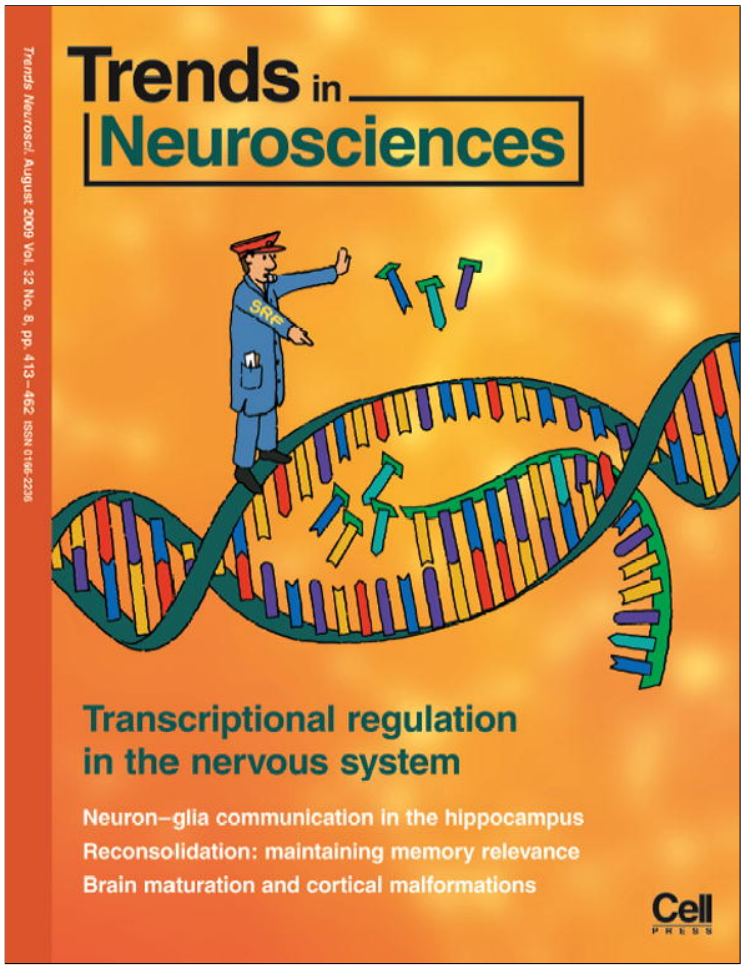

Review

# Tripartite synapses: astrocytes process and control synaptic information

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**The term 'tripartite synapse' refers to a concept in synaptic physiology based on the demonstration of the existence of bidirectional communication between astrocytes and neurons. Consistent with this concept, in addition to the classic 'bipartite' information flow between the pre- and postsynaptic neurons, astrocytes exchange information with the synaptic neuronal elements, responding to synaptic activity and, in turn, regulating synaptic transmission. Because recent evidence has demonstrated that astrocytes integrate and process synaptic information and control synaptic transmission and plasticity, astrocytes, being active partners in synaptic function, are cellular elements involved in the processing, transfer and storage of information by the nervous system. Consequently, in contrast to the classically accepted paradigm that brain function results exclusively from neuronal activity, there is an emerging view, which we review herein, in which brain function actually arises from the coordinated activity of a network comprising both neurons and glia.**

## Introduction

Ten years ago the term 'tripartite synapse' was proposed to conceptualize the evidence obtained by many laboratories during the 1990s that revealed the existence of bidirectional communication between neurons and astrocytes (Figure 1). It represents a new concept in synaptic physiology wherein, in addition to the information flow between the pre- and postsynaptic neurons, astrocytes exchange information with the synaptic neuronal elements, responding to synaptic activity and regulating synaptic transmission [1] (Figure 2). The biology of astrocyte–neuron interaction has emerged as a rapidly expanding field and has become one of the most exciting topics in current neuroscience that is changing our vision of the physiology of the nervous system. The classically accepted paradigm that brain function results exclusively from neuronal activity is being challenged by accumulating evidence suggesting that brain function might actually arise from the concerted activity of a neuron–glia network.

Here, we briefly summarize early evidence that led to the establishment of the concept of a tripartite synapse and then discuss more recent data regarding the properties and physiological consequences of the astrocyte $Ca^{2+}$ signal, which has a fundamental role in neuron–astrocyte communication as the cellular signal triggered by the neuronal activity and responsible for transmitter release from astrocytes and the consequent neuromodulation. Although astrocytes have important roles in key aspects of brain development and function, such as neuronal metabolism, synaptogenesis, homeostasis of the extracellular milieu, or cerebral microcirculation [2], we focus on the role of astrocytes in synaptic physiology, discussing data indicating that astrocytes integrate and process synaptic information and finally regulate synaptic transmission and plasticity through the release of gliotransmitters (i.e. transmitters released by glial cells implicated in rapid glial–neuron and glial–glial communication) [3].

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## $Ca^{2+}$-mediated cellular excitability of astrocytes

The astrocytic revolution in current neuroscience began in the early 1990s when pioneering studies used the fluorescence imaging techniques to monitor intracellular $Ca^{2+}$ levels in living astrocytes. Those studies revealed that cultured astrocytes display a form of excitability based on variations of the intracellular $Ca^{2+}$ concentration [4,5]. Until then, astrocytes had been considered as non-excitable cells because, unlike neurons, they do not show electrical excitability (e.g. see Refs [6–9]). Since these pioneering findings, subsequent studies performed in cultured cells, brain slices and, more recently, *in vivo* have firmly established the astrocyte excitability, which is manifested as elevations of cytosolic $Ca^{2+}$ mainly as a result of the mobilization of $Ca^{2+}$ stored in the endoplasmic reticulum. The elevated $Ca^{2+}$ then acts as a cellular signal [10]. Whereas neurons base their cellular excitability on electrical signals generated across the plasma membrane [11], astrocytes base their cellular excitability on variations of $Ca^{2+}$ concentration in the cytoplasm.

