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# GABAergic interneuron to astrocyte signalling: a neglected form of cell communication in the brain

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GABAergic interneurons represent a minority of all cortical neurons and yet they efficiently control neural network activities in all brain areas. In parallel, glial cell astrocytes exert a broad control of brain tissue homeostasis and metabolism, modulate synaptic transmission and contribute to brain information processing in a dynamic interaction with neurons that is finely regulated in time and space. As most studies have focused on glutamatergic neurons and excitatory transmission, our knowledge of functional interactions between GABAergic interneurons and astrocytes is largely defective. Here, we critically discuss the currently available literature that hints at a potential relevance of this specific signalling in brain function. Astrocytes can respond to GABA through different mechanisms that include GABA receptors and transporters. GABA-activated astrocytes can, in turn, modulate local neuronal activity by releasing gliotransmitters including glutamate and ATP. In addition, astrocyte activation by different signals can modulate GABAergic neurotransmission. Full clarification of the reciprocal signalling between different GABAergic interneurons and astrocytes will improve our understanding of brain network complexity and has the potential to unveil novel therapeutic strategies for brain disorders.

## 1. Background

Our knowledge of how the brain computes incoming sensory signals and governs our cognitive and motor functions has faced an exponential increase in the last decades. This was made possible thanks to a number of technological advances in molecular biology, brain imaging and optogenetics. It is now clear that the complexity of brain activity relies on fast dynamic interactions between different cell types that are finely and constantly regulated in time and space. The greatest challenge for neuroscientists is represented by the speed, the number and the heterogeneity of cellular signals that relentlessly occur in our brain. Over the last years our understanding of the functional role of two heterogeneous cell populations, i.e. GABAergic interneurons and astrocytic glial cells, has been marked by a significant improvement. Whether and how these cell types interact with each other and what functional significance such a signalling may have in the brain network remain, however, largely undefined.

Astrocytes are the major class of glial cells in the brain and play essential roles in brain homeostasis and metabolism. They are coupled in a syncytium through gap junctions and exert a homeostatic control of the extracellular space by regulating the pH, the water content and the extracellular concentration of different neurotransmitters and ions. In addition, astrocytes supply neurons with nutrients, trophic factors, cytokines and neuromodulators [1,2], contributing to the neurovascular coupling mechanism [3,4] and to the defensive reaction to tissue insult. Only recently the role of astrocytes has been extended to functions that were once considered an exclusive domain of neurons, such as short- and long-term modulation of synaptic transmission. This has led to the concept of the

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tripartite synapse in which the astrocytic fine processes, together with the pre- and postsynaptic neuronal elements, actively participate in the regulation of information transfer and integrative process [5,6]. Indeed, astrocytes express a wide variety of receptors for neurotransmitters, neuromodulators, cytokines and trophic factors that are used by these cells to sense the synaptic release of neurotransmitters as well as other extracellular signals [7]. In response to these signals, astrocytes can release molecules that regulate fundamental events in brain function by acting on neurons, glia and vascular cells. Molecules such as glutamate, ATP, D-serine, cytokines and GABA have been collectively termed gliotransmitters as they are similar to neurotransmitters and target similar receptors activated by neurotransmitters. Gliotransmitters have been shown to exert distinct modulatory actions on synaptic activity and network excitability through different mechanisms [7,8]. Evidence for a $Ca^{2+}$-dependent exocytotic release mechanism has been provided for these gliotransmitters, but $Ca^{2+}$-independent mechanisms have also been identified and characterized [9–11], including efflux through plasma membrane ion channels [12]. While we cannot draw a definitive conclusion, it seems conceivable that gliotransmitters are released from astrocytes through $Ca^{2+}$-dependent and $Ca^{2+}$-independent not mutually exclusive mechanisms.

