressed in barrels and septa: they are highly expressed in barrel astrocytes (dark blue) and weakly expressed in septal astrocytes (light blue)[31]. **d** | In the olfactory bulb the cell bodies of glomerular astrocytes are located at the periphery whereas their processes have a centripetal orientation[142,143]. In **c** and **d** astroglial communication is focused towards the centre of the functional unit, which enhances communication in neuronal compartments[31]. The red triangles or circles indicate the location of neuronal somata in the indicated structures but do not reflect their density or size.

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Box 2 | **Hemichannels can contribute to paracrine pathways**

Under certain conditions, connexins (Cxs) can operate as hemichannels, allowing exchanges between the cytoplasm and the extracellular medium[11,12,127]. Under basal conditions, the hemichannels that have been described in various cell types are inactive, maintaining cellular integrity[11]. In astrocytes, hemichannels become activated under several experimental conditions, such as Ca^{2+}-free medium, metabolic inhibition, moderate increase in intracellular Ca^{2+} concentrations or pro-inflammatory treatments, resulting in their opening, as indicated by various dye-uptake assays. Interestingly, astrocyte hemichannels were shown to be permeable to gliotransmitters such as glutamate, ATP[128,129], glucose[130] and glutathione[131,132]. Thus, hemichannel activation and ATP release may support the propagation of intercellular Ca^{2+} waves[88,133]. Alternatively, hemichannels can affect nearby neuronal activity through the release of gliotransmitters or can affect neuronal survival through the release of neuroprotective or deleterious agents.

Cx43 and Cx30 have been reported to form functional hemichannels in HeLa transfected cells[134] indicating that both Cxs can account for membrane permeabilization. However, so far it is not known whether mixed Cx43 and Cx30 hemichannels operate in astrocytes. Another family of membrane proteins, the pannexins might form hemichannels (but not gap junction channels[13]) in astrocytes. Nevertheless, except in the case of Bergmann glia[135], their *in situ* expression in glia remains to be proved. Sequence analysis of pannexins indicates that they have a transmembrane topology similar to that of Cxs but a great divergence in primary sequence[13,136] that might explain their distinct unitary conductance and pharmacology.

Properties of astroglial networks

Besides individual astrocytic domains, communicating networks constitute another level of spatial organization of these cells. Some clues to the rules that govern such multicellular organization are provided by recent findings.

Dye-coupling experiments. Dye-coupling experiments (BOX 1), in which dye is injected into one cell and its intercellular diffusion is monitored, indicate that more than 80% of all coupled cells are astrocytes, identified by using either specific astrocyte markers or transgenic mice that carry labelled astrocytes (hGFAP–eGFP mice, in which the expression of enhanced green fluorescent protein (eGFP) is under the control of the human GFAP promoter, with GFAP being a marker for astrocytes)[20,22,35,36]. However, these findings do not exclude the possibility that heterotypic gap junctions may occasionally occur between astrocytes and either neurons[37–39] or oligodendrocytes[40,41]. Interestingly, certain interactions between astrocytes and oligodendrocytes could be linked to the expression of astroglial Cxs, as deletion of Cx43 and Cx30 is associated with dysmyelination as well as to hippocampal vacuolation[42]. Nevertheless, although the existence of such heterotypic junctions has been demonstrated, they are rare and limited to certain brain regions and developmental stages. Moreover, astroglial networks exhibit a degree of selectivity, for example in the hippocampus of hGFAP–eGFP mice several eGFP-positive cells (which are assumed to be astrocytes) are located within the coupling domain of an injected astrocyte, but are not dye coupled[43] (BOX 1). Also, in the somatosensory cortex of rodents, astrocytes that are located between barrels are weakly or not coupled, whereas coupling of astrocytes within a barrel is extensive and oriented towards the barrel's centre[31] (FIG. 1c). In the olfactory bulb, dye-coupling experiments have shown restricted communication between intra- and extraglomerular astrocytes[44] (FIG. 1d). Consequently, in addition to differences in the expression of receptors, transporters and ion channels[45], Cx-mediated coupling provides a criterion with which to discriminate subpopulations of astrocytes in one region.

Factors influencing the shape and extent of astrocyte networks. The extent and shape of these astrocytic networks are variable and under the control of several factors, including the developmental stage. Indeed, astroglial coupling increases in the hippocampus and the cortex during the first postnatal weeks[31,36], a property that is probably linked to the onset of Cx30 expression and the increase in Cx43 level. By contrast, the number and size of Cx43 and Cx30 plaques as well as the level of coupling decline in the ageing brain[46,47]. Heterogeneity in Cx43 and Cx30 expression between brain regions and even in a single region[17,18,31,48] may also explain differences in the extent of dye coupling[31]. For instance, astrocytes are more dye coupled in CA1 than in CA3 of the hippocampus[30]. The functional relevance of this difference in coupling has not yet been elicited. However, as CA1 is poorly supplied with capillaries compared with CA3 (REF. 49), it has been proposed that the substantial astroglial coupling in CA1 is attributable to the need to convey metabolic signals across this less vascularized region (see below). Although the coupling area of an astrocyte is in general spherical, in some cases it is asymmetrical, as shown in upper cortical layers or CA1 (REF. 43). In these cases, the shape of astroglial networks results from the organization of neuronal layers and is correlated to the Cx expression pattern (FIG. 1b). Moreover, the shape and extent of astroglial networks can be tightly linked to neuronal functional units, as recently reported for the barrels in the somatosensory cortex[31] and for olfactory glomeruli[44] (FIG. 1c,d). Therefore, astroglial networks seem to coincide with functional units of the neuronal network.

