Review

Emerging role for astroglial networks in information processing: from synapse to behavior

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Astrocytes contribute to neurotransmission through a variety of mechanisms ranging from synapse isolation to active signaling. Astroglial involvement in neurophysiology has been mostly investigated at the single-cell level. However, a unique feature of astrocytes is their high level of intercellular connectivity mediated by connexins, the proteins forming gap junction (GJ) channels. These astroglial GJ circuits enable the rapid intercellular exchange of ions, metabolites, and neuroactive substances. Recent findings have suggested that, despite their extensity, astroglial networks are also selective, preferential as well as plastic, and can regulate synapses, neuronal circuits, and behavior. The present review critically discusses the impact of astroglial networks on normal and pathological neuronal information processing as well as the underlying mechanisms.

Introduction

Brain information processing is traditionally viewed as a neuronal performance. However, crucial advances in the molecular and physiological characterization of glial cells have recently identified astrocytes as part of the tripartite synapse (see Glossary), playing active roles in neurotransmission. These cells display dynamic signaling, which enables them to sense neuronal inputs through ion channels, neurotransmitter receptors or transporters, and to respond via elaborate calcium (Ca^{2+}) signaling, morphological plasticity, and uptake or release of numerous neuroactive factors that modulate neighboring pre- and postsynaptic elements. For instance, astrocytes are classically thought to tune synaptic efficacy by controlling the homeostasis of essential factors such as energy metabolite supply [1], clearance of extracellular potassium (K^+) [2], and glutamate through plastic physical coverage of neurons [3]. Such synapse enwrapping also allows control of extracellular space volume, thereby regulating extracellular levels and diffusion of neuroactive substances [4].

In addition, astrocytes also modulate the formation and stability of synapses, receptor trafficking, and the moment-to-moment synaptic activity by releasing various molecules such as proteoglycans [5], cytokines, and neurotransmitters, respectively, as recently described in several comprehensive reviews [6–9]. The involvement of astrocytes in these processes has primarily been investigated at the level of a single astrocyte, largely overlooking the tremendous network connectivity of these cells via GJ channels. Nevertheless, in the past few years much pioneering information has been acquired on the original properties of GJ-mediated astroglial circuits and their potential role in neurotransmission and cognition. Thus the current model of astroglial regulation of neuronal activity, which ranges from the very local modulation of individual synapses to the more global regulation of neuronal populations by release or uptake of neuroactive substances, should be reconsidered in light of emerging evidence that GJ-mediated astroglial network communication also contributes to local and distal neuronal activity. Here we review the most recent developments on astroglial networks, highlighting their major features, and describing how they likely regulate neuronal activity at the synaptic and circuit level in relation to cognitive functions.

Glossary

Connexin: GJ channel protein subunit, with four transmembrane domains, two extracellular loops, one cytoplasmic loop and intracellular N- and C-terminal regions.
Gap junction (GJ) channel: aqueous channel between the cytoplasm of two neighboring cells formed by the docking of two hemichannels or connexons, each composed of six connexins. GJ channels are poorly selective and mediate direct electrical and metabolic coupling between adjacent cells by allowing cytoplasmic exchange of a wide variety of small molecules with a molecular mass of up to 1.5 kDa. These include ions (K^+, Ca^{2+}, Na^+), second messengers (cAMP, IP3), neurotransmitters (glutamate), and energy metabolites (glucose, lactate).
Schaffer collaterals: axonal projections from hippocampal CA3 pyramidal cells forming excitatory synapses onto CA1 pyramidal cell dendrites.
Gliotransmission: release of neuroactive substances such as glutamate, ATP, D-serine, or lactate from astrocytes to modulate neuronal activity.
Neurometabolic coupling: mechanisms by which the supply of energy substrates to neurons depends on neuronal activity. Astrocytes are thought to play a crucial role in this coupling.
Tripartite synapse: revised concept of the synapse defined as a functional unit formed by the pre- and postsynaptic elements as well as the surrounding astrocyte. The astrocyte integrates the moment-to-moment synaptic activity via its ion channels, neurotransmitter receptors and transporters, and provides feedback modulation of neurotransmission through uptake or release of neuroactive substances.

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GJ-mediated astroglial networks: extensive, but selective, preferential, and plastic

Astrocytes express high levels of connexins (Cxs), the GJ channel subunits (Box 1). As a result, extensive network communication via GJs is a prominent functional property of astrocytes. Throughout the brain GJ channels mediate the formation of large cellular ensembles, involving hundreds of astrocytes, which are able to exchange information through direct intercellular diffusion of a wide variety of small molecules. However, GJs not only provide the molecular basis for extended astroglial circuits, but also confer selective and preferential inter-astroglial connections. Indeed, dye-coupling experiments show that not all neighboring astrocytes are functionally connected by GJs [10–12]. This may be due to (i) heterogeneous expression of the GJ-forming proteins, Cx43 and Cx30, in astrocytes, (ii) short-term regulation of astroglial GJ coupling by various molecules, or (iii) functionally distinct glial populations resulting from astrocyte allocation to specific spatial domains during development [13]. Thus, akin to neuronal assemblies, astroglial networks are finely organized into anatomical and functional compartments, as shown in strongly compartmentalized structures such as the somatosensory cortex or the olfactory bulb [10,12]. Confinement of astroglial networking within a well-defined spatial domain might contribute to the precision of local neuronal information processing by favoring astrocytic interactions with neighboring neurons.

Another important property of astroglial networks is their functional plasticity. The extent of astroglial networks is determined by the permeability and selectivity of GJ channels, which are regulated in the short and long-term by a variety of endogenous factors such as endothelins, cannabinoids, and neurotransmitters [14]. Of particular interest is the regulation of astroglial networks by neuronal activity, as shown in several preparations including cultured striatal [15,16] or cerebellar astrocytes [17], optic nerve [18], and acute slices from the hippocampus [19] or the olfactory bulb [12]. Although the underlying mechanisms are still elusive, the depolarizing action of K^+ has been suggested to increase astroglial GJ coupling [12,20,21], likely via Cx43 phosphorylation by Ca^{2+}/calmodulin-dependent protein kinase II [20]. Alternatively, glutamate also modulates astroglial GJ coupling [14], although various actions have been reported depending on the preparation and the receptor subtype involved. Interestingly, in the hippocampus, synaptically-released glutamate selectively increases the trafficking of bioactive molecules such as glucose through astroglial GJ channels via activation of postsynaptic AMPA receptors [19]. This effect is not mediated by a direct regulation of GJ channel permeability because glutamate does not alter GJ coupling for dyes. Thus, glucose diffusion through astroglial networks might follow a gradient from sites of high levels, near blood vessels, to sites of lower levels associated with high consumption by active neurons. In accordance with this hypothesis, local evoked neuronal activity triggers glucose trafficking through astroglial networks from distal sites towards the region of evoked activity [19] (Figure 1C). This suggests that excitatory synaptic activity induces a local energy demand resulting in an activity-dependent reshaping of astroglial metabolic networks.

Box 1. Connexins: properties and mouse models

Connexins form a family of 20 genes in mice and 21 in humans, with 11 being expressed in vertebrate brains. They are abbreviated 'Cx' followed by their molecular mass in kDa. Cxs display three conserved extracellular cysteine residues that are important for docking and vary mostly in their cytoplasmic regions. In the adult brain, astrocytes express mainly Cx43, from embryonic stages to adulthood, and Cx30, expressed from postnatal day 10 [56]. Cx functions extend beyond the classic intercellular GJ communication and include hemichannel-mediated exchange with the extracellular space as well as channel-independent functions involving protein interactions, cell adhesion, and intracellular signaling [23,88]. Until now the functions of GJ-mediated astroglial networks have been investigated using either non-selective GJ channel blockers or Cx knockout mouse models chronically disrupting Cx channel and non-channel functions, hence complicating our comprehension of the direct functions of astrocytic networks.