According to these studies, the brain can be conceived as a network of interactive neurons and astrocytes. This novel view is, however, mainly based on results from *in vitro* and *in vivo* studies that focused on glutamatergic synaptic transmission. These studies revealed that astrocytes respond to glutamate with metabotropic glutamate receptor (mGluR)-mediated $Ca^{2+}$ elevations and can signal back to neurons by releasing gliotransmitters. The excitatory amino acid glutamate is probably the most widely studied gliotransmitter. Glutamate of astrocytic origin was shown to potentiate excitatory synaptic transmission through presynaptic NMDA and mGlu receptor activation [13–15] and to favour neuronal synchronies by targeting postsynaptic NMDA receptors [16]. These are only some examples of the role of astrocytes in the modulation of neuronal activity (for review, see [7]). Whether the reciprocal signalling revealed at glutamatergic synapses can be extended to GABAergic synapses is unclear. Although several works reported that astrocytes are sensitive to GABA, the literature on this topic is very limited [17].

In the last decade, our knowledge of GABAergic interneuron physiology has grown exponentially, in parallel with that on the astrocytes. GABAergic interneurons are only one quarter of all neurons and yet their control of cortical excitability is crucial [18]. Their role is not only to inhibit glutamatergic pyramidal neurons and maintain in the neural network a proper excitatory/inhibitory balance, but also to coordinate and synchronize neuronal activity generating specific oscillations [19–24] that characterize neuronal network function in different brain regions [25]. Interneurons represent a very heterogeneous class of cells (for reviews, see [26–31]). They differ in membrane and firing properties, in morphology and in biological markers expressed. This great heterogeneity allows a division of the labour that is now intensively investigated. For example, somatostatin (Sst) positive Martinotti cells preferentially target pyramidal neuron distal dendrites to control synaptic integration and plasticity [32–34]. In parallel, parvalbumin (Pv) expressing basket and chandelier cells innervate proximal dendrites, soma and the axonal initial segment in pyramidal neurons, exerting an efficient control of the firing discharge in these cells that guarantees temporal precision and synchronization at various frequencies [22,35]. Reciprocal inhibition between different interneuronal subtypes and autaptic self-inhibition are two important aspects of interneuron physiology that have been addressed only recently [36,37]. The strong glutamatergic innervation of GABAergic interneurons allows a continuous and efficient feed-forward inhibition in response to physiological inputs, including sensory stimuli [38–41]. Notably, cortical interneurons, and especially Pv interneurons, receive a strong glutamatergic input. Accordingly, glutamatergic transmission generated either locally or at distant regions is invariably accompanied by a stimulation of GABA release, making inhibition and excitation *de facto* inseparable [18].

Given both the emerging modulatory role of astrocytes on neuronal network activities and the fast GABAergic response to local excitatory inputs, it is of interest to provide a framework that summarizes what we know about the reciprocal interactions between GABAergic interneurons and astrocytes. Currently available literature on astrocytic response to GABAergic signals refers mainly to two different measurable effects on astrocytes of GABA applications: a membrane depolarization and an intracellular $Ca^{2+}$ rise. Besides these functional effects, GABA significantly increases GFAP content and astrocytic branching [42], suggesting a GABA role on astrocytic maturation. In addition, a number of studies revealed that GABA inhibits proinflammatory cytokine release from astrocytes, suggesting an involvement of GABA signalling also in the modulation of inflammatory astrocytic response [43].

In our review, we will critically discuss currently available data that could help us to answer the following central questions: (i) Are astrocytes responsive to GABA signals? (ii) Do GABA-activated astrocytes signal back to either interneurons and/or principal neurons? (iii) Do astrocytes activated by non-GABAergic signals affect GABAergic transmission?