Connexins dictate properties of gap junctions

Selectivity of gap junction channels in astrocytes. To elucidate the potential role of Cxs in the physiology and pathology of the nervous system, it is important to understand the properties of GJCs that govern their selectivity and intercellular exchange. Cx channels have traditionally been viewed as poorly selective channels that are permeable to ions and small molecules. As GJCs are permeable to second messengers, they are considered to be mediators of intercellular signalling[50]. However, only recently has the permeability of GJCs to endogenous bioactive cytoplasmic molecules been tested, as it was realized that this does not necessarily correlate with permeability to fluorescent tracers.

The rules that dictate the ability of a molecule to permeate through GJCs are complex. The permeability of GJCs is not a simple function of the molecular weight and size of the exchanged molecule — it depends also on the molecule's shape, charge and specific interactions (electrostatic or binding) with the Cxs in the channels.

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Cx43 channels are selective for several endogenous molecules, including second messengers (cyclic AMP, inositol-1,4,5-trisphosphate ($InsP_3$) and Ca^{2+}), amino acids (glutamate, aspartate and taurine), nucleotides (ADP, ATP, CTP and NAD), energy metabolites (glucose, glucose-6-phosphate and lactate), small peptides (glutathione) and RNA (24mer)[51,52], but not for large molecules, such as nucleic acids, proteins and lipids. Less data are available on the bioactive molecules that can permeate through Cx30 GJCs, but ATP, $InsP_3$, aspartate, glutamate, glucose and lactate have been reported to do so[52]. In addition, Cx30 channels have been shown to be selective for cations over anions[53]. Although GJC permeability has often been thought to maintain homeostasis between coupled cells, differences in the rate of junctional flux can affect the speed of diffusion and the effective concentration of second messengers (such as $InsP_3$ or cAMP) that have a restricted cytoplasmic diffusion and lifetime. As most of the signalling molecules are charged at physiological pH, their diffusion through GJCs occurs along an electrochemical gradient. However, it is conceivable that the cationic selectivity of Cx30 channels could generate polarized pathways of intercellular signalling in astrocytes[53].

Voltage dependence of GJCs. The transjunctional and transmembrane voltage dependence of GJC conductance can also act as a selectivity filter to limit and compartmentalize the diffusion of biological signalling molecules. This is relevant to certain pathological conditions, as discussed later. Homotypic Cx30 and Cx43 channels and heterotypic Cx43–Cx30 channels are voltage dependent[54] (but see also REF. 55 for astrocytes). There are two lines of evidence for this: first, both Cx30 and Cx43 channel conductances are dependent on transmembrane voltage; and second, Cx30 is more sensitive than Cx43 to transjunctional voltage sensitivity, conferring a pronounced rectification in heterotypic channels[56]. These properties of GJCs may allow cells to isolate themselves from depolarized, damaged cells in pathology. Interestingly, even when physiological conditions are dramatically altered to mimic pathological situations, GJCs (or at least part of them) remain open[57]; however, it is not known whether their selectivity is modified.

The network as a tool

The network organization of astrocytes can be used to deliver pharmacological or molecular tools to specific populations of communicating astrocytes. Whole-cell patch-clamp of a single astrocyte allows the dialysis of this astrocyte, and molecules permeable to GJCs will diffuse in the associated network. Applying the Ca^{2+} chelator BAPTA with this strategy showed that Ca^{2+} signalling in astroglial networks affects hippocampal heterosynaptic depression[58]. It was also shown that astroglial networks supply glucose and lactate to sustain hippocampal synaptic transmission[22], and studies investigating the role of $InsP_3$ in hippocampal function suggested that this molecule can induce glutamate release from gap junction-coupled astrocytes, triggering transient depolarizations and epileptiform discharges in CA1 pyramidal neurons[59]. In the future, this network property could be exploited to silence specific molecular targets in a population of astrocytes by application of GJC-permeable biochemical tools or small interfering RNAs[60] in Cx-connected networks. Such a directed pharmacological strategy could allow testing of the impact of altering specific signalling components (for example kinase or phosphatase pathways, vesicular secretion and ionic channels) in a population of astrocytes on neuronal signalling.

Neuronal activity shapes astroglial networks

The permeability of GJCs is regulated by several factors that act on neuronal membrane receptors (TABLE 1). But does neuronal activity regulate communication in astrocytic networks?

Neuronal activity effects on astrocytic dye coupling. Only a few studies have addressed this question. Dye coupling is increased in cerebellar and striatal astrocytes that were co-cultured with neurons[61,62] and in glial cells of the frog optic nerve when neurons are stimulated[63]. The underlying mechanisms remain largely unknown, but recent studies have shed some light. Elevated levels of extracellular K^+ depolarize astrocytes and increase the permeability of gap junctions[64], an effect that is potentially mediated by phosphorylation of Cx43 by calcium/calmodulin-dependent protein kinase II[65]. The effects of glutamate are more complex and depend on the brain area studied (cortex[64], striatum[61,66], hippocampus[67] or cerebellum[68]), the type of preparation used (cultures or brain slices) and the type of glial receptor involved.