Total knockout of either *Cx43* (*Cx43*$^{-/-}$) [89] or *Cx30* (*Cx30*$^{-/-}$) [90] induces several abnormalities due to systemic expression of both Cxs [91], but brain development of both knockout mice is indistinguishable at the macroscopic level from that of wild type mice [52,89,90]. Mice carrying Cx point mutations specifically targeting GJ channel permeability, but without affecting GJ assembly, such as the human deafness-associated *Cx30*^{T5M} mutation [92], should help understanding of the direct function of GJ networks. To target Cx deletion in astrocytes, conditional knockout (cKO) mice have been generated by expressing the Cre recombinase under GFAP or S100β promoters, which are primarily, but not exclusively, expressed in astroglia. Intriguingly, cKO mice exhibit different phenotypes depending on their genetic background. In particular, *Cx43* cKO mice with the 129SVEV, but not C57Bl/6J, background exhibit a severe phenotype characterized by malformation of several brain areas accompanied by behavioral impairments [93]. GFAP promoters from different species, used to activate the Cre recombinase [94], can also lead to different mouse phenotypes, most likely due to distinct times of promoter expression onset during development. For example, the human, but not the mouse GFAP promoter, targets oligodendrocyte progenitors [95].

Crossbreeding *Cx30*$^{-/-}$ with *Cx43*$^{fl/fl}$ GFAP–Cre mice results in a mouse devoid of astroglial GJ communication [26,51,60]. The data obtained with such KO models must be carefully interpreted because deletion of *Cx43* and *Cx30* can lead to: (i) disruption of GJ communication between astrocytes and oligodendrocytes due to loss of heterotypic GJs formed by Cx43/Cx47 and Cx30/Cx32 astrocytes/oligodendrocytes channels; although such junctions are rare and are limited to specific brain areas and developmental stages, their disruption in adult mice is thought to cause dysmyelination and vacuolation [51]; (ii) suppression of transmitter release via Cx hemichannels – this may abolish local signaling, although hemichannel opening probability is low under physiological conditions; (iii) alteration of the aforementioned Cx channel-independent functions; (iv) compensatory alterations and off-target effects such as down-regulation of non-astroglial Cxs genes located in the same chromosomal locus. Therefore, to relate precisely specific functions to astroglial network communication one would need to ensure that the GJ-independent functions of Cxs are unaltered and to block GJ channels acutely and reversibly using either novel selective pharmacological inhibitors or inducible knockout mice targeting only the GJ channel function of astroglial Cxs (Table 1).

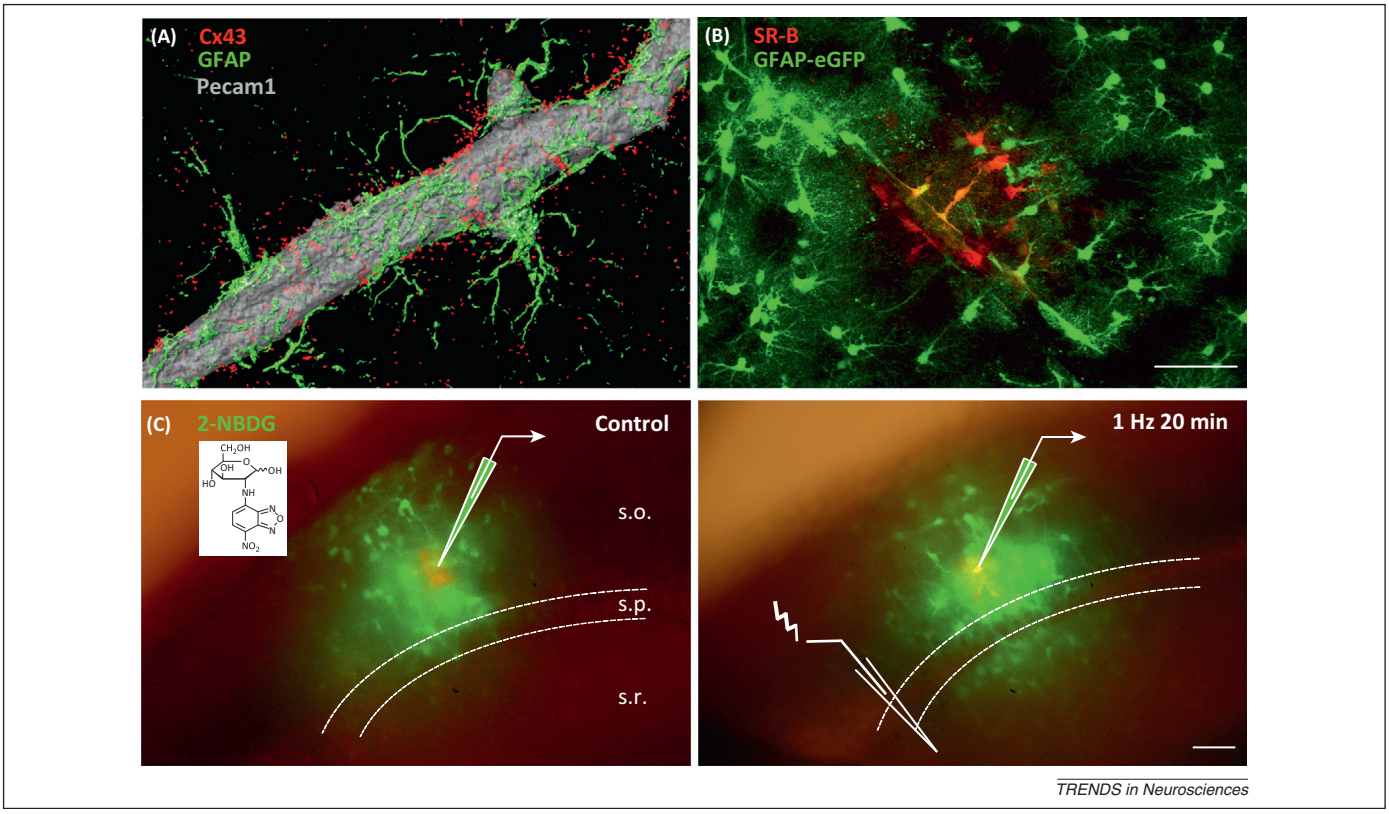


Figure 1. Shaping of connexin-mediated functional metabolic networks of perivascular astrocytes. **(A)** Cx43 (red) is enriched in perivascular astrocytic endfeet (GFAP labeling, green) and delineates blood vessel walls (Pecam1 labeling, grey), as revealed by confocal microscopy projection of a 20 μm blood vessel in the adult mouse cortex. **(B)** Functional coupling of perivascular astrocytes in GFAP–eGFP (enhanced green fluorescent protein) mice visualized by diffusion of sulforhodamine-B (SR-B, red), dialyzed for 5 min by whole-cell recording of a perivascular astrocyte, revealing preferential diffusion along the vessel walls [20]. Scale bar: 20 μm (A) and 100 μm (B). **(C)** A fluorescent glucose derivative (2-NBDG, green) was injected through a patch pipette in a single astrocyte located in stratum oriens (s.o.) (with coinjection of rhodamine–dextran to localize the injected cell, red), as shown in the sample pictures. Without stimulation of the Schaffer collaterals, such injection results in 2-NBDG diffusion into neighboring astrocytes located mainly in the stratum oriens (left image). However, prolonged stimulation of the Schaffer collaterals (1 Hz, 20 min) induces a change in the shape of the metabolic network by recruiting additional astrocytes located over the pyramidal layer in the stimulated stratum radiatum (s.r.) (right image) [20]; s.p., stratum pyramidale; scale bar, 50 μm. Adapted, with permission, from [86] (A) and [19] (B,C).