## 2. GABAergic interneuron signalling to astrocytes

### (a) GABA-mediated depolarization

The first evidence that astrocytes can sense GABA comes from electrophysiological experiments on cultured or acutely isolated astrocytes [44–47] and later on hippocampal, retinal and cerebellar slices [48–51]. These studies revealed that astrocytes express functional $GABA_A$ receptors that are similar in many, though not all, aspects to those expressed by neurons. The first difference is that activation of astrocytic $GABA_A$ receptors leads to a depolarizing current in mature astrocytes, as opposed to mature neurons, due to the $Na^+/K^+/Cl^-$ cotransporter (NKCC1) expression and activity that maintains a larger intracellular $[Cl^-]$ in astrocytes. The roles of GABA-mediated $Cl^-$ efflux and astrocytic depolarization are still under investigation. It was proposed that during intense GABAergic interneuron firing, $Cl^-$ efflux from astrocytes helps to maintain a certain $[Cl^-]_o$ level that could counteract $Cl^-$ entry into neurons [52]. Thus, GABA would act on astrocytes to ultimately buffer $[Cl^-]_o$ and preserve the inhibitory driving force of $Cl^-$ ions in neurons. A recent work supports this concept and shows that gap-junction coupling is necessary to maintain astrocytic $[Cl^-]_o$ buffering capacity [53]. The authors revealed that blockade of gap junctions during intense stimulation of GABAergic transmission to CA1 neurons

induced a collapse of the $Cl^-$ gradient in these neurons. While we have to keep in mind that neurons are more sensitive to internal than external [$Cl^-$] changes, the $Cl^-$ efflux from astrocytes may significantly contribute to the control of $[Cl^-]_o$ at the restricted extracellular space surrounding the GABAergic synapse.

Another difference of $GABA_A$-mediated currents in astrocytes compared to those in neurons is the effect of some allosteric modulators. In particular, the benzodiazepine site inverse agonist methyl-4-ethyl-6,7-dimethoxy-β-carboline-3-carboxylate (DMCM) acts in astrocytes, but not in neurons, as pure agonist by increasing $GABA_A$-mediated depolarizing currents [46,54]. This result hints at a different subunit composition of the $GABA_A$ receptor complex in astrocytes with respect to neurons.

### (b) GABA-mediated $Ca^{2+}$ response in astrocytes

The advent of $Ca^{2+}$ imaging technique revealed an unexpected responsiveness of astrocytes to several neurotransmitters and molecules [55,56]. It was thus found that GABA evokes astrocytic $Ca^{2+}$ events through different intracellular pathways mediated by both ionotropic $GABA_A$ and metabotropic $GABA_B$ receptors as well as GABA transporters (GATs). Some groups observed exclusively $GABA_A$-mediated responses that, by depolarizing the astrocytic membrane as reported above, activate voltage-sensitive $Ca^{2+}$ channels [47], while other groups reported GABA-evoked $Ca^{2+}$ events in astrocytes that were mediated exclusively by $GABA_B$ receptors [57,58]. Furthermore, other studies in cultured astrocytes [59] and rat hippocampal slices [60] described GABA-evoked astrocytic $Ca^{2+}$ oscillations mediated by both $GABA_A$ and $GABA_B$ receptor activation. In the latter work, the authors observed a conserved $GABA_A$-mediated response during development, while $GABA_B$-mediated response peaked during the second postnatal week and then progressively decreased. The mechanism of $GABA_B$-mediated $Ca^{2+}$ events was shown to involve G proteins and $Ca^{2+}$ release from internal stores [60,61]. It is unclear, however, which G protein is responsible for the $Ca^{2+}$ response, because $GABA_B$ receptors are known to be coupled to Gi/o proteins, at least in neurons [62], while $Ca^{2+}$ release from internal stores usually requires Gq protein activation. Further experiments are needed to address this important aspect of astrocytic physiology. Beside this mechanism, once GABA activates $Ca^{2+}$ oscillations in astrocytes it may, in turn, activate gliotransmission. A point of interest here is that GABA-induced $Ca^{2+}$ oscillations in astrocytes are comparable to those induced by glutamate or others excitatory transmitters. Given that $Ca^{2+}$ elevations represent a form of $Ca^{2+}$-based excitation in astrocytes, it turns out that in the neuron–astrocyte network an inhibitory GABA signal has the potential to become an excitatory signal through astrocyte activation. This leads to a number of questions: (i) Do GABA and glutamate trigger a similar $Ca^{2+}$ response in astrocytes? (ii) Do individual astrocytes respond to both neurotransmitters or do astrocyte subpopulations exist that respond exclusively to either GABA or glutamate? In the case that an individual astrocyte can respond to both neurotransmitters, what is the ultimate effect of a simultaneous activity of the two signal pathways? In other words, does this astrocyte integrate the signals, as was observed in hippocampal astrocytes activated by glutamate and acetylcholine [5,63]? Interestingly, Meier *et al.* [60] observed that challenging astrocytes with a subthreshold stimulation using a $GABA_B$ agonist (baclofen) increased the $Ca^{2+}$ response to t-ACPD, i.e. an mGluR agonist. This observation suggests that a spatial and temporal summation of different receptor-mediated $Ca^{2+}$ signals can occur in astrocytes. For example, GABAergic and glutamatergic inputs can occur very closely in time, allowing a summation of the intracellular $Ca^{2+}$ response that may evoke gliotransmission or the modulation of important astrocytic functions.