Neuronal activity effects on GJC permeability to bioactive molecules. All of the above studies have investigated the effect of neuronal activity on astroglial GJC permeability to passive dyes or tracers. But what about bioactive molecules? Glucose trafficking through GJCs was recently studied in hippocampal slices using fluorescent glucose derivatives[22]. This study demonstrated that glutamate released from neurons increases glucose trafficking in astroglial networks by activating AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors. As hippocampal astrocyte networks lack AMPA receptors[69,70], it remains unclear whether glutamate itself or a downstream effector of postsynaptic AMPA receptors regulates glucose trafficking through astroglial GJCs. Surprisingly, this activity-dependent regulation of glucose trafficking is not observed with passive dyes or tracers. This suggests that, at least in the hippocampus, glutamatergic activity does not regulate GJCs but triggers a local energy demand that generates a diffusion gradient for glucose to be trafficked to the sites of high neuronal demand.

Astrocytic networks regulate neuronal function

Astrocytes are now considered to be active elements of the brain circuitry: they integrate neuronal signals, exhibit Ca^{2+} excitability and process information[71,72]. Indeed, Ca^{2+} signalling in activated astrocytes has been proposed to trigger the release of many neuroactive molecules, named gliotransmitters, such as glutamate, ATP and

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Table 1 | **Regulation of gap junctional permeability between astrocytes**

Models	Effectors	Effect on GJC function	Techniques	Refs
Endogenous substances (or analogues)				
Mouse cerebellum	Kainate	↓	Junctional current recordings in Bergmann glia	68
hGFAP–eGFP mouse hippocampus	NMDA application associated with neuronal action potentials	↑	Dye coupling (biocytin)	67
Rat hippocampus	Endothelins	↓	Dye coupling (biocytin)	20
			Junctional current recordings	145
Drugs that have an effect on neuronal activity				
Mouse olfactory glomeruli	TTX	↓	Dye coupling (sulforhodamine B)	44
Mouse hippocampus	TTX	↓	Dye coupling (2-NBDG)	22
		No effect	Dye coupling (biocytin or sulforhodamine B)	22
	Mg^{2+} + picrotoxin (epileptic activity)	↑	Dye coupling (2-NBDG)	22
		No effect	Dye coupling (biocytin or sulforhodamine B)	22
	CNQX	↓	Dye coupling (2-NBDG)	22
	CNQX + CPP (in epileptic activity or not)	↓		
Stimulations				
Frog optic nerve	Nerve impulses	↑	Dye coupling (Lucifer Yellow)	63
Conscious rat inferior colliculus	Acoustic stimulation	↓	Microinfusion ([1-^{14}C]glucose)	92
		↑	Microinfusion ([U-^{14}C]lactate)	92
		↑	Microinfusion ([U-^{14}C]glutamine)	92
Others				
Rat striatum	Ethanol	↓	Dye coupling (Lucifer Yellow)	146

Note that only brain slices and *in vivo* experiments are listed here; data obtained from cell cultures were previously reviewed and discussed[147]. CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; CPP, 3-(-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid; eGFP, enhanced green fluorescent protein; GJC, gap junction channel; hGFAP, human glial fibrillary acidic protein; NMDA, *N*-methyl-D-aspartate; TTX, tetrodotoxin.

D-serine, which can modulate neuronal excitability, synaptic activity and plasticity. These findings initiated the exciting concepts of the tripartite synapse, astrocyte Ca^{2+} excitability, gliotransmission and astrocytic vesicular release[73–77], some of which (such as Ca^{2+}-dependent gliotransmission, vesicular or lysosomal release) are still controversial. Alternative approaches, including the use of transgenic mice, might resolve these controversies by using more specific molecular tools to selectively target astrocytes[78–81].

Up to now, the involvement of astrocytes in CNS functions has mostly been considered on a single-cell basis rather than as an active partnership between cell populations. The latter view is supported by the network organization of astrocytes and their permeability to signalling molecules.

Here we posit that, owing to the proximity of astrocyte gap junctions and neuronal synapses[25] (FIG. 2a,b), astrocytic networks could be actively associated with a group of synapses. Hence, specific astrocytic networks, thanks to their permeability to the potential gliotransmitters glutamate, glutamine[82] and, possibly, D-serine, could coordinate the activity of local groups of synapses (FIG. 2c,d). In addition, the coordinated release of gliotransmitters from astrocytes of one network could span a large area and hence could affect neuronal network activity. This working hypothesis can be tested by infusing astrocytic networks with molecules involved in gliotransmission and by using Cx-knockout mice. In fact, recent studies have shown that, owing to their Ca^{2+}-signalling properties, astroglial networks in the hippocampus are involved in heterosynaptic depression: ATP is released by astrocytes beyond the area of activated synapses and converted into adenosine, which causes inhibition of transmitter release in nearby synapses[58]. In addition, hippocampal astroglial networks that were infused with $InsP_3$ released glutamate that in turn triggered neuronal depolarizations and epileptic discharges[59]. Moreover, astrocytic networks were also reported to increase the threshold for generation of epileptic discharges by contributing to the buffering of extracellular K^+ (REF. 21). In summary, although these data suggest that astroglial networks have a role in regulating neuronal network activity in the hippocampus, this remains to be directly demonstrated.

Tripartite synapse
A concept in synaptic physiology based on the existence of communication between the pre- and postsynaptic terminal and a surrounding astrocyte.