Physiological functions of astroglial networks: from homeostasis to signaling

Astroglial networking adds another level of complexity to astrocyte functions by requiring the coordination of cellular ensembles for a specific task. Such interconnectivity has been proposed to contribute to several well-known functions of astrocytes, including ionic homeostasis, control of cellular volume, and proliferation or survival, as previously reviewed [14,22,23]. Hence, we hereafter focus this review on astroglial network functions related to modulation of neurophysiology. We furthermore critically discuss the current approaches used to assign directly specific functions to astroglial networks (Box 1).

Neuronal activity induces pre- and postsynaptic K^+ release, and rapid removal of extracellular K^+ is necessary to maintain neuronal excitability [24]. This is partly achieved by spatial buffering (see Figure 4A, below), which consists of K^+ uptake by astroglia via densely expressed inwardly rectifying potassium channels [25], and redistribution to sites of lower extracellular K^+ concentration. Although a single astrocyte can redistribute K^+ within its own microdomains, GJ channels appear to contribute to K^+ spatial buffering within the astrocytic network at more distal sites, particularly in the hippocampal layer stratum lacunosum moleculare [26], but also in different preparations [25,27,28]. However, other mechanisms enabling K^+ spatial buffering are clearly at play in the hippocampus because radial relocation of K^+ is independent of GJs in the stratum radiatum region [26].

Neuronal activity can also trigger intracellular Ca^{2+} signaling in astrocytes. This is believed to represent the astrocytic form of excitability because these cells are electrically non-excitable. However, recent data obtained either in transgenic mice where astroglial Ca^{2+} signaling is selectively targeted [29,30], or in adult mice, where the major mGluR5 receptor-dependent Ca^{2+} signaling pathway reported in young mice is undetectable [31], questioned the physiological relevance of astrocyte Ca^{2+} signaling. Spatial distributions of activity-dependent Ca^{2+} signals are diverse because they can be confined to astroglial microdomains [32–35] or to a single astrocyte, and can propagate as slow and long-range inter-astroglial Ca^{2+} waves. Such waves, initially discovered *in vitro* [36], and later *in situ* [37] and *in vivo* [38], have been proposed to modulate neuronal activity by inducing the release of numerous neuroactive molecules [39]. Although astroglial Ca^{2+} waves have been mostly associated with pathological situations, such as epilepsy [40] or Alzheimer's disease [41], their existence in physiological conditions, long questioned, has recently been shown [38]. The mechanisms underlying Ca^{2+} waves are complex, and were initially proposed to involve GJ channels mostly because (i) they

are permeable to Ca^{2+} and inositol 1,4,5-trisphosphate (IP3), (ii) various GJ blockers or knockout of Cxs in astrocytes reduce astroglial Ca^{2+} wave propagation, and (iii) the slow timescale of astroglial Ca^{2+} waves is compatible with propagation through GJ channels. However, independently of the GJ pathway, single-point or regenerative release of extracellular messengers such as ATP, via Cx hemichannels or Ca^{2+}-dependent processes, has since been shown to mediate astroglial Ca^{2+} waves [39]. Hence, because GJ blockers and Cx knockout mice also target Cx hemichannels, there is no solid evidence that astroglial GJs contribute to Ca^{2+} waves. However, whether astroglial GJs amplify Ca^{2+} waves mediated by extracellular pathways is still an open question, which can only be addressed by blocking specifically astroglial GJ channel permeability (Box 1).

Astroglial networks have also been proposed to contribute to inter-astroglial sodium (Na^+) waves in cultured cortical astrocytes [42] and hippocampal slices [43]. Na^+ waves are coupled to a spatially correlated increase in glucose uptake [42], occurring in parallel to Ca^{2+} waves *in vitro* but not *in situ*, and might therefore contribute to neurometabolic coupling. Intercellular exchange of ions such as Na^+, Ca^{2+}, or K^+ through GJs could also enable astroglial populations to equalize their intracellular ion levels, as shown for Na^+ [44] or Ca^{2+} [45,46]. This might allow the coordination of ion-dependent physiological or pathological responses in astroglial networks. Finally, GJs enable intercellular trafficking of energy metabolites such as glucose, lactate, glyceraldehyde-3-phosphate, NADH, or NADPH through astroglial networks [19,47,48], and might thus directly contribute to neuronal energy supply [19].

A role for astroglial networks in synaptic transmission and plasticity

The impact of GJ-mediated astroglial circuits on brain activity was initially proposed at the behavioral [49–52] and neuronal network levels [26], but not at synapses. Instead, the role of individual astrocytes at tripartite synapses has been extensively studied in synaptic physiology, most likely because a single astrocyte already controls and modulates ~140 000 synapses from 100 neurons [53]. However, recent data have suggested an important contribution of astroglial networks to basal synaptic transmission and plasticity through multiple mechanisms.

Metabolic support

One of the fundamental functions of astrocytes consists in providing metabolic support to neurons. Indeed, *in vivo*, astrocytes take-up most of the glucose during neuronal activity [54]. Furthermore, lactate, which is metabolized from glucose by astrocytes, has been proposed to be the main form of energy used by neurons [1] and is notably required for hippocampal memory formation [55]. Because the two main GJ subunit proteins in astrocytes, Cx43 and Cx30, are markedly enriched at perivascular astrocytic endfeet and delineate blood-vessel walls [19,56,57] (Figure 1A), the connectivity of perivascular astroglial networks (Figure 1B) might contribute to neurometabolic coupling. It was indeed demonstrated that selective glucose or lactate delivery into astroglial networks inhibits, independently of Cx hemichannels, the depression of synaptic transmission induced by extracellular glucose deprivation [19]. These data suggest that GJs directly contribute to the metabolic supportive function of astrocytes by providing an activity-dependent intercellular pathway for glucose delivery from blood vessels to neurons. Whether this regulation targets only excitatory neurons or also involves local circuit inhibition remains unknown. These results called for a revision of the classical model of neurometabolic coupling in which astrocytes were generally considered as single entities [58]. Such pathways may be particularly critical to sustain neuronal activity and survival in pathological conditions associated with a decrease in energy production, such as hypoglycemia, anoxia or ischemia, in which GJ channels are still operational [59].

Control of extracellular ion and neurotransmitter homeostasis

In addition to energy metabolites, many other small molecules, including neurotransmitters and ions, can transit across GJ channels. Thus, one may ask what is the predominant function of astroglial networks in the moment-to-moment synaptic transmission and plasticity in physiological conditions? Unexpectedly, recent data suggest that disconnection of astrocytes by inactivation of the *Cx30* and *Cx43* genes (Figure 2A) massively increases hippocampal excitatory synaptic transmission in CA1 pyramidal neurons, pointing toward an inhibitory role of astroglial networks (Figure 2C) [60]. This effect, that is independent of Cx hemichannel disruption [61], is likely attributable to insufficient K^+ (Figure 2B) and glutamate removal by disconnected astroglia during synaptic activity [60]. In fact, the extracellular accumulation of glutamate and K^+ during synaptic activity may not only be due to the reduced uptake or buffering capacity of uncoupled astrocytes but might also result from a reduction in the extracellular space volume caused by massive swelling of disconnected astrocytes [60]. Hence, these data suggest that astroglial networks dictate extracellular concentrations of glutamate and K^+ during synaptic activity.