## Astrocyte $Ca^{2+}$ signal is controlled by synaptic activity

Astrocyte $Ca^{2+}$ elevations can occur spontaneously as intrinsic oscillations in the absence of neuronal activity [12–15], and they can also be triggered by neurotransmitters released during synaptic activity [10] (Table 1), which is of crucial importance because it indicates the existence of neuron-to-astrocyte communication (Figure 3a).

The synaptic control of the astrocyte $Ca^{2+}$ signal is based on the fact that astrocytes express a wide variety of functional neurotransmitter receptors. Many of these receptors are of metabotropic type, being associated with G proteins that, upon activation, stimulate phospholipase C and formation of inositol (1,4,5)-triphosphate (Ins(1,4,5)$P_3$), which increases the intracellular $Ca^{2+}$ concentration through the release of $Ca^{2+}$ from intracellular Ins(1,4,5)$P_3$-sensitive $Ca^{2+}$ stores [16–21]. Early studies using cultured cells showed that the astrocyte $Ca^{2+}$ signal can propagate to

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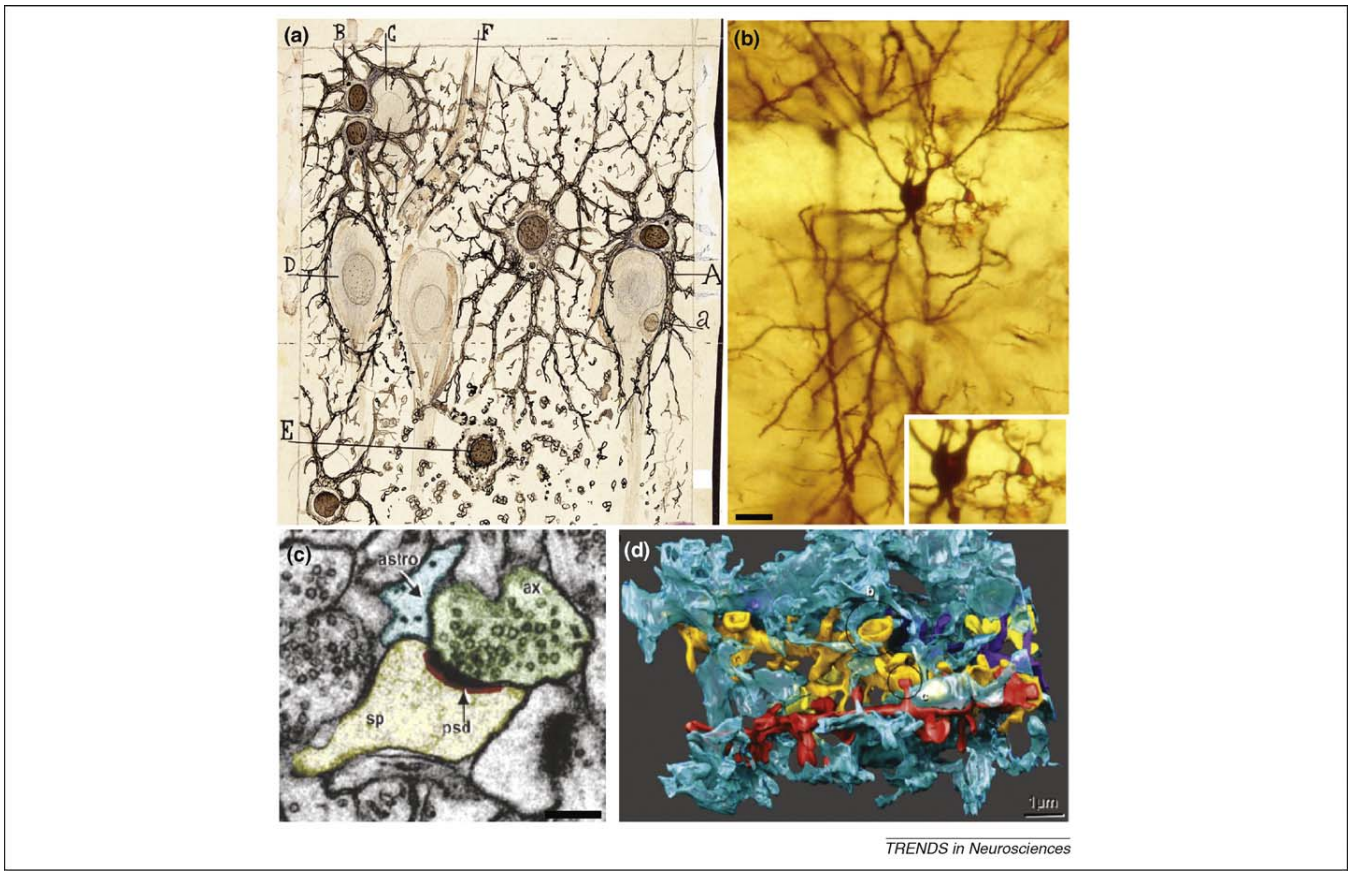