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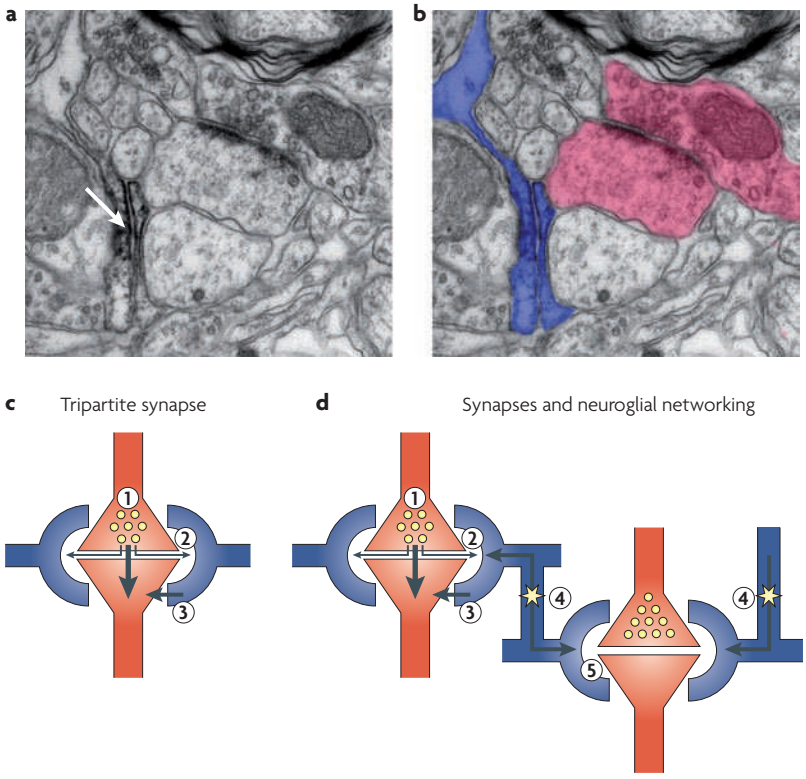

Figure 2 | **Tripartite synapses and neuroglial networks: possible consequences for synaptic transmission. a**,**b** | Electron micrographs showing the closeness of two modes of communication: a synapse for neuronal communication and gap junctions between astrocytes. Note that pre- and postsynaptic elements (pink) are surrounded by astrocytic processes (blue). Immunostaining with a Cx30-specific antibody indicates the presence of a gap junction (arrow) between two astrocytic processes. **c**,**d** | The role that astrocytic processes (blue) could have nearby a glutamatergic synapse (red). In **c**, only neuroglial interactions occurring at the tripartite synapse are taken into consideration. The different steps involved in this dynamic interaction are: the release of neurotransmitter (step 1), its action on receptors and transporters in astrocytes (step 2) and the release of gliotransmitter (step 3) that in turn influences neuronal activity. In **d**, in addition to these three steps, glutamate that has been taken up by a neighbouring astrocyte, and also its derivative, glutamine, can diffuse and permeate through gap junction channels (step 4) of astrocytic networks (yellow stars). This trafficking may result in subsequent release of gliotransmitter at a distant synapse (step 5) or even extrasynaptic sites and hence affect the activity of the underlying neuronal network. Images in part **a** are courtesy of C. Genoud and E. Welker, University of Lausanne, Switzerland.

Astrocyte networks contribute to energy supply

Processing of information in the brain is metabolically expensive. Indeed, the brain accounts for only 2% of our body mass but 20% of our oxygen and glucose consumption[83,84]. The role of astrocytes in the supply of energy substrates to neurons is one of their oldest known functions. Astrocytes are located at a strategic position between blood capillaries and neurons (first reported in the nineteenth century[85]) and have a key role in coupling neuronal activity to the use of glucose in the brain (neurovascular coupling)[86].

Neuronal glutamate release stimulates glucose supply by astrocytes. Astrocytes provide neurons that release glutamate with metabolic substrates, as neuronal activity is indicative of an increase in energy demand. This activity-dependent mechanism involves Na^+-coupled glutamate uptake in astrocytes and activation of the Na^+/K^+ ATPase, which triggers glucose uptake from the blood and its glycolytic processing, resulting in the release of lactate from astrocytes (FIG. 3a). Lactate, as well as glucose, can in turn be used as fuel by neurons to meet their energy demand[84]. In this model, also called the astrocyte–neuron lactate shuttle, astrocytes are considered as single entities. However, recently glutamate released from neurons has also been shown to generate metabolic waves in cultured astrocytes, resulting in coordinated uptake of glucose by gap junction connected astrocytes[87], thereby amplifying the metabolic response.

Glutamate increases glucose diffusion through GJCs. This amplification system, in which Na^+ has been proposed as a second messenger in neurometabolic coupling, requires intercellular Ca^{2+} waves to trigger glutamate release from cultured astrocytes. Interestingly, the network organization of astroglia may affect the astrocyte–neuron lactate shuttle (FIG. 3a), as intercellular calcium waves are dependent on Cx channels[88] (FIG. 3b). Whether Ca^{2+} waves propagate through astrocyte networks *in situ* and *in vivo* under physiological conditions remains unclear[89], but this pathway might be involved in pathological conditions — intercellular Ca^{2+} waves in astrocytes have been reported *in vivo* and in mouse models of Alzheimer's disease[90].