As major determinants of extracellular homeostasis, hippocampal astroglial circuits, similarly to astroglial coverage of neurons [3], were proposed to regulate synaptic transmission. However, astroglial networks were suggested to control the function of both pre- and postsynaptic terminals as well as their bidirectional synaptic plasticity. This is supported by investigations suggesting that astroglial network disruption increases neuronal excitability, release probability, glutamate spillover, and activity-dependent insertion of postsynaptic AMPA receptors, leading to synapse unsilencing [60]. The latter effect likely strongly alters synaptic plasticity (Figure 3A) because the threshold balance between long-term potentiation (LTP) and long-term depression (LTD) is shifted, favoring synaptic depression [60]. Hence, astroglial network communication is thought to be crucial for precise spatial and temporal synaptic information transfer, processing, and storage. Among the various molecules which cross GJ channels, ions and neurotransmitters may thus predominate to regulate basal synaptic activity and plasticity in physiological conditions. This would imply that the aforementioned metabolic supportive function [19] mostly operates in situations

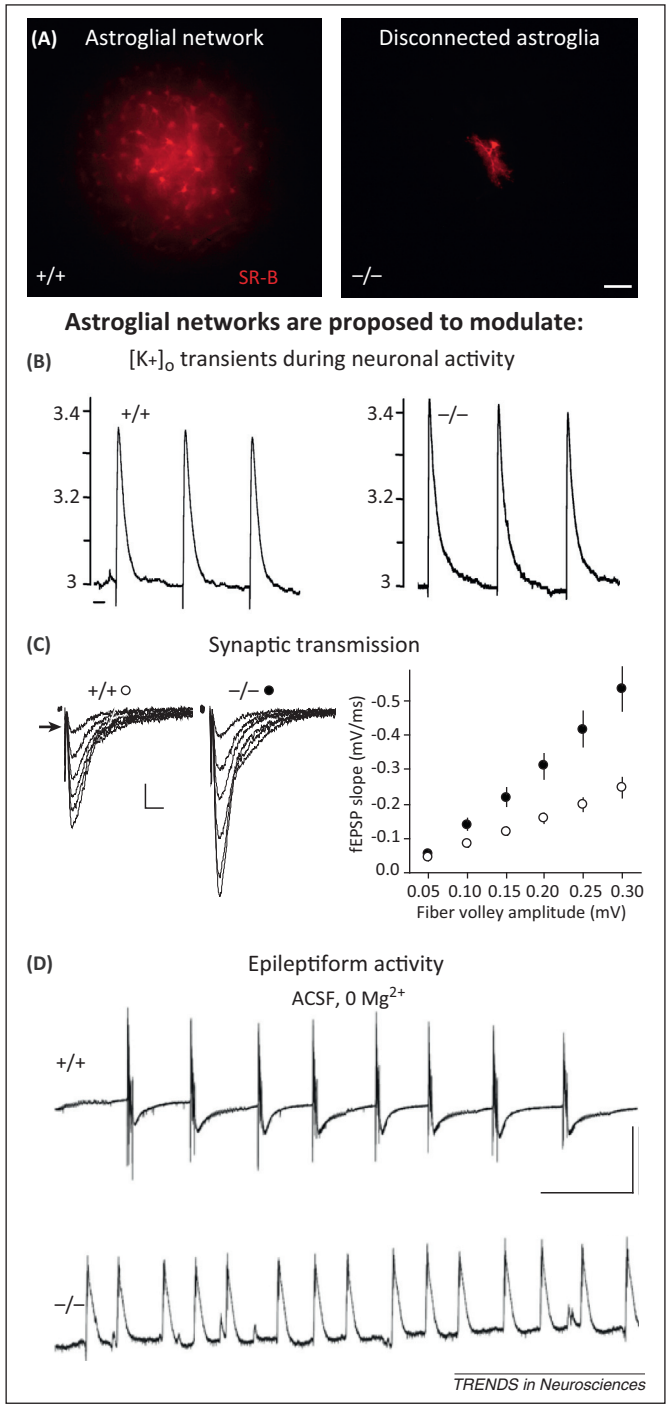


Figure 2. Contribution of astroglial circuits to synaptic and neuronal network activity. **(A)** Deficiency in the GJ-forming subunits, Cx43 and Cx30, results in astroglial uncoupling as assessed by dye-coupling experiments. Astroglial GJ communication is visualized by the diffusion of sulforhodamine B (SR-B), initially dialyzed in a single astrocyte from wild type (+/+) and *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ (–/–) hippocampal slices [60]. Scale bar, 50 μm. **(B)** Increased extracellular potassium levels ($[K^+]_o$) induced by neuronal activity in *Cx30*$^{-/-}$ *Cx43*$^{-/-}$ (–/–) and wild type (+/+) hippocampal slices. Sample traces of $[K^+]_o$ elevations induced by three paired pulses (0.1 ms, 50 ms interval, 30 s interspike interval) at 100% stimulation intensity in wild type (middle panel) and *Cx30*$^{-/-}$ *Cx43*$^{-/-}$ (right panel) hippocampal slices [26]. Scale bar, 5 s. **(C)** Increase in basal excitatory synaptic transmission in *Cx30*$^{-/-}$ *Cx43*$^{-/-}$ (–/–) hippocampal slices, as assessed by input–output curves. As illustrated in the sample traces and the graph below, for each input (fiber volley, arrowed), the output (fEPSP) is increased compared to wild type mice (+/+) [60]. Scale bar, 0.2 mV, 10 ms. **(D)** Enhanced epileptiform activity in CA1 stratum pyramidale of *Cx30*$^{-/-}$ *Cx43*$^{-/-}$ mice. Epileptiform field potentials were induced by Mg^{2+} washout. Representative traces of wild type (+/+, top) and *Cx30*$^{-/-}$ *Cx43*$^{-/-}$ (–/–, bottom) are shown [26]. Scale bar 0.5 mV, 4 s. Adapted, with permission, from [60] (A,C) and [26] (B,D).

of high neuronal activity provoking strong energy demand, or in pathological conditions associated with impaired energy production, such as hypoglycemia. Alternatively, the potential role of astroglial metabolic networks in basal synaptic activity may be masked in these experiments because glucose was exogenously supplied to brain slices, thereby bypassing the astroglial metabolic pathway. Consequently, *in vivo* recordings are required to decipher whether both clearance of neuroactive substances and delivery of energy molecules by astroglial networks are key regulators of synaptic activity in physiological conditions.

Astroglial network ability to tune neuronal circuits

Astroglial networks, through coordinated uptake or release of neuroactive molecules, are also thought to modulate the activity of neuronal circuits.

Astroglial calcium signaling and neuronal ensemble coordination

Astroglial Ca^{2+} signaling has been suggested to contribute to neuronal circuit coordination involved in particular forms of synaptic plasticity and in physiological or pathological network activity. For instance, Ca^{2+} signaling in astroglial networks was recently hypothesized to underlie hippocampal heterosynaptic depression, a form of synaptic plasticity involved in learning and memory [62]. Indeed, astroglial network Ca^{2+} signaling, induced by GABA release from interneurons, was proposed to spread neuronal information beyond the directly activated synapses by releasing ATP which, subsequently converted into adenosine, inhibits glutamate release in distal presynaptic elements [62,63]. Astroglial Ca^{2+} signaling was also recently suggested to synchronize neuronal ensembles during cortical UP states, likely via purinergic signaling, although the precise underlying mechanism remains unclear [64]. Finally, artificially induced Ca^{2+} signaling in astroglial networks by IP3 infusion into single, but connected astrocytes, can trigger glutamate release that is capable of producing neuron depolarization and epileptiform bursts [65].

Altogether these data, all obtained by infusion of molecules into astroglial networks inducing either Ca^{2+} chelation or release (Table 1), suggest that coordinated gliotransmitter release mediated by astroglial network Ca^{2+} signaling synchronizes physiological and pathological neuronal network activity (Figure 4). However, it is experimentally and conceptually difficult to differentiate between the effects of individual astrocytes versus GJ-mediated astroglial networks. Indeed, regulation of neuronal activity by infusion of molecules in a single but connected astrocyte might not be related to the astroglial network, but instead to the activity of individual astrocytes that are known to control thousands of synapses within their own domain. Thus the involvement of Ca^{2+} signaling in astroglial networks remains to be directly demonstrated by alternative approaches such as the specific inactivation of GJ channels (Box 1).