**Figure 1**. Views of the neuron–astrocyte interaction at the tripartite synapse. **(a)** Cajal's drawing showing 'neuroglia' of the pyramidal layer and stratum radiatum of the Ammon horn (from adult man autopsied three hours after death). Original labels: A, large astrocyte embracing a pyramidal neuron; B, twin astrocytes forming a nest around a cell, C, while one of them sends two branches forming another nest, D; E, cell with signs of 'autolysis'; F, capillary vessel. Reproduced from an original drawing, with permission of the Instituto Cajal [106]. **(b)** Neuron and astrocyte stained with the Golgi method from a rat hippocampus. Inset: astrocyte and neuronal somas. Image generously given by Dr Lopez-Mascaraque (Instituto Cajal). **(c)** Electron microscopy image of astrocyte process at the axon–spine interface: astrocyte process (astro, blue); postsynaptic density (psd, red); dendritic spine head (sp, yellow); axonal bouton (ax, green). Reproduced, with permission, from Ref. [107]. (d) 3D reconstruction of a single astrocyte process (blue) interdigitating among four dendrites (gold, yellow, red and purple). Reproduced, with permission, from Ref. [107].

neighboring astrocytes as an intercellular $Ca^{2+}$ wave involving dozens of cells [4,5,22]. By contrast, in brain slices such waves seem to involve few astrocytes, and their actual existence in more intact preparations is currently under debate [23]. The synaptically evoked as well as the spontaneous $Ca^{2+}$ signal originates in spatially restricted areas – called 'microdomains' – of the astrocyte processes [24,25] from where it can eventually propagate intracellularly to other regions of the cell [20,25,26]. As a single astrocyte might contact ~100 000 synapses [27], the control of the spatial extension of the $Ca^{2+}$ signal could have relevant functional consequences for the physiology of the nervous system, because not all synapses covered by a single astrocyte are necessarily functionally locked to be similarly and simultaneously modulated (see below). Therefore, differential neuromodulation of specific synapses would provide an extraordinary increase of the degrees of freedom to the system [28,29].

## Astrocyte $Ca^{2+}$ signal *in vivo*

For many years, technical constraints limited astrocyte $Ca^{2+}$-signal studies to cultured cells and brain slices. The recent use of novel imaging techniques, that is, two-photon microscopy and specific fluorescent dyes that selectively label astrocytes *in vivo* [30], which enable the study of astrocyte $Ca^{2+}$ signals in the whole animal, has revealed important findings (Figure 3b). First, reports from studies of rat, mouse and ferret have demonstrated that astrocytes *in vivo* exhibit intracellular $Ca^{2+}$ variations, indicating that astrocyte $Ca^{2+}$ excitability is not a peculiarity of slice preparations. Second, like in brain slices, astrocyte $Ca^{2+}$ variations occur spontaneously [30–33] and are also evoked by neurotransmitters released during synaptic activity [31,33–37], indicating that neuron-to-astrocyte communication is present *in vivo*. Finally, and of special relevance, astrocyte $Ca^{2+}$ elevations might be triggered by physiological sensory stimuli. Indeed, stimulation of whiskers increased the astrocyte $Ca^{2+}$ in mouse barrel cortex [33] (Figure 3b). Astrocytes of the sensory cortex also elevate their $Ca^{2+}$ in response to a robust peripheral stimulation that is known to activate the locus coeruleus or to direct electrical stimulation of this nucleus [34], as well as during running behavior in alert mice [35]. Astrocytes from other brain regions also respond to stimuli of corresponding sensory modalities. Astrocytes in the visual cortex not only show $Ca^{2+}$ elevations in response to visual stimuli but also the properties of these responses indicate the existence of distinct spatial receptive fields and reveal an even sharper

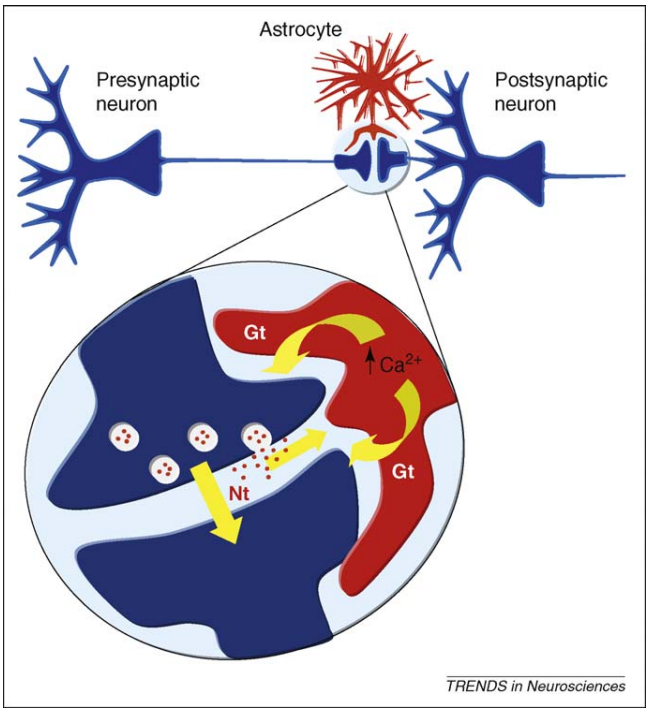


**Figure 2.** Scheme of the tripartite synapse. Cartoon representing the transfer of information between neuronal elements and astrocyte at the tripartite synapse. Astrocytes respond with $Ca^{2+}$ elevations to neurotransmitters (Nt) released during synaptic activity and, in turn, control neuronal excitability and synaptic transmission through the $Ca^{2+}$-dependent release of gliotransmitters (Gt).

tuning than neurons to visual stimuli [37]. In summary, astrocytes *in vivo* display $Ca^{2+}$ excitability and respond to neuronal activity. Furthermore, because astrocytes in specific sensory areas respond to a variety of sensory stimuli, it is feasible that astrocytes participate in the brain representation of the external world.