In slices of the olfactory bulb, a region where astrocytes enwrap mainly GABAergic synapses, GATs have been shown to indirectly activate $Ca^{2+}$ events in astrocytes from P2–7 mice [64]. In particular, Doengi *et al.* [64] found that GABA-evoked astrocytic $Ca^{2+}$ events are fully prevented by GAT blockers, but only partially by $GABA_B$ antagonists and they are not affected at all by $GABA_A$ antagonists. GAT activation leads to intracellular $Na^+$ increase (that is cotransported with GABA), which indirectly inhibits the $Na^+/Ca^{2+}$ exchanger. The proposed mechanism is that the consequent $Ca^{2+}$ increase is sufficient to induce $Ca^{2+}$ release from internal stores in an inositol triphosphate-dependent manner. The authors provide also evidence that GABA mediates a blood vessel constriction that is blocked by GAT inhibitors, providing evidence for an important functional effect of this phenomenon.

## 3. GABA-activated astrocytes signal back to the neural network

What is the functional effect of GABA astrocyte activation on the neuronal network? A main consequence of cytosolic $Ca^{2+}$ elevations in astrocytes is the release of gliotransmitters. The frequency, amplitude, kinetics and spatial extension of the $Ca^{2+}$ change appear to be crucial factors that determine the responsiveness of astrocytes to neuronal signals. The functional consequences of GABA signalling to astrocytes are, however, undefined and it is not conclusively proved that GABA-mediated $Ca^{2+}$ elevations trigger the release of gliotransmitters. Furthermore, both the nature and the ultimate modulatory action on the neuronal network of gliotransmitters released from astrocytes upon their activation by inhibitory signals are also unclear. Our current understanding of astrocyte-mediated modulation at inhibitory synapses is, thus, very defective, at least with respect to our knowledge of the gliotransmitter effects at excitatory synapses. Considering this important issue, we should not, however, under-evaluate a few studies that provided important clues for the potential of GABA-activated astrocytes to regulate network activities. For example, in hippocampal slices, Kang *et al.* [57] reported that stimulation of an intense interneuron firing induced $Ca^{2+}$ oscillations in astrocytes and, in parallel, increased the probability of evoked unitary inhibitory postsynaptic currents (IPSCs). A similar increase was observed after a direct activation of individual astrocytes by mechanical stimuli or by stimulation with the selective $GABA_B$ receptor agonist baclofen. Notably, the effect was blocked by inserting the $Ca^{2+}$ chelator BAPTA in the astrocytic syncytium. The authors suggested that GABA activation of astrocytes leads to a release of glutamate onto the presynaptic elements that increases inhibitory synaptic transmission onto pyramidal neurons. This study provided the first evidence that synaptically released GABA activates astrocytes that, in turn, modulate synaptic activity in the hippocampus. A role of GABA-activated astrocytes in modulating heterosynaptic depression in the hippocampus has been revealed by Serrano *et al.* [58]. These authors found that tetanization- or NMDA-induced heterosynaptic depression