Role of metabolic support and K^+ homeostasis in aberrant network activity

Astroglial networks, through activity-dependent delivery of energy metabolites to neurons, can also sustain epileptiform activity. Such activity, although being inhibited by

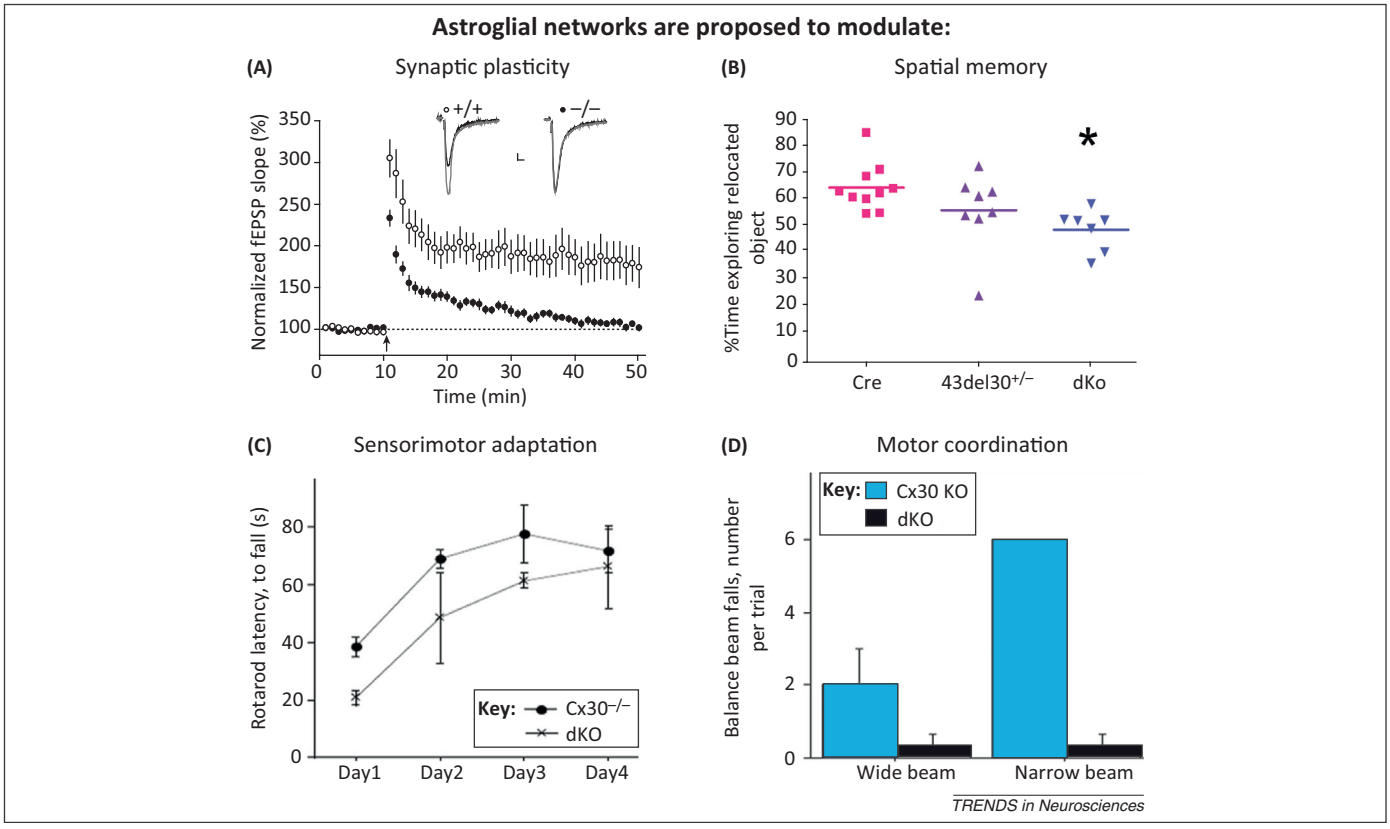


Figure 3. Involvement of astroglial networks in synaptic plasticity, spatial learning, and sensorimotor performance. **(A)** Long-term synaptic plasticity in the hippocampus is altered in the absence of astroglial networking. LTP, induced by two 100 Hz tetani separated by 20 s (arrow), is abolished in hippocampal slices from *Cx30*$^{-/-}$ *Cx43*$^{-/-}$ mice (–/–). Representative traces of averaged fEPSP recordings in slices before (black) and 40–50 min after tetanic stimulation of Schaffer collaterals (grey) are shown above the graph [60]. Scale bar, 0.05 mV, 10 ms. **(B–D)** Behavioral impairments in single knockout (KO) and double knockout *Cx30*$^{-/-}$ *Cx43*$^{-/-}$ mice (dKO). dKO mice, compared to mGFAP–Cre mice, fail to explore preferentially a relocated object, indicating impaired spatial memory (43del30$^{+/-}$ denotes *Cx43*$^{fl/fl}$ GFAP–Cre *Cx30*$^{+/-}$ mice) (B), fall off of the rotarod more quickly (C), and have more slips while crossing wide and narrow balance beams than *Cx30*$^{-/-}$ mice (D), indicating impaired motor coordination [51]. Adapted, with permission, from [60] (A) and [51] (B–D).

extracellular glucose deprivation, was indeed partially restored by selective glucose delivery into astroglial networks [19]. By contrast, using mice deficient for both astroglial Cx30 and Cx43, astrocytic networks were proposed to increase the threshold for the generation of epileptiform discharges (Figure 2D) [26,61]. Similarly, using astrocyte-targeted *Cx43* knockout mice, astrocytic networks were suggested to decrease *in vivo* spreading depression, a wave of depolarization followed by extensive neuronal inactivation, which is associated with epileptifom activity and migraine [52]. Such effects of astroglial networks are likely attributable to their contribution to ion and neurotransmitter homeostasis, as detailed above [60], although this has not been directly demonstrated. As highlighted above, further *in vivo* work is needed to unravel which function of astroglial networks, metabolic support or extracellular homeostasis, predominates in the regulation of coordinated neuronal burst discharges in normal and pathological conditions.

Evidence for an astroglial network contribution to behavior

The relevance of astroglial networking for brain information processing is suggested by the altered behavior of mice devoid of astroglial GJ communication (*Cx30*$^{-/-}$ *Cx43*$^{fl/fl}$ GFAP–Cre) (Tables 1 and 2; Box 1).

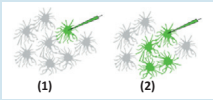
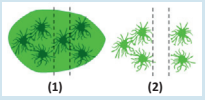
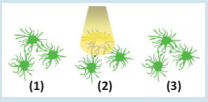
Spatial memory

Although these mice display normal exploratory behavior, they fail to preferentially explore a relocated object (Figure 3B), indicating impaired hippocampal-dependent spatial working memory [51]. Such deficits may specifically relate to Cx30 deficiency because *Cx30*$^{-/-}$ mice also show altered exploratory activity in novel environments [49], in contrast to astrocyte-targeted *Cx43*$^{-/-}$ mice [50]. Because astroglial networks are crucial for the temporal and spatial restriction of extracellular K^+ and glutamate [26,60,61], they might directly regulate the occurrence of hippocampal high-frequency oscillations related to exploratory behavior [66]. Consistent with this idea, intra-hippocampal injections of KCl also result in exploratory behavioral impairments [67]. Thus, investigating endogenous neuronal network oscillation patterns in mice devoid of astroglial network communication is of particular interest (Box 2).