Glucose and some of its metabolites, such as lactate, traffic through the GJCs of astroglial networks, as shown in cultures[91], slices[22] and *in vivo*[92]. In hippocampal astroglial networks, this traffic is increased by glutamate release and activation of neuronal AMPA receptors but not astroglial glutamate transporters[22]. Synaptic more than spiking activity seems to control this glucose trafficking in astrocyte networks. Interestingly, this is also the case for neurometabolic coupling in the visual cortex[93] and for odour-evoked oxidative metabolism in the olfactory bulb[94]. Glucose trafficking that is dependent on neuronal activity could also sustain neuronal survival in pathological conditions that alter energy production, such as hypoglycaemia, anoxia or ischaemia, in which GJCs are still functional[57].

Therefore, glutamatergic synaptic activity enhances both glucose uptake and glucose trafficking in astroglial networks and might serve to efficiently supply energy metabolites to remote sites of high neuronal demand (FIG. 3b–d). Whether only glutamatergic neurons, or also distal GABA (γ-aminobutyric acid)-ergic and cholinergic neurons, are supplied by these astroglial network pathways remains unknown. Indeed, although astrocytes also take up GABA through Na^+-dependent transporters, inhibitory activity has not been shown to be coupled to the use of glucose[95].

What is the role of coordinated astroglial metabolic networks? First, it is not rare in biology for more than one mechanism or molecule to fulfil an essential function, and therefore glucose trafficking through GJCs might be an alternative or parallel pathway to glucose uptake. Second, these pathways might ensure that astrocytes can meet the increased glucose demand resulting from high neuronal activity. Furthermore, when glucose demand

Neurovascular coupling
The link between neuronal activity and energy supply from blood flow, in which astrocytes participate.

Astrocyte–neuron lactate shuttle
Activity-dependent fuelling of neuronal energy demand, consisting of glucose uptake at astrocyte endfeet, its glycolysis and the delivery of lactate to neurons by astrocytes.

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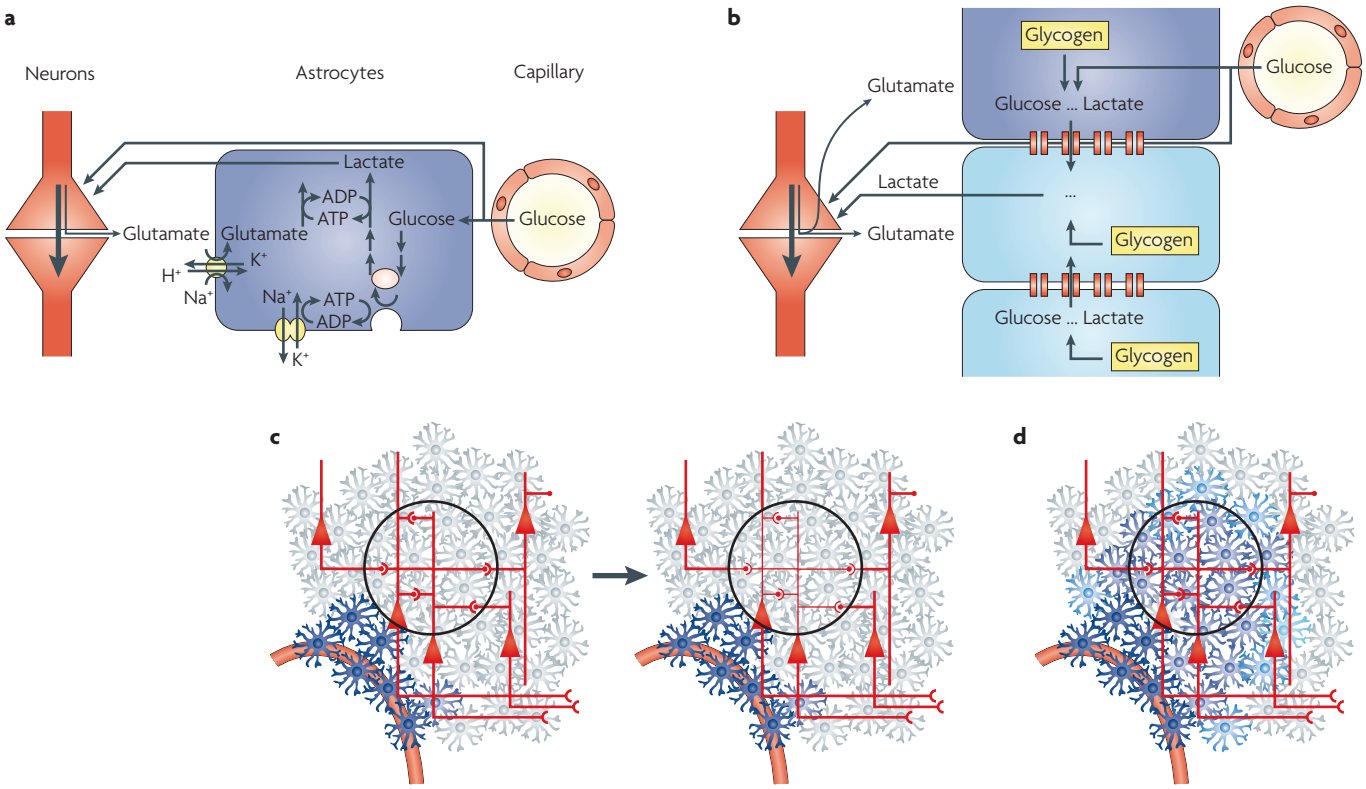