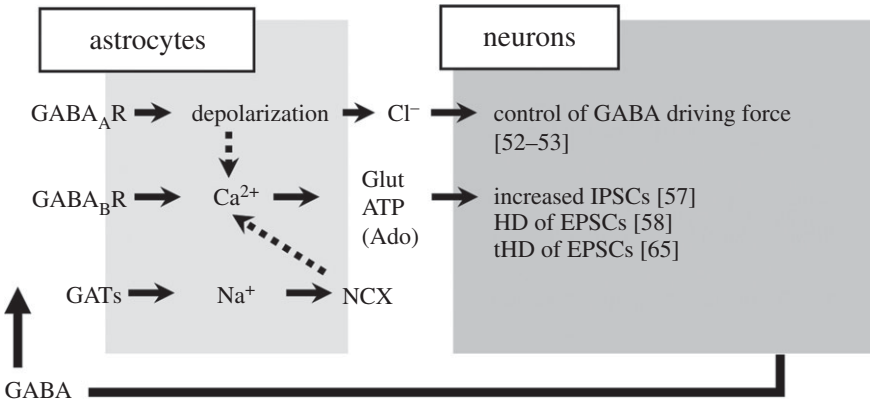


**Figure 1.** GABA-activated astrocytes modulate neuronal activity. Summary of the astrocytic response to GABA and the consequent signalling to neurons; dotted arrows refer to contradictory observations or limited brain regions (see §§1 and 2). Glut, glutamate; Ado, adenosine; HD, heterosynaptic depression; tHD, transient heterosynaptic depression; IPSCs, inhibitory postsynaptic currents; EPSCs, excitatory postsynaptic currents; GATs, GABA transporters; NCX, $Na^+$ $Ca^{2+}$ exchanger.

also evoked astrocytic $Ca^{2+}$ oscillations, and that inhibition of astrocytic $Ca^{2+}$ responses by BAPTA abolished the heterosynaptic depression indicating that astrocyte activation was necessary for this form of synaptic plasticity. More relevant for the issue that we discuss here, the authors reported that activation of astrocytes was dependent on $GABA_B$ receptors because $GABA_B$ receptor blockade prevented both $Ca^{2+}$ responses in astrocytes and heterosynaptic depression in neurons, whereas stimulation with baclofen evoked both events. Finally, the authors found that ATP released from GABA-activated astrocytes was rapidly degraded to adenosine that inhibited glutamate release through presynaptic A1 receptor activation. The crucial role of astrocytes in mediating another form of hippocampal plasticity, i.e. the transient heterosynaptic depression, has been demonstrated by Andersson *et al*. [65]. In this case, the authors suggested that GABA-activated astrocytes release glutamate that induces this form of synaptic depression by acting on group II/III mGluRs.

Although these studies provide compelling evidence for a contribution of GABA-activated astrocytes in the modulation of synaptic activity, our knowledge of GABA-mediated gliotransmission remains unsatisfactory. It is likely that future studies will unveil that GABA-activated astrocytes are involved in other forms of synaptic modulations (figure 1).

## 4. Astrocytes activated by non-GABAergic signals modulate GABAergic transmission

Astrocytes can modulate GABAergic transmission through different mechanisms. GATs expressed on astrocytic and neuronal membrane have an important functional significance in the control of the extracellular GABA concentration that sets the tone of GABAergic inhibition in local neural circuits [66–68]. In the neocortex, GAT-1 and GAT-3 are the most abundantly expressed, with GAT-1 mainly expressed in GABAergic interneurons [69,70] and less on astrocytes, while GAT-3 is located exclusively on astrocytic processes in the proximity of synapses [71]. Recent works show that astrocytic GAT-3 is important to control extracellular [GABA] also *in vivo*, particularly during periods of intense neuronal activity [72], and to shape $GABA_B$ postsynaptic currents in the thalamus [73]. Notably, in this brain region, GAT-1 is selectively expressed on astrocytes [74,75], as opposed to other regions, and its role is crucial in regulating GABA tonic inhibition and thalamocortical seizures that characterize absence epilepsy [76,77].