## Synaptic information processing by astrocytes

In contrast to the view of astrocytes as passive elements that provide the adequate environmental conditions for appropriate neuronal function and that respond to neurotransmitters, simply performing a linear readout of the synaptic activity, experimental evidence supports the idea that astrocytes integrate and process synaptic information elaborating a complex nonlinear response to the incoming information from adjacent synapses (Box 1). As described earlier, it is firmly established that astrocytes respond with $Ca^{2+}$ elevations to synaptic activity [25]. However, to understand the actual role of astrocytes in brain information processing, it is necessary to define whether the astrocyte $Ca^{2+}$ signal passively results from different neurotransmitter concentrations attained during synaptic activity or, alternatively, whether neuron-to-astrocyte communication presents properties of complex information processing that are classically considered to be exclusive to neuron-to-neuron communication. In Box 1 and in the following discussion we will elaborate the evidence that supports the idea that astrocytes are cellular processors of synaptic information.

### *Astrocytes discriminate the activity of different synaptic pathways*

The astrocyte $Ca^{2+}$ signal does not result from a nonspecific spillover of neurotransmitters; instead, it is selectively mediated by the activity of specific synaptic terminals (Figure 4). Astrocytes located in the *stratum oriens* of the CA1 area of the hippocampus respond to the stimulation of the alveus (which contains glutamatergic and cholinergic axons) with $Ca^{2+}$ elevations that are specifically mediated by acetylcholine (ACh) but not by glutamate [16]. By contrast, these astrocytes do respond to glutamate when it is released by different glutamatergic synapses, that is, the Schaffer collateral (SC) synaptic terminals [25]. Hence, astrocytes selectively respond to different synapses that use different neurotransmitters (i.e. glutamate and ACh), and they discriminate between the activity of different pathways that use the same neurotransmitter (i.e. glutamatergic axons of SC and alveus) [25]. Likewise, astrocytes in the ventrobasal thalamus respond to the

**Table 1. $Ca^{2+}$ signaling in astrocytes**

| | Neurotransmitter | Experimental model | Brain area | Refs |
|---|---|---|---|---|
| Spontaneous activity | Non-applicable | Brain slices | Thalamus | [12,14] |
| | | | Hippocampus | [12,13] |
| | | | Cerebellum | [12,24] |
| | | | Cortex | [15] |
| | | | Striatum | [12] |
| | | *In vivo* | Cortex | [31,32,34–36] |
| Synaptically evoked | Norepinephrine | Brain slices | Cerebellum | [19] |
| | | *In vivo* | Cortex | [34] |
| | ATP | Brain slices | Hippocampus | [81] |
| | | | Cerebellum | [82,83] |
| | | | Retina | [84] |
| | | | Olfatory bulb | [85] |
| | GABA | Brain slices | Hippocampus | [18,70] |
| | Glutamate | Brain slices | Hippocampus | [16,17,20,25,86] |
| | | | Cortex | [20,39] |
| | | | Nucleus accumbens | [61] |
| | | | Cerebellum | [82,83] |
| | | | Olfatory Bulb | [85] |
| | | *In vivo* | Cortex | [33,37] |
| | Acetylcholine | Brain slices | Hippocampus | [16,25] |
| | Nitric Oxide | Brain slices | Cerebellum | [87] |
| | Endocannabinoids | Brain slices | Hippocampus | [64] |