Figure 3 | **Astroglial metabolic networks sustain neuronal activity.** Astroglial gap junctions contribute to metabolic pathways in the brain. **a** | The mechanism underlying glutamate-induced glycolysis during glutamatergic synaptic activity. In the classical "astrocyte-neuron lactate shuttle"[144], in addition to the direct extracellular route, glucose is taken up by astrocytes in response to glutamatergic neuronal activity that is followed by glutamate uptake in astrocytes. The energy required for glutamate uptake is provided by glucose metabolism leading to lactate production that is delivered to neurons. **b** | The contribution of the astrocyte network to the glucose–lactate shuttle shown in **a**. Note that in the example shown, glutamate spillover after presynaptic release stimulates a distant astrocyte (dark blue), close to a blood vessel. The metabolic cascade illustrated in **a** applies but is not detailed for reasons of clarity. After glucose uptake from the blood by the dark blue astrocyte, glucose and its metabolites permeate gap junction channels and reach adjacent astrocytes (light blue) that are in contact with the active neurons and provide these with energy substrates. Parts **c** and **d** show the contribution of astroglial metabolic networks to synaptic activity. **c** | Astroglia networks with closed gap junction channels do not support increased neuronal activity (thick red lines and symbols) (although they still get extracellular glucose directly from their transporters) and with time, the activity of neuronal networks can be reduced owing to a minimal energy supply (thin red lines and symbols). **d** | By contrast, in the presence of an open metabolic network (dark to light blue), an intercellular route is provided, allowing the trafficking of energy substrates from their source, blood vessels, to the site of high energy demand and use, neurons (red triangles). Part **a** is modified, with permission, from REF. 144 © (1999) International Union of Physiological Sciences and the American Physiological Society.

exceeds supply, for example in situations of altered substrate availability (such as in hypoglycaemia, ischaemia and glucose transporter deficiency), astrocytes can convert their glycogen stores into glucose and lactate, which can spread through GJCs to fuel distal neurons. Third, although every capillary is a local source of glucose, the capacity to take up glucose and deliver it to neurons may differ between astrocytes in a given brain area. The capacity for glucose uptake will depend on the capillary coverage by astrocytic endfeet, their density of glucose transporters, the astroglial metabolic machinery and the strength of neuroglial interactions (which is determined by the density of receptors on astrocytes and astrocytic coverage of neurons). These parameters are likely to be heterogeneous among astrocytes, and therefore astrocytic networks may constitute a pathway to equilibrate glucose supply to neurons.

Astrocyte networks and blood flow control

Astroglial endfeet that enwrap blood vessels are characterized by high levels of Cx expression[18,22]. As a consequence, gap junctional communication between astrocytes is favoured close to the vasculature[22].

Emerging evidence implicates an increase in intracellular Ca^{2+} concentrations ($[Ca^{2+}]_i$) in astrocytes in either vasodilation or vasoconstriction[96,97], depending on the nature of the signal that triggered the $[Ca^{2+}]_i$ increase. Indeed, a local $[Ca^{2+}]_i$ increase in a single perivascular digit spreads throughout the entire process of the astrocyte, including the endfoot, and then propagates to adjacent endfeet[98]. As Cx channels contribute to the intercellular propagation of Ca^{2+} waves[88] *in vivo*[99] and in pathological situations[90], it is suggested that they participate in the regulation of blood flow by increasing the number of endfeet involved in the response. As the

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production of vasoactive molecules is Ca^{2+}-dependent, their efficacy in blood flow control is expected to be related to the extent of the perivascular propagation of Ca^{2+} signalling through endfeet of the same astrocyte or different astrocytes connected by reflexive or intercellular gap junctions, respectively[100]. Additionally, hemichannels could be involved in the regulation of blood flow, as they have also been proposed to contribute to the propagation of intercellular Ca^{2+} waves through ATP release[88].

Astroglial networks in brain dysfunction

In pathological situations, from acute injury to neurodegenerative disease, astrocytes undergo profound morphological and functional remodelling that is dependent on the type of insult or pathology, timing and the distance from the site of injury[101]. During this process, reactive astrocytes can lose their non-overlapping domains[102]. For example, after multiple epileptic seizures the processes of neighbouring astrocytes interdigitate, leading to 'mixed' territories. Such remodelling at the single-cell level is likely to have an impact on astroglial network organization.

In addition, changes in Cx expression have been reported in diverse pathological situations that may also affect the extent of astroglial networks. (TABLE 2; Supplementary information S1 (table)). In general, in most neurodegenerative diseases and in ischaemia, reactive astrocytes have increased levels of Cx43 expression, although this may also result from the internalization of gap junctions[103] and hence does not necessarily reflect an increase in the numbers of GJCs or in astroglial networking. Moreover, in brain injury, changes in the level of Cx expression are dependent on the proximity of the reactive astrocytes to the damaged site[104–106]. In epileptic tissue, decreased, unchanged and increased Cx43 expression have been reported (TABLE 2; Supplementary information S1 (table)). These contradictory results may in part be due to the diversity of epilepsy models — for example, acute versus chronic — and also to the duration of seizures.