The efficacy of inhibitory synapses in the hippocampus was recently revealed to be finely regulated by the dynamics of GAT-3 expression in astrocytes [78]. These authors found that transient receptor potential A1 channels (TRPA1) mediate frequent and localized $Ca^{2+}$ events in astrocytes that contribute to set the resting $[Ca^{2+}]_i$. Blocking TRPA1 channels reduced resting $[Ca^{2+}]_i$ and the $Ca^{2+}$-dependent membrane insertion of GAT-3. The consequent increase in extracellular GABA concentration desensitized $GABA_A$ receptors, leading to a reduction of IPSCs on hippocampal interneurons. This work shows the importance of astrocytic TRPA1 channels in regulating the inhibitory signalling in the hippocampus. It also shows the importance of resting $Ca^{2+}$ levels for effective GAT trafficking to the cell membrane.

Under certain conditions, GATs can reverse their function to release GABA in the extracellular space. In two separate studies, Heja *et al*. [79,80] found that astrocytes convert excitation to tonic inhibition of neurons in hippocampal slices and in *in vivo* experiments. In the presence of reduced $Mg^{2+}$ to increase network activity, they show that glutamate uptake is coupled to the reversal of GAT2/GAT3 that induces a GABA tonic current. The proposed mechanism is that in the presence of elevated glutamatergic activity, excitatory amino acid transporters that co-transport $Na^+$, as well as glutamate, inside the cell (1 glutamate$^-$, 3$Na^+$ and 1 $H^+$ inside/1 $K^+$ outside) lead to an intracellular $Na^+$ increase. As also GATs use the $Na^+$ gradient for uptake of GABA, this intracellular $Na^+$ increase may be sufficient to reverse GAT transport and start extrusion of GABA. These studies provide evidence that this glutamate-induced GABA release via EAAT/GAT transporters is also present *in vivo*. This mechanism represents a compensatory feedback that may be protective under excessive excitatory events. Indeed, the authors show that epileptic-like discharges in slices are prolonged in the presence of GAT blockers, suggesting that their activity during epileptic-like activity is reversed and increases network inhibition [80].

Several recent works showed that astrocytes not only control $[GABA]_o$ through GATs but they can also directly affect $[GABA]_o$ by releasing GABA as a gliotransmitter. GABA in the brain is mainly synthesized in neurons by glutamic acid decarboxylases activity (GAD-65 and -67) [81]. Astrocytic GABA content is believed to be mainly due to GATs that capture the neurotransmitter from the external space. However, several studies have reported an astrocytic expression of