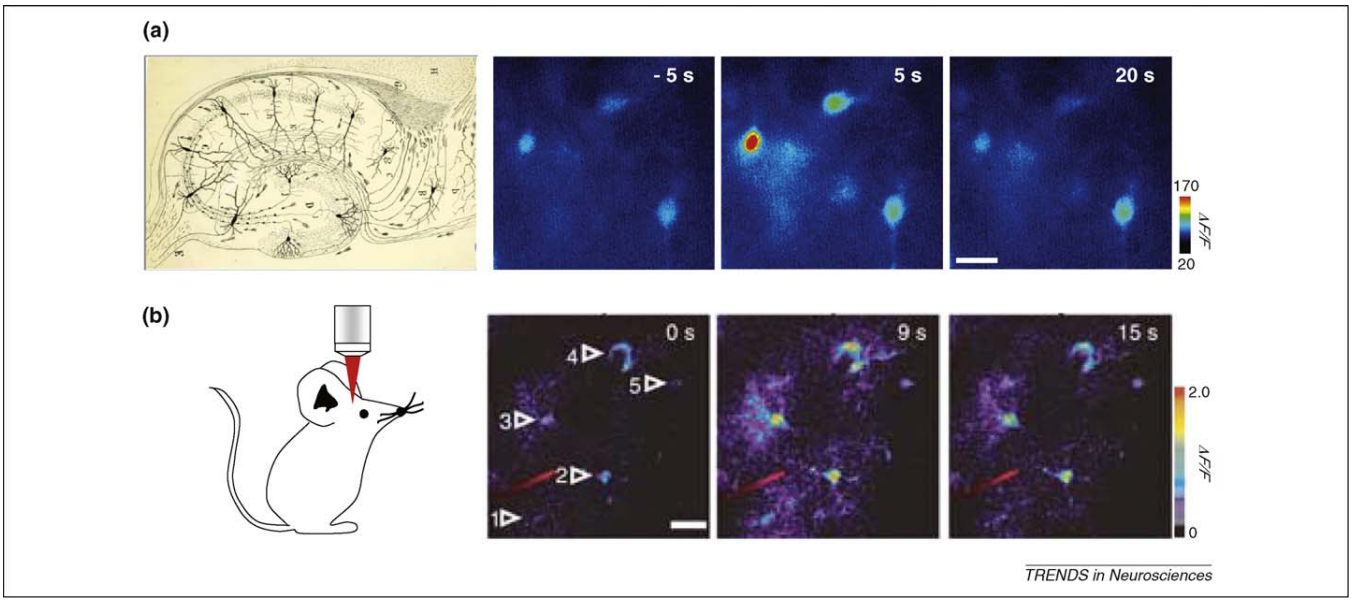


**Figure 3.** Astrocyte $Ca^{2+}$ signaling in brain slices and *in vivo*. (a) Cajal's drawing of the mammalian hippocampus (reproduced from an original drawing with permission of the Instituto Cajal) and pseudocolor images from rat hippocampal slices representing fluorescence intensities indicative of astrocyte $Ca^{2+}$ levels before (−5 s) and after (5 s, 20 s) electrical stimulation of Schaffer collaterals. Scale bar, 10 μm. (b) Two-photon microscopy images of the *in vivo* astrocyte $Ca^{2+}$ signal in the barrel cortex. Pseudocolor images represent fluorescence intensities indicative of astrocyte $Ca^{2+}$ levels before (0 s) and after (9 s, 15 s) evoked by whisker stimulation. Scale bar, 20 μm. Reproduced, with permission, from Ref. [33]. Note the astrocyte $Ca^{2+}$ elevations evoked by electrical synaptic and sensory stimulation in hippocampal slices (a) and *in vivo* barrel cortex (b), respectively.

stimulation of either sensory or corticothalamic pathways, but very few respond to the activity of both [38]. Furthermore, astrocytes in the barrel cortex also respond selectively to the activity of different neuronal inputs, because astrocytes in layer 2/3 respond to glutamatergic inputs from layer 4 in the same column but not to glutamatergic projections from layer 2/3 of adjacent columns [39] (Figure 4b). Therefore, astrocytes show selective responses that discriminate the activity of specific synapses.

### *Astrocyte $Ca^{2+}$ signals show a nonlinear relationship with the synaptic activity*

The analysis of the astrocyte $Ca^{2+}$ signal evoked by the activity of different synaptic terminals that release ACh and glutamate indicates that astrocytes integrate synaptic information [25]. In hippocampal slices, the simultaneous stimulation of alveus and SC (that elicit $Ca^{2+}$ elevations mediated by ACh and glutamate, respectively) evokes astrocytic responses that are inconsistent with a linear readout of the synaptic activity. The amplitude of the $Ca^{2+}$ elevations elicited by simultaneous stimulation of both pathways is not equivalent to the linear summation of the $Ca^{2+}$ signals evoked by independent stimulation [25] (Figure 4a). Therefore, the astrocyte $Ca^{2+}$ signal is nonlinearly modulated by the simultaneous activity of cholinergic and glutamatergic synapses. Moreover, while the $Ca^{2+}$ signal evoked by simultaneous stimulation at high frequencies (30 and 50 Hz) displays a sublinear summation of the responses evoked independently, it shows a supralinear summation after stimulation at relatively low frequencies (1 and 10 Hz); that is, the $Ca^{2+}$ signal is relatively depressed or potentiated at relative high and low frequencies of neuronal activity, respectively. Therefore, the astrocyte $Ca^{2+}$ signal is nonlinearly modulated by the simultaneous activity of different synaptic inputs, and the sign of this modulation depends on the synaptic activity level [25].

### *Astrocytes have cell-intrinsic properties*

The astrocyte $Ca^{2+}$ signal evoked by exogenously applied neurotransmitters can have synergistic effects [40–42]. Furthermore, the modulation of the $Ca^{2+}$ signal in hippocampal astrocytes described earlier occurs in the absence of synaptic activity when the transmitters glutamate and ACh are applied [25]. Interestingly, the $Ca^{2+}$ signal evoked by the simultaneous application of glutamate and γ-aminobutyric acid (GABA) is equal to the linear summation of the $Ca^{2+}$ elevations evoked independently, indicating that the astrocyte $Ca^{2+}$ signal modulation depends on the transmitters involved, probably owing to the activation of different intracellular signaling cascades. Indeed, the intracellular signaling pathways of both metabotropic ACh and glutamate receptors converge at the activation of the phospholipase C, whereas $GABA_B$ receptors are coupled to different intracellular pathways that involve adenylate cyclase regulation [29]. Hence, the astrocyte $Ca^{2+}$-signal modulation is a specific phenomenon that depends on the neurotransmitters involved and, consequently, might be selectively induced by specific synaptic pathways. Therefore, astrocytes are endowed with cell-intrinsic properties that grant the nonlinear responses to the synaptic activity and that are probably determined by the intracellular signaling events, like intrinsic properties of neurons are based on the electrical properties of the membrane.