What are the consequences of altered Cx expression levels for the function of astroglial networks? Most of the studies to date that have examined the expression and function of astroglial Cxs in a pathological context have used culture models that focus on only selected signals and do not reproduce all events occuring *in vivo* in a chronological manner. *In situ* models have started to provide expression–function correlations for astroglial Cxs. For example, in brain tissues from patients with Alzheimer's disease, the expression of Cx43 is increased in reactive astrocytes surrounding amyloid plaques[107]. Accordingly, in mouse models of Alzheimer's disease, an increase in dye coupling between cortical astrocytes was recently reported[47], and intercellular Ca^{2+} waves, initiated in astrocytes at the periphery of amyloid plaques, were shown to propagate over longer distances than in control animals[90]. In epileptic situations, an increase in Cx43 expression and gap junctional communication was described in hippocampal organotypic cultures[108]. Interestingly, prolonged exposure to Cx43-mimetic peptides attenuates epileptiform activity, indicating that enhanced coupling in astroglial networks might support or trigger epileptic activity[109]. By contrast, in a mouse model of tuberous sclerosis complex that exhibits epileptic-like seizures, Cx43 expression is reduced and dye coupling is impaired[110]. Hence, the role of astroglial networks in epileptic situations requires further investigation.

What are the consequences of astroglial network changes for neuronal function and/or survival? Two strategies have been adopted to investigate this question[111]. One is a pharmacological approach using agents (such as carbenoxolone and octanol) that block GJCs. However, the results obtained are difficult to interpret: first, most pharmacological agents are not specific and have side effects on neuronal activity; second, most inhibit all GJCs, and therefore the relative contributions of neuronal and glial gap junctions cannot be discriminated; and third, most block hemichannels as well as GJCs and do not allow their respective roles to be distinguished. Because of these limitations, alternative strategies, such as using Cx-knockout mice or molecular tools, were developed. However, most of the results of these studies, which mainly concern acute injury (ischaemia and trauma), are controversial (Supplementary information S2 (table)).

Given the role of gap junctions in the buffering of ions, long-range signalling and exchange of small molecules in astroglial networks, a neuroprotective role for astroglial GJCs has been proposed. However, it remains controversial whether enhanced coupling through GJCs is beneficial or injurious under pathological conditions. Some studies have proposed that Cx43-containing GJCs or hemichannels may have a role in neuronal survival after ischaemia. First, Cx43-knockout mice exhibit a larger infarct volume than wild-type mice after induced ischaemia[112–115]. Moreover, it has recently been shown that after hypoxic preconditioning, Cx43-containing hemichannels play a key part in neuroprotection. These hemichannels release ATP, which in turn is rapidly converted into the potent neuroprotective agent adenosine[112].

By contrast, gap junctional communication in astrocytes can propagate and amplify cell injury by allowing intercellular diffusion of death signals that kill adjacent cells[116]. Such a 'bystander effect' could account for secondary effects at sites distant from the brain injury after cerebral ischaemia. Indeed, in organotypic hippocampal slices submitted to hypoxic injury, hypoglycaemic injury or traumatic insult, treatment with Cx channel blockers or Cx43-mimetic peptides decreased cell death[117,118]. Also, in *ex vivo* and *in vivo* models of spinal cord injury, the application of mimetic peptides that suppress transient Cx43 upregulation after trauma resulted in a reduction of neuronal death and/or damage spread[119,120]. As mimetic peptides prevent activation of hemichannels but may also, after longer exposure, prevent their docking to form GJCs[109], inhibition of one or both of the functions of Cx43 channels — that is, their functions as hemichannels or GJCs — may be involved in the protective effect observed.

Reactive astrocytes
Astroglia that, after brain injuries or during pathology, are characterized by functional and morphological changes that can be associated with cell migration and proliferation.

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Table 2 | **Changes in connexin expression associated with pathological situations in the CNS**

Brain pathologies and injuries	Effects on connexin expression	Effects on connexin function
Neurodegenerative diseases	↑ Cx43 IR in AD, PD and HD ↓ Cx43 IR, protein and mRNA levels in EAE	↑ Dye coupling in AD NT
Epilepsy	↑, unchanged or ↓ Cx43 IR, protein and mRNA levels, according to animal models and seizure duration	↑ and ↓ dye coupling
Ischaemia	↑ or no change in Cx43 protein and mRNA Cx43 dephosphorylation Cx43 IR redistribution	↓ dye coupling
Autism	↑ Cx43 protein	NT
Pain	↑ Cx43 IR	NT
Excitotoxic injury	↓ Cx43 and Cx30 IR at zone of neuronal death ↑ Cx43 and Cx30 IR at periphery	NT

Details and references are presented in Supplementary information S1,S2 (tables). AD, Alzheimer's disease; Cx, connexin; EAE, experimental autoimmune encephalomyelitis; HD, Huntington's disease; IR, immunoreactive; NT, not tested; PD, Parkinson's disease.

Conclusions and perspectives

It is now clear that astroglial networks, like neuronal circuits, have multiple levels of complexity. First, communication between astrocytes is favoured in specific brain regions that are characterized by compartmentalized functional neuronal units, such as the barrel cortex[31] or the olfactory glomeruli[44]. Second, gap junctional communication is controlled by endogenous compounds, including neurotransmitters, and therefore depends on neuronal activity (TABLE 1). Third, the permeation through GJCs of inactive tracers obeys the law of passive diffusion, which is not the case for endogenous biologically active molecules[22]. Indeed, several parameters (lifetime, buffering by binding to intracellular sites and regenerative processes) influence the trafficking of bioactive molecules through GJCs. Thus, the rules governing intercellular trafficking of these molecules through GJCs are likely to be more complex than for dyes[22], and therefore the number of astrocytes that are functionally coupled for a given bioactive molecules is expected to be smaller than for a dye. Astroglial networks are likely to be independent from each other and are probably not as elaborate as neuronal circuits in terms of size and the specificity of their connections. However, it is conceivable that their shape and extent vary with time and the activity of their environment.