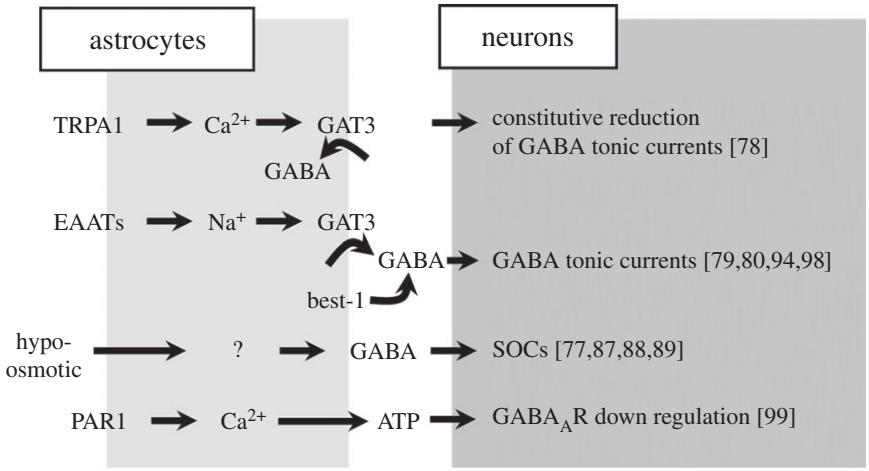


**Figure 2.** Astrocytes activated by different signals modulate GABAergic transmission. Summary of the astrocytic modulation of GABAergic transmission in response to different signals (see §3). TRPA1, transient receptor potential A1; GAT3, GABA transporter type 3; EAATs, excitatory amino acid transporters; Best-1, bestrophin-1; SOCs, slow outward currents; PAR1, protease-activated receptor type-1.

GAD-67 and -65 [43,82,83]. For example, Lee *et al.* [43] found in human adult tissue a GAD-65 expression in astrocytes that was comparable to that in inhibitory interneurons. In addition, GABA can also be synthesized in astrocytes starting from the polyamine putrescine [80,84–86]. In astrocytes, GABA can be degraded by GABA-α-ketoglutaric acid aminotransferase (GABA-T) to glutamine, which is then released and subsequently captured by neurons. Most relevant to the focus of this review is the finding that GABA itself is released by astrocytes in many brain regions, including the olfactory bulb [87], the ventro-basal thalamus [77,88] and the hippocampus [89]. In the olfactory bulb, Kozlov and co-workers reported the first evidence that astrocytic GABA can induce slow outward currents (SOCs) in neurons. SOCs share common features with the slow inward currents (SICs) evoked by astrocytic glutamate that were observed in neurons from different brain regions [16,90–93]. Similarly to SICs, SOCs are tetrodotoxin insensitive, occur at low frequency and have significantly slower rise and decay time with respect to synaptic currents. Notably, astrocytes in the olfactory bulb were able to release both GABA and glutamate to inhibit or activate synchronously groups of specific cell populations, revealing a complex astrocytic modulation of local network activity [87]. A similar dual action of astrocytes was observed in the hippocampus [89]. All in all, these results raise a number of questions on the ultimate effects of astrocytic signalling in local networks. Do glutamatergic SICs and GABAergic SOCs derive from the same activated astrocytes or do they come from different ones? As to the GABA and glutamate release, Le Meur *et al.* [89] suggest that different astrocytes were probably involved because simultaneous SICs and SOCs were extremely rare. It is possible, however, that the same astrocyte may release both GABA and glutamate, but from distinct releasing sites in contact with different synapses. The mechanism of astrocytic GABA release is unclear. The fact that both in ventro-basal thalamus and hippocampal slices SOCs were increased in number upon hypo-osmotic challenge suggests a release mechanism sensitive to cell volume [88,89].

A different form of GABA release has been described in astrocytes from cerebellar slices [94,95]. In this region, GABA appears to be released by astrocytes through the bestrophin-1 channel, a large channel that may also allow glutamate efflux [96,97]. This astrocytic GABA release may contribute to GABA tonic inhibition of neurons that is particularly relevant in the cerebellum. As tonic inhibition can be crucial for neuronal excitability, this work opens a novel aspect in the astrocytic control of local circuit activity. Notably, GABA release has been described also in human astrocytes [98].

A recent study revealed an additional mechanism by which astrocytes regulate GABAergic inhibitory transmission. Lalo *et al.* [99] reported that exocytosis of ATP from astrocytes modulated both phasic and tonic inhibition in somato-sensory cortex. The authors showed that $Ca^{2+}$ elevations in astrocytes evoked by TFFLR, a peptide agonist of the protease-activated receptor 1, lead to a vesicular ATP release that evoked P2X receptor-mediated currents in neurons. $Ca^{2+}$ entry in neurons through P2X receptor openings lead, in turn, to a phosphorylation-dependent downregulation of $GABA_A$ receptors. In a transgenic mouse with impaired astrocytic ATP release, i.e. the dn-SNARE mouse, the IPSCs and tonic GABA currents were significantly larger. These data show that a $Ca^{2+}$-dependent release of ATP from astrocytes can affect the responsiveness of neurons to synaptic and extrasynaptic GABAergic signals.