In summary, these findings indicate that astrocytes are cellular elements involved in the information processing by the nervous system. Although our current knowledge of the

**Box 1. Astrocytes integrate and process synaptic information**

One of the most relevant functional properties of neurons, which are responsible for their role in the brain information processing, is based on the fact that neuronal electrical excitability is nonlinearly regulated by the simultaneous activity of different converging synaptic inputs. Neurons receive thousands of input signals in the form of synaptic potentials that are integrated nonlinearly in the soma and dendrites to elaborate a single output signal in form of action potentials. This nonlinear integration of the multiple incoming input signals is considered to represent the fundamental basis of the information processing by neurons and is the heart of the nervous system activity. Neuronal information processing is based on two key functional properties of neurons: (i) selective responsiveness to different specific synaptic inputs, and (ii) neuronal intrinsic properties determined by the expression of a plethora of voltage- and ligand-gated channels and membrane electrical properties. These properties account for the complex nonlinear input–output relationships that are responsible for the integrative properties of neurons [108,109].

The demonstration that astrocytes are excitable cells that base their excitability on variations of the intracellular $Ca^{2+}$ signal that can be triggered by neurotransmitters released during synaptic activity [25] raises the question of whether astrocytes integrate and process synaptic information, challenging the classical idea that synaptic information processing is exclusive to neurons.

Two possible views arise from the synaptic regulation of the astrocyte $Ca^{2+}$ signal. (i) While the duration, amplitude and frequency of the astrocyte $Ca^{2+}$ signal are regulated by different levels of synaptic activity [29], the different responses might passively result from different neurotransmitter concentrations attained during different levels of synaptic activity. Consequently, astrocytes would perform a linear readout of the synaptic activity, where the astrocyte $Ca^{2+}$ signal would simply reflect the synaptic activity level. (ii) Alternatively, astrocytes might integrate and process synaptic information, elaborating a complex nonlinear response to the incoming input signals received from adjacent synapses.

To distinguish between both alternative views, we propose the following simplest criteria that astrocytes must meet to be considered as cellular processors of synaptic information:

(i) To have cellular excitability.
(ii) To show selective responsiveness to specific synaptic inputs.
(iii) To display nonlinear input–output relationships.
(iv) To have cell-intrinsic properties.

As detailed in the text, several pieces of evidence indicate that astrocytes satisfy these requirements:

(i) the astrocyte cellular excitability based on intracellular calcium variations has been firmly established in culture, slices and *in vivo* preparations; this $Ca^{2+}$ excitability might be present as spontaneous intrinsic oscillations [12–14] and might be triggered by neurotransmitters released from synaptic terminals [16,18,20,24,86] as well as from postsynaptic neurons [64].
(ii) Hippocampal astrocytes selectively respond to different synapses that use different neurotransmitters [25]. Furthermore, astrocytes in the hippocampus [25], ventrobasal thalamus [38] and barrel cortex [39] can discriminate between the activity of different synaptic pathways that use glutamate as neurotransmitter, selectively responding to specific neuronal pathways. Therefore, astrocytes show selective responses that discriminate the activity of specific synapses.
(iii) The amplitude of the astrocyte $Ca^{2+}$ signal is nonlinearly modulated by the simultaneous activity of different synaptic pathways that use glutamate and acetylcholine as neurotransmitters, showing sublinear or supralinear summation at relatively high or low levels of synaptic activity, respectively [25]. Hence, astrocytes accommodate nonlinearly their $Ca^{2+}$ signal to the different simultaneously active synapses and to their activity level.
(iv) The astrocyte $Ca^{2+}$ signal is nonlinearly modulated by simultaneous exogenous application of different neurotransmitters. Therefore, astrocytes are endowed with cell-intrinsic properties that grant the nonlinear responsiveness to the synaptic activity. These cell-intrinsic properties of astrocytes probably reside in the intracellular signaling events, just like the intrinsic properties of neurons are determined by the electrical properties of their membranes.

These data indicate that astrocytes fulfill the requirements proposed and that, in addition to neurons, astrocytes too are cellular processors of information. Indeed, the properties of the astrocyte $Ca^{2+}$ signal reveal that astrocytes integrate and process synaptic information, indicating that neuron-to-astrocyte communication presents attributes that were classically considered to be exclusive to neuron-to-neuron communication. Consequently, astrocytes are cellular elements involved in the information processing by the nervous system.

ability of astrocytes to process synaptic information has been gained from analysis performed in brain slices, *in vivo* studies are still required to appreciate the actual extent and importance of these properties on brain function.

## Gliotransmission and modulation of synaptic transmission

One of the most stimulating topics in current neuroscience is the functional consequences of the astrocyte $Ca^{2+}$ signal on neuronal physiology. Evidence obtained during the past 15 years has demonstrated that signaling between neurons and astrocytes is a reciprocal communication, where astrocytes not only respond to neuronal activity but also actively regulate neuronal and synaptic activity. Therefore, according to the concept of the tripartite synapse, to fully understand synaptic function, astrocytes must be considered as integral components of synapses where they have crucial roles in synaptic physiology.