The concepts of the tripartite synapse and of non-overlapping territories of single astrocytes are essential for our understanding of dynamic neuroglial interactions. However, the existence of astroglial networks may extend the neuroglial dialogue by allowing information processing and integration from a large number of neurons. Also, astroglial networks might have a role in providing metabolites to remote sites during high neuronal demand and in buffering ion or neurotransmitter concentrations. Such functions are likely to be important in pathological situations, when neuronal hyperactivity — for example, in the case of epilepsy — consumes more energy and leads to increased concentrations of K^+ and glutamate in the extracellular space. Interestingly, at the level of the single cell and in the particular context of the tripartite synapse, astrocytes are thought to boost neuronal activity by releasing glutamate[121,122]. By contrast, when considered at the network level, especially during episodes of high neuronal activity, astroglia have been shown to attenuate neurotransmission by buffering surplus K^+ (for example, to reduce epileptic discharges[21]) and releasing ATP (for example, to produce neuroprotective adenosine and inhibit presynaptic neurotransmitter release[112]). In most of the brain pathologies or injuries that have been studied using animal models, changes in Cx expression and network coupling have been reported (Supplementary information S1 (table)) that have beneficial or deleterious effects. More studies that combine expression and function analysis are required to understand the complex role of astroglial Cxs in brain pathologies.

An important question is also whether astroglial networks affect neuronal activity. So far, this question has been rather difficult to address owing to the lack of specific tools for studying astroglial Cx channels (BOX 1). Moreover, only a few studies have investigated which molecules permeate through GJCs *in vivo* under physiological conditions and what functional significance this type of intercellular signalling has.

Mathematical modelling of neuroglial interactions could help to determine the role of independent astrocytes and astroglial networks in this dialogue (BOX 3). Several studies have indeed attempted to reproduce *in silico* astroglial Ca^{2+} signals and responses to neuronal activity. By contrast, very few studies have modelled the feedback of astrocytic activity on neurons. Further modelling attempts could prompt significant advances in our understanding of neuroglial interactions.

Defects in astroglial Cxs and therefore in the exchange of information at the network level have been reported in several neurological pathologies, but it remains unknown whether these changes are the cause or the consequence of neuronal dysfunction and death. New pharmacological and genetic approaches that control the expression and function of astroglial Cx channels might provide answers to these questions[111]. In addition, the identification of the molecules that can permeate

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Box 3 | **The why and how of modelling neuroglial networks**

Modelling approaches (biophysical models and mathematical analysis) offer an alternative method to understand how astrocytes contribute to information processing in CNS function and dysfunction. Two levels of interaction can be considered: the single-cell level, with the concept of the tripartite synapse[137], and the network level. Here, we briefly illustrate emerging concepts by focusing on glutamatergic transmission and the permeability of astroglial networks to Ca^{2+}, inositol-1,4,5-trisphosphate ($InsP_3$), glutamine and glutamate.

Combining classical equations for the neuronal voltage (Hodgkin Huxley equations) and the Li-Rinzel model to account for the Ca^{2+}–$InsP_3$ signalling in astrocytes[138] has allowed several predictions to be made concerning the tripartite synapse, such as persistent neuronal spiking occurring if glutamate receptors are overexpressed in astrocytes[139] or Ca^{2+} oscillations persisting in astrocytes even when neuronal activity is minimal[140]. At the network level, it is conceivable that coupling between astrocytes will interfere with neuronal activity. Under the assumption that both Ca^{2+} and glutamate can be redistributed and dissipated throughout astroglial networks, both signalling molecules might ultimately contribute to neuronal activity far away from the initial source. Several examples of a neuronal activity dependence of gap junctional communication in astrocytes suggest that the sphere of influence of astroglial networks on neuronal synchrony can vary. We propose that for low neuronal spiking frequencies, moderate glutamate release from restricted astroglial networks might lead at proximal sites to synaptic depression through the activation of extrasynaptic metabotropic glutamate receptors, as astrocytes first contact extrasynaptic sites[77,141] and consequently desynchronize neurons. However, at a higher frequency, larger glutamate release from extensive astroglial networks could reach distal sites and activate postsynaptic AMPARs (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors) and NMDARs (*N*-methyl-D-aspartate receptors), resulting in an increase in neuronal activity. Conversely, this model predicts that when the extent of glutamate release from astrocytic networks decreases, distal synapses should desynchronize, rendering astrocytes unable to modulate large neuronal networks.

Future models should address two aspects of neuronal activity: first, the contribution of astroglial networks to a single synapse, and second, the possibility of synchronization or at least coordination at a slow timescale between several synapses. As several of the parameters mentioned above are likely to affect pathological situations, a modelling approach could ultimately be used to determine the contribution of astroglial networks to dysfunctions in neuronal activity, such as during epileptic seizures.

through GJCs represents another important challenge for fully understanding the physiology of astrocyte networks. Indeed, the level of Cx expression in astrocytes is unique in the nervous system and certainly plays a crucial part in their contribution to brain metabolism and processing. The existence of astroglial networks has prompted us to reconsider neuroglial interactions at a more integrated level.

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