Motor coordination

Additionally, *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice show defects in motor coordination and sensorimotor adaptation, as assessed using rotarod and balance beam assays (Figure 3C,D) [51]. Moreover, these deficits are most likely underestimated because in this study control mice were not wild type but were instead mice deficient for Cx30 and with floxed *Cx43* alleles, and these mice already display severe impairment in astroglial GJ coupling. Motor coordination

Table 1. Tools to study astroglial networks[a]

Study target	Technique/tool[b]	Description	Useful for	Advantages	Disadvantages
Network connectivity	Dye coupling		Visualizing the extent of astroglial networks for various inactive and bioactive fluorescent molecules [14]	Assesses specifically the connectivity of the targeted cell	Invasive (cytoplasm dialysis) Assesses the connectivity of only one cell Technically demanding, especially in adult animals
	Scrape-loading		Screening for functional GJs in cell culture [96]	Simple and fast assay Assessment of the overall connectivity of cells Potentially also usable in slices [97]	Invasive (scrape) Mostly adapted to cell culture
	Fluorescence recovery after photobleaching (FRAP)		Monitoring GJ connectivity of one cell in real time [98]	Adapted to various types of preparation (culture, slices, *in vivo*) in young and adult animals	Invasive (high level of free radicals in the targeted cell)
	Electrical coupling		Investigating ionic coupling in two cells of interest [99]	Online and sensitive assay for ionic coupling between isolated pairs of connected cells in culture	Not adapted to highly connected cells owing to the incomplete space-clamp and high access resistance which bias conductance measurements Technically demanding
Impact of network connectivity	GJ inhibitors	Carbenoxolone, octanol, halothane, etc [91]	Investigating the occurrence of GJ coupling	Easy to use	Unspecific block of Cx hemichannels and voltage-gated channels; direct alteration of neuronal activity [100]
	Small interfering RNAs	Cx43 siRNA [101]	Selective spatial and temporal knockdown of specific Cxs Targeted knockdown of any gene of interest in an astroglial network [102]	Selectivity Spatial and temporal control of gene knockdown	Only local knockdown (depending on transfection/infection efficiency) Off-target effects [91]
	Knockout mouse models	*Cx43*$^{fl/fl}$ GFAP–Cre [52,93], *Cx30*$^{-/-}$ [90], *Cx43*$^{fl/fl}$ *Cx30*$^{-/-}$ GFAP–Cre [26,51,60], *Cx43*$^{fl/fl}$ S100β–Cre [68], *Cx30*^{T5M} [92] for full list see [91]	Studying the impact on neuronal functions [19,26,49–51,60,61,68,74]	Investigation *in vivo* and in acute brain slices is possible	Possible developmental effects [88,93,94] Irreversible
	Calcium chelators	BAPTA, EGTA (caged and uncaged)	Studying Ca^{2+}-dependent astroglial network gliotransmission [62,64]	Reveals functional neuronal domains modulated by specific astroglial networks [62,64]	Impact on all Ca^{2+}-dependent intracellular pathways [6,7]
	GJ-permeable molecules	Ca^{2+}, IP3, glutamate, GABA, glucose (caged and uncaged)	Triggering gliotransmission [19,65,81,103,104]	Allows investigation of the impact of astroglial network signaling on neuronal activity	Artificial concentration of infused substances, unspecific side-effects [6,7]

[a]Abbreviations: BAPTA, 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid; Cre, Cre-recombinase; Cx, Connexin; EGTA, ethylene glycol tetraacetic acid; fl, floxed; GFAP, glial fibrillary acidic protein; GJ, gap junctions; IP3, inositol 1,4,5-trisphosphate; Rec, recording electrode; T5M, substitution of a threonine by methionine at position 5 of the N terminus.

[b]Dye coupling: cell membrane-impermeable tracer molecules injected by patch-clamp in a single astrocyte (1) diffuse via GJs to all connected neighboring astrocytes (2). Scrape-loading: a scrape or scratch introduced in a cell monolayer or slice in the presence of a GJ permeable tracer (1) results in the cellular uptake of the dye around the scrape and the subsequent diffusion into neighboring GJ-connected cells (2). Fluorescence recovery after photobleaching (FRAP): after cell loading with a fluorescent dye (1) a targeted cell is photobleached by a laser beam (2) and the redistribution of bleached and unbleached dye molecules via GJs is monitored (3). Electrical coupling is assessed by dual patch-clamp of two cells of interest and by applying test pulses only to one cell while recording the subsequent membrane potential dynamics of the other cell, which will, if they are connected, reflect the test pulses introduced.

and learning are not altered by reduced Bergmann glial GJ coupling mediated by postnatal deletion of *Cx43* in the cerebellum, a brain area directly involved in motor performance [68]. Thus, the motor deficits are most likely related to the myelin pathology observed in adult *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice [51] (Box 1). Strikingly, conditional *Cx43*$^{-/-}$ mice targeting astrocytes from all brain regions also show altered motor capacities with impaired rotarod performance [50], but increased locomotor and exploratory activity in novel environments [50,52], in contrast to *Cx30*$^{-/-}$ mice [49].