Astrocytes release several neuroactive molecules, such as glutamate, D-serine, ATP, adenosine, GABA, tumor necrosis factor α (TNFα), prostaglandins, proteins and peptides, that can influence neuronal and synaptic physiology [3]. The mechanisms and consequences of this process, called gliotransmission, have attracted considerable interest. Several mechanisms of transmitter release from astrocytes have been proposed. Compelling evidence demonstrates that some transmitters are released in a $Ca^{2+}$-dependent manner [10,43–48] through vesicle [47–51] and lysosome [52–54] exocytosis. Furthermore, ultrastructural studies have shown that astrocytic processes contain small synaptic-like vesicles, which are located in close proximity to synapses, apposed either to presynaptic and postsynaptic elements [49,50]. Alternative release mechanisms, including reversal of glutamate transporters, connexin/pannexin hemichannels, pore-forming P2X7 receptors and swelling-induced activation of volume-regulated anion channels, have also been proposed (for a review, see Ref. [55]). Whether $Ca^{2+}$-dependent and -independent mechanisms coexist and under what physiological or pathological conditions they occur remain unclear.

The original demonstration of astrocyte-induced neuromodulation in cultured cells [43,44,56] has been considerably expanded by later studies on acute brain slices (for reviews, see Refs [57–59]; Table 2). Glutamate was one of the first gliotransmitters released from astrocytes to be identified and has been reported to exert many effects on

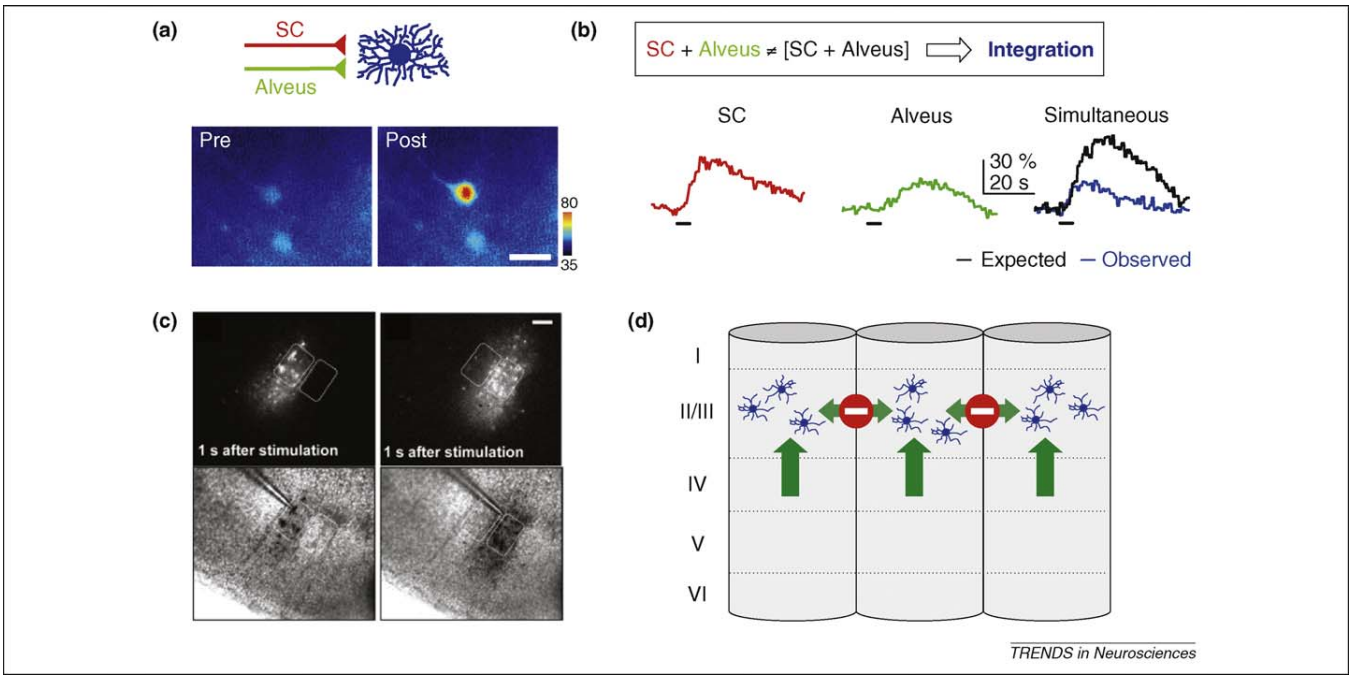


**Figure 4**. Astrocytes integrate synaptic information. **(a)** Schematic drawing and pseudocolor images of astrocyte $Ca^{2+}$ elevations evoked by stimulation of Schaffer collaterals (SC, red) or alveus (green). Astrocytes integrate synaptic information from different synaptic inputs. Scale bar, 15 μm. **(b)** Hypothesis of astrocyte integration of synaptic information induced by SC and alveus activity (top) and astrocyte $Ca^{2+}$ signals evoked by independent and simultaneous stimulation of SC and alveus (bottom). Blue and black traces correspond to the observed and expected responses (i.e. the linear summation of the responses evoked by independent stimulation of both pathways), respectively. Horizontal lines at the bottom of each trace represent the stimuli. Modified from Ref. [25]. Note the lack of correspondence between observed and expected responses, that is, the relative reduction of the observed response versus the linear summation of the responses evoked independently, which is indicative of synaptic integration. **(c)** Top, fluorescence images showing astrocytic $Ca^{2+}$ signals evoked after electrical stimulation (responding cells are displayed in white) of two contiguous barrel cortex. Bottom, images showing an overlay of the bright-field image with the location of the stimulating pipette and the responding astrocytes (shown in black). The barrels in layer 4 are outlined by dotted white lines. Scale bar, 100 μm. Note that astrocytes that respond to the stimulation of the barrel column are located within the stimulated barrel column, and no astrocytes respond to the stimulation of the adjacent barrel column, which indicates the selectivity of astrocyte responses. Reproduced, with permission, from Ref. [39]. **(d)** Schematic drawing illustrating the discrimination and response selectivity of barrel cortex astrocytes to neuronal activity from layer IV but not from layer II/III of neighboring barrels.