Finally, astrocytes can modulate inhibition in local circuits through a direct action on GABAergic interneurons. For example, by regulating, through the glutamate transporters, the occupancy of the mGluRs in oriens-lacunosum moleculare interneurons, astrocytes modulate the excitability of these hippocampal interneurons [100]. A $Ca^{2+}$-dependent release of glutamate has also been reported to activate presynaptic kainate receptors at GABAergic synapses onto inhibitory interneurons, ultimately decreasing inhibitory transmission in the hippocampus [101]. An opposing effect was described for another gliotransmitter, such as ATP, that increased inhibitory synaptic transmission in the hippocampus through activation of P2Y1 receptors in interneurons [102].

Although our understanding of how GABAergic interneurons and astrocytes communicate in the neuronal network is largely undefined, these few studies hint at a richness of different mechanisms by which astrocytes can modulate GABAergic inhibition in local circuits (figure 2).

## 5. Open questions and conclusion

A large variety of neuronal signals, including the neurotransmitter GABA, are now recognized to trigger intracellular $Ca^{2+}$ transients in astrocytes. Astrocytes are proposed to act

as space and time integrators that decode the information deriving from different neuronal signals into dynamic $Ca^{2+}$ signal changes that either remain spatially restricted to individual and multiple processes or recruit the entire astrocyte in a global $Ca^{2+}$ response [7]. Based on this hypothesis, a number of questions regarding a possible reciprocal signalling between GABAergic interneurons and astrocytes need to be answered. First, we need to understand whether GABA released from a given class of interneurons establishes with astrocytes a specific signalling or whether all the different classes of interneurons can similarly activate astrocytes. Second, do all astrocytes respond to GABA or do different astrocyte subpopulations respond selectively to either GABA or other neurotransmitters? Third, how does the GABA-mediated response integrate with other neurotransmitter-mediated responses? This is a crucial issue since glutamategic and GABAergic signalling, as reported above, are intimately linked. Finally, has the same astrocyte the potential to release GABA as well as other gliotransmitters? Technological advances are now providing new powerful tools to address these questions and help us to fully understand the role of neuron–astrocyte communication in the brain. For example, the existence of a specific GABAergic signalling pathway between different interneuron classes and astrocytes can be investigated in mice that express the light-gated cation channel channelrhodopsin-2 selectively in a distinct class of interneurons. Similarly, the use of novel genetically encoded $Ca^{2+}$ indicators will allow to study astrocytic $Ca^{2+}$ responses with unprecedented time and space resolution with respect to that achieved after bulk loading with classical fluorescence $Ca^{2+}$ indicators. This could also make it possible to study neuron–astrocyte crosstalk at fine astrocytic processes located at different subcellular sites, for example at dendritic versus somatic inhibitory synapses onto principal neurons.

Given the plethora of functions played by astrocytes in brain function, it is no surprise that their involvement in most neurological disorders is increasingly documented also from the very early stages of diseases such as epilepsy, Alzheimer's disease, Parkinson's disease, Hungtington's disease, amyotrophic lateral sclerosis, stroke and brain injury (for reviews, see [2,103–105]). Under pathological conditions, interneuron–astrocyte reciprocal interactions may also be affected. For example, it has been reported that astrocytes activated by an epileptogenic insult increase their synthetic machinery to produce neurosteroids, potent $GABA_A$ receptors modulators [106–108], that temporally prevent seizure generation and prolong the latent period in animal models of temporal lobe epilepsy [109].

In conclusion, we are only beginning to understand the dynamic interactions between distinct classes of GABAergic interneurons and astrocytes. Future studies are expected to greatly improve our knowledge in this field and have the potential to unveil novel mechanisms in brain physiology and pathology.

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