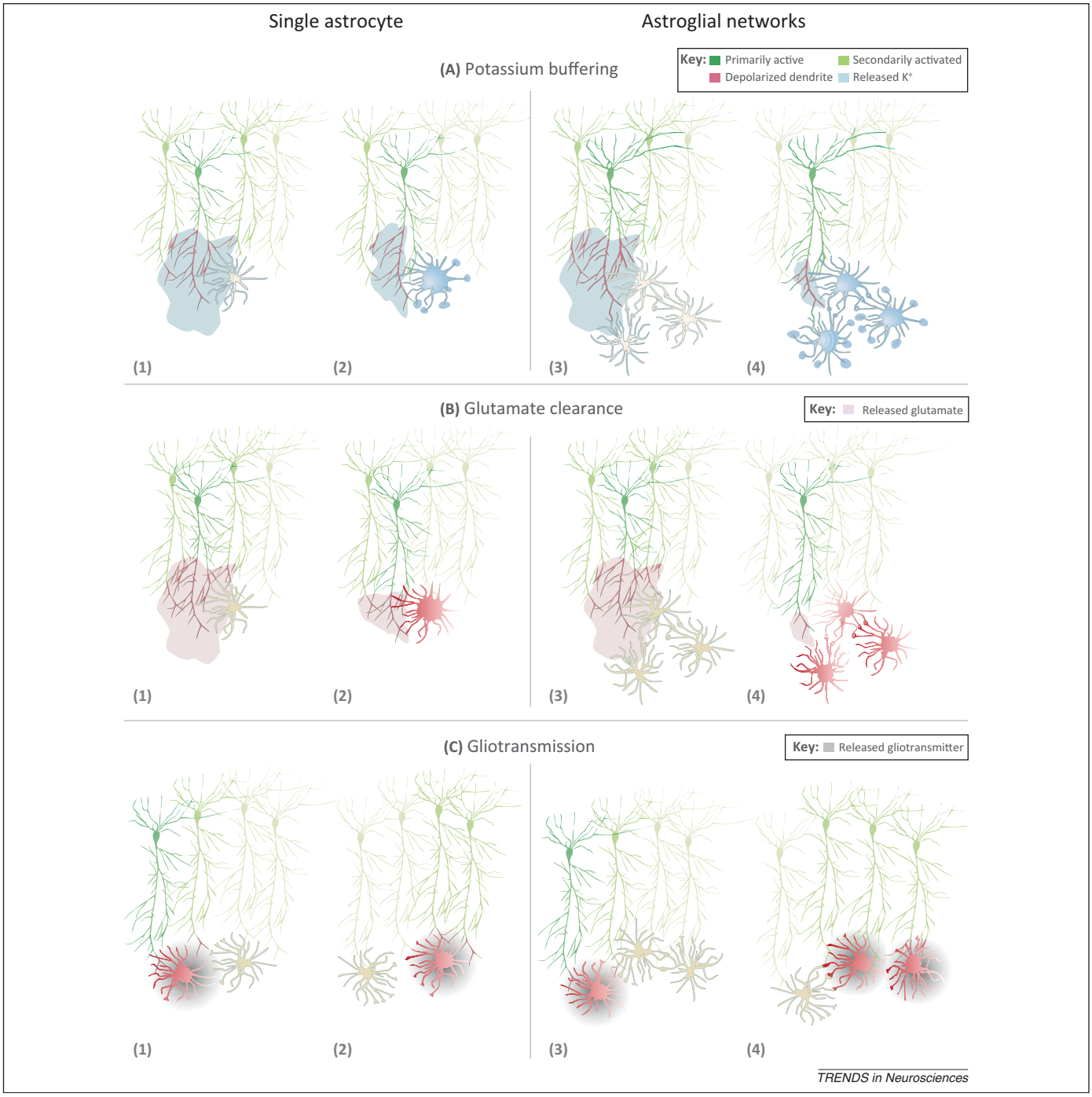


Figure 4. Comparison of proposed functional effects of single astrocytes versus astroglial networks on neuronal activity. Modulation of neurotransmission by astroglial K^+ buffering **(A)**, glutamate clearance **(B)**, and gliotransmission **(C)**. Illustration of the role of a single astrocyte (left panels) versus astroglial networks (right panels). Sequential schematics depicting interactions between pyramidal neurons (green) and adjacent astrocytes (beige/blue/red). Neuronal activity results in the release of K^+ (A1 and A3) and/or glutamate (B1 and B3) which can diffuse in the extracellular space and activate adjacent primarily inactive neurons. Astrocytes can buffer K^+ (A2) and clear up glutamate (B2), which limits nonspecific activation of neighboring neurons. However, astroglial networking further facilitates K^+ buffering and prevents nonspecific activation of adjacent neurons (A4) by enabling the redistribution of K^+ to adjacent connected astrocytes and by preventing prolonged reduction of the extracellular space volume due to astrocytic volume changes following K^+ uptake. Similarly, glutamate clearance is facilitated by astroglial networking (B4), and thus restricts nonspecific background activity. Neuronal activity can also trigger gliotransmitter release (such as glutamate, ATP, D-serine) in an adjacent astrocyte (C1), which activates neighboring neurons that were not initially recruited and can diffuse to adjacent astrocytes triggering subsequent gliotransmission (C2). Astroglial networking might enable the spread of gliotransmitter release from the initially activated astrocyte to the GJ-connected cells due to intercellular trafficking of molecules (ions, second messengers) (C3). Thus astroglial networking might coordinate neuronal networks by activating distal neurons (C4).

Anxiety, stress, and pain

Cx30$^{-/-}$ mice also display anxiogenic behavior and higher emotionality in novel environments [49], in contrast to astrocyte-targeted *Cx43*$^{-/-}$ mice, which were suggested to exhibit anxiolytic behavior [50]. Interestingly, human brains of patients with major depression [69] and of suicide completers [70] show reduced expression of *Cx30*. The link between astroglial networks and emotion is still unclear, but may rely on astroglial receptors and peptides involved in anxiety behaviors, such as benzodiazepine or serotonin

Table 2. Summary of the impact of astroglial networks on neuronal information processing[a]

Effect shown/effect proposed	Proposed mechanism	Experimental system and tools employed	Refs
Synapses			
↓ Excitatory synaptic transmission ↓ Neuronal excitability ↓ Release probability ↓ Silent synapses ↓ Postsynaptic AMPAR density ↑ LTP ↓ LTD	Facilitation of extracellular glutamate and K^+ removal during synaptic activity through increased astroglial clearance rate and extracellular space volume regulation	Acute hippocampal slices from *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice	[60]
↑ Excitatory synaptic transmission under hypoglycemic conditions	Metabolic support through lactate delivery	Acute hippocampal slices from *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice and infusion of energy metabolites into astrocytes	[19]
↓ Epileptiform activity	Maintenance of low extracellular K^+ and glutamate levels	Acute hippocampal slices from *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice	[26,61]
↑ Epileptiform activity	Metabolic support through glucose delivery	Acute hippocampal slices from *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice and infusion of energy metabolites into astrocytes	[19]
Circuits			
↑ Epileptiform activity	Astroglial network Ca^+-induced gliotransmission (glutamate)	IP3 infusion into a GJ-connected astrocyte to trigger ↑$[Ca^+]_i$ in acute hippocampal slices	[65]
↑ Heterosynaptic depression	Astroglial network Ca^+-induced gliotransmission (ATP)	Chelation of astroglial $[Ca^+]_i$ in acute hippocampal slices	[62]
↑ Neuronal synchrony during cortical UP states	Astroglial network Ca^+-induced gliotransmission (likely ATP)	Chelation of astroglial $[Ca^+]_i$ in acute neocortical slices	[64]
↓ Spreading depression	Facilitation of extracellular glutamate and K^+ removal through efficient astroglial glutamate clearance and K^+ buffering	Acute hippocampal slices from *Cx43*$^{-/-}$ mice	[52]
Behavior			
↑ Spatial working memory	Modulation of high-frequency oscillations through temporal and spatial restriction of extracellular K^+ and glutamate	*Cx43*$^{-/-}$ *Cx30*$^{-/-}$ and *Cx30*$^{-/-}$ mice	[49,51]
↑ Motor coordination and sensorimotor adaptation	K^+ buffering on myelinated axons by the heterotypic GJ network of astrocytes and oligodendrocytes	*Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice	[51]
↓ Anxiety	Astroglial network Ca^+-induced gliotransmission through activation of astrocyte benzodiazepine or serotonin receptors	*Cx30*$^{-/-}$ mice	[49,70,105]
↑ Pain perception after spinal cord injury	Astroglial network Ca^+-induced gliotransmission (proinflammatory cytokines and pronociceptive substances)	*Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice	[74]

[a]Abbreviations: ATP, adenosine-triphosphate; Cx, Connexin; $[Ca^+]_i$, intracellular calcium concentration; *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice, *Cx43*$^{fl/fl}$ GFAP-Cre *Cx30*$^{-/-}$ mice; GLT, glutamate transporter; IP3, inositol 1,4,5-trisphosphate; K^+, potassium; LTD, long-term depression; LTP, long-term potentiation.

receptors, known to induce astroglial Ca^{2+} signaling [71] or S100β release [72], respectively. Additionally, Cx43 may be specifically involved in adaptative processes following stress exposure because a strong decrease in hippocampal Cx43 expression occurs in response to inescapable aversion situation [73]. Finally, *Cx43*$^{-/-}$ *Cx30*$^{-/-}$ mice display normal pain perception in baseline conditions, but do not show heat hyperalgesia or mechanical allodynia after spinal cord injury, in contrast to wild type and *Cx30*$^{-/-}$ mice [74]. This suggests that Cx43-mediated astroglial networks and/or hemichannels are involved in the development of chronic neuropathic pain. In particular, Ca^{2+} waves in astroglial networks induced by spinal cord injury might coordinate the release of pro-inflammatory cytokines and pro-nociceptive substances, such as glutamate, ATP, or prostaglandins, in trauma tissue.

Box 2. Outstanding questions

- What are the molecular determinants of GJ formation and targeting during development? Because astroglial coupling is restricted to anatomical and functional neuronal units in particular brain regions [10,12], specific signals likely coordinate astroglial network formation. Identification of Cx interaction partners, as well as promoters of GJ formation, should improve understanding of the underlying molecular mechanisms.
- Why do the extensively connected astrocytes have non-overlapping domains? Because this domain organization is lost in pathological situations linked to neuronal hyperexcitability [106], it will be interesting to ascertain whether such organization is necessary for the fine-tuning of neuronal activity.
- What is the timescale of the dynamic regulations of astroglial GJ coupling by endogenous molecules? Answering this question will reveal whether neurons can modulate astroglial GJ communication at the timescale of fast synaptic transmission (i.e., milliseconds).
- What is the moment-to-moment action of astroglial intercellular communication on neurotransmission? In addition to neuronal supply of energy metabolites and clearance of neuroactive molecules, a possible scenario might be the exchange of signaling molecules such as Ca^{2+} or IP3 in the network, which can activate distant and formerly inactive astrocytes to modulate adjacent synapses (Figure 4C). This might create glial-modulated neuronal units defined by the extent of astrocytic network communication.
- What are the mechanisms of astroglial network plasticity? In different physiological and pathological situations associated with changes in brain function, alterations in Cx expression have been observed [14,49–52,69,70,73,107]. Thus, it will be interesting to determine whether the associated changes in astroglial connectivity are a cause or a consequence of altered brain physiology.
- What are the specific properties and roles of each Cx-mediated astroglial network? Because Cx43 and Cx30 differ in their (i) biophysical properties and C-terminal domain, (ii) regulation by neuronal activity [12], and (iii) contribution to behavior [49,50,52], it is now necessary to determine whether each Cx confers specific features to astroglial networks, and to unravel the underlying differential regulations of neurotransmission.
- Does astroglial GJ coupling regulate physiological brain oscillatory rhythms? Because astroglial spatial K^+ buffering significantly contributes to extracellular K^+ transients, and oscillations strongly depend on membrane potential dynamics, astroglial network communication might play a role in brain oscillations.
- What are the properties and roles of astroglial networks *in vivo* during specific tasks? Although a few behavioral studies have been performed on knockout mice for astroglial Cxs [49–52], most data acquired on astroglial networks have been obtained in cell cultures or brain slices. Deciphering *in vivo* the dynamics of intercellular communication for bioactive molecules in astroglial networks and their involvement in brain performance is an important issue to address.

Key questions however remain about the GJ molecular substrates involved in behavior (Box 2). Indeed, data suggest that astroglial Cx43 and Cx30 surprisingly induce opposite exploratory and emotional behaviors, although they both contribute to ~50% of astroglial network communication [19]. However, astrocytes deficient for Cx43 show a compensational cortical increase in Cx30 [52], which may underlie the unexpected behavioral phenotype of $Cx43^{-/-}$ mice which contrasts in several aspects with that of $Cx30^{-/-}$ mice. Alternatively, Cx30- and Cx43-mediated astroglial networks might display differential properties in terms of intercellular communication for bioactive molecules, and this may result in divergent physiological functions. Therefore, the specific role of Cx43- or Cx30-mediated networks should be investigated in future studies (Box 2). Finally, GJ-independent functions of Cxs [23] may complicate the behavioral phenotype of the various Cx-deficient mice (Box 1).

What role do astroglial networks play in cognitive and neurological disorders?

Dual role of astroglial networks

Astroglial network communication, owing to its prominent role in regulating extracellular neurotransmitter and ion homeostasis, is predestined to play a crucial role in pathologies associated with enhanced release of ions and neurotransmitters. Indeed, released K^+ or glutamate can induce an activity-dependent plasticity of astrocytic networks [12,19]. This results in an increased connectivity, which subsequently potentiates astrocytic clearance capacities, as previously mentioned [60]. Such plasticity might help preserve synapse independence and protect from excitotoxicity, and thereby prevent altered information processing in brain disorders. With this in mind, the increased Cx expression and/or GJ coupling reported in some human epileptic patients and animal models of epilepsy [19,40,75–78] may be interpreted as an adaptative change to clear-up the excess neuroactive substances released in the extracellular space, and thus may limit the development of aberrant bursting activity. However, inconsistencies about Cx expression and astroglial GJ coupling in human and experimental epilepsy, probably due to differences in models [79], prevent the generalization of a protective role of astroglial networks in epilepsy.

In sharp contrast, astrocytic networks also exhibit pro-epileptic aspects. Indeed, neuronal hyperactivity during epileptic states creates an enhanced metabolic demand, which can be sustained, at least partially, by nutrient supply through astroglial networks [19]. In addition, intercellular Ca^{2+} waves in astroglial networks during epileptiform events [40] may also contribute to the generation of aberrant bursting by promoting neuronal synchronization through release of glutamate at extrasynaptic sites [80–83]. Thus, the overall role of astroglial GJ communication in epilepsy, and in other brain disorders associated with excitotoxicity, remains unclear.

Dysfunction of astroglial networks, the cause of brain disorders?

A variety of pathological situations, such as multiple sclerosis or infections, are associated with a reduction in Cx expression and astroglial coupling [14]. As mentioned above, disrupting astroglial networks alters the spatial and temporal precision of information processing by synapses, and may result ultimately in excitotoxicity. Accordingly, mice with reduced astrocytic networks display a more severe outcome in ischemia/reperfusion paradigms [84], enhanced stroke volume after middle cerebral artery occlusion [85], as well as accelerated spreading depression and motor impairments [50,52]. Furthermore, complete loss of astroglial network communication results in impaired sensorimotor and spatial memory, as well as reduced blood–brain barrier integrity and hypomyelination [51,86]. Thus, alterations in astroglial GJ coupling might

be an underlying cause of diverse brain disorders linked to imbalances in energy supply, ion homeostasis, or neurotransmitter clearance. Remarkably, altered expression of astroglial Cxs was reported in human brains of patients with psychiatric disorders including autism [87], major depression [69], and suicide completers [70]. This suggests that astroglial Cxs may be involved in cognitive functions and psychopathologies. Thus, Cxs and the underlying GJ communication in astrocytes might be interesting therapeutic targets to restore normal brain information processing.

Concluding remarks

The high connectivity of astrocytes mediated by Cx channels has long been recognized as one of their typical characteristics. However, the properties and roles of astroglial networks in brain information processing by integrated systems have only recently started to be addressed. Here we review data revealing the extensive, but selective, preferential and plastic nature of astroglial networks that is strongly regulated by neuronal activity. We also illustrate the dual roles for astroglial networks in regulating neurotransmission (i.e., sustaining or down-tuning neuronal activity through delivery of energy metabolites or clearance of neuroactive molecules, respectively). However, these apparent opposite roles at first sight most likely converge to endow neurons with an optimal state enabling them to process information with temporal and spatial accuracy. Indeed astroglial networks fuel metabolically active synapses and simultaneously preserve synapse independence by efficiently clearing up the released neuroactive molecules.

However, our understanding of the role of astroglial networks, especially at the behavioral level, remains limited. In particular, most studies have been performed in brain slices, thus raising the question of the real functions of astroglial networks *in vivo* during specific tasks. In addition, the acute role of dynamic astroglial GJ coupling is still unknown because no selective GJ channel blockers are yet available. Also, although the repertoire of molecules crossing GJ channels is theoretically huge, given their poor selectively, the absence of visualization of such molecules in the network prevents the discovery of novel unexpected roles. Finally, given that Cx functions include channel and non-channel functions, this research field needs new pharmacological and molecular tools specifically targeting Cx intercellular channels. Thus, the development of fluorescent bioactive molecules to identify novel pathways mediated by astroglial networks, and the generation of novel conditional mouse models, targeting selectively and temporally the GJ channel function of astroglial Cxs, are required to decipher the moment-to-moment action of astroglial networking on neuronal information processing, and to understand better brain functions and dysfunctions.

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