neuronal excitability. Astrocytic glutamate evokes slow inward currents (SICs) through activation of postsynaptic *N*-methyl-D-aspartate (NMDA) receptors [25,44,60–65] and synchronously excites clusters of hippocampal pyramidal neurons, indicating that gliotransmission increases neuronal excitability and operates as a nonsynaptic mechanism for neuronal synchronization [60,62]. By contrast, astrocytic glutamate might also activate receptors localized at presynaptic terminals. Through activation of group I metabotropic glutamate receptors (mGluRs) [46,26] or NMDA receptors [50], astrocytes enhance the frequency of spontaneous and evoked excitatory synaptic currents. Alternatively, astrocytes induce the potentiation [20] or depression of inhibitory synaptic transmission by activation of presynaptic kainate [66] or II/III mGlu [67] receptors, respectively. Therefore, a single gliotransmitter can exert multiple effects depending on the sites of action and the activated receptor subtypes, which provides a high degree of complexity to astrocyte–neuron communication. This complexity becomes even higher when considering that other gliotransmitters, such as GABA, ATP, adenosine (a metabolic product of ATP) or D-serine, could act on the same neuron or act on different cell types, thus evoking distinctive responses [63,68–72]. Moreover, in hippocampal astrocytes, $Ca^{2+}$ elevations induced by activation of PAR-1 receptors, but not $P2Y_1$ receptors, evoke NMDA-receptor-mediated SICs in pyramidal neurons [65], indicating that the $Ca^{2+}$ signal evoked by activation of different receptors might not be equally competent to stimulate gliotransmitter release. A great effort has been made so far to identify different gliotransmitters and their potential modulatory actions, but it remains unknown whether different gliotransmitters are co-released or whether different gliotransmitters are released by different astrocytes or by different astrocytic processes or domains. It is also crucial to elucidate the specific incoming inputs, the molecular mechanisms and the physiological conditions that govern the precise release of each gliotransmitter. Intracellular regulatory mechanisms of release and spatially defined specific intercellular signaling pathways seem to be present to grant a coherent astrocyte–neuron communication (see later).

Besides glutamate, ATP and its product adenosine of astrocytic origin also control synaptic transmission [68–71]. Indeed, heterosynaptic depression of hippocampal synaptic transmission requires astrocyte release of ATP/adenosine [69–71], which is stimulated by the $GABA_B$-mediated astrocyte $Ca^{2+}$ signal elicited by interneuron activity evoked by SC [70]. This represents a paradigmatic example of the consequences of coordinated neuron–glia network on synaptic function. Furthermore, it also shows that synaptically evoked astrocytic ATP might signal to other synapses, thus spreading neuronal information beyond activated synapses [70]. Likewise, glutamate from

**Table 2. Gliotransmitters and synaptic transmission**

| Gliotransmitter | Experimental preparation | Neuromodulation | Refs |
|---|---|---|---|
| Glutamate | Hippocampus | Depression of evoked EPSCs and IPSCs | [43,67] |
| | | Frequency increase of miniature PSCs | [44] |
| | | Frequency increase of miniature IPSCs | [18] |
| | | Frequency increase of spontaneous EPSCs | [50,26] |
| | | Frequency increase of spontaneous IPSCs | [66] |
| | | Postsynaptic SIC | [25,43,60,62,64,65,88–94] |
| | | Increase of neuronal excitability | [17] |
| | | Heterosynaptic depression | [95] |
| | Cortex | Postsynaptic SIC | [96] |
| | Ventro basal thalamus | Postsynaptic SIC | [14] |
| | Nucleus accumbens | Postsynaptic SIC | [61] |
| | Olfactory Bulb | Postsynaptic SIC | [63] |
| | Retina | Light-evoked neuronal activity | [97] |
| ATP/Adenosine | Cerebellum | Depression of spontaneous EPSCs | [98] |
| | Hippocampus | Heterosynaptic depresion of EPSCs | [70,71] |
| | | Modulation of LTP | [69] |
| | | Synaptic depression | [69] |
| | Hypothalamic paraventricular nucleus | Insertion of AMPA receptors | [99] |
| | Retina | Depression of light-evoked EPSCs | [100] |
| D-Serine | Hippocampus | Modulation of LTP | [101] |
| | Hipothalamic supraoptic Nucleus | Modulation of LTP | [72] |
| | Retina | Potentiate NMDA receptor transmission | [102] |
| TNFα | Hippocampus | Insertion of AMPA receptors | [74] |
| | | Increase of synaptic scaling | [76] |
| GABA | Olfactory bulb | Postsynaptic SOC | [63] |
| Undefined (glutamate and/or nitric oxide) | Neuromuscular junction | Synaptic depression | [103,104] |
| | | Synaptic potentiation | [105] |

Abbreviations: EPSCs, excitatory postsynaptic currents; IPSCs, inhibitory postsynaptic currents; LTP, long-term potentiation; PSCs, postsynaptic currents; SIC, slow inward current; SOC, slow outward current.

astrocytes stimulated by endocannabinoid released during neuronal activity could signal to adjacent unconnected neurons [64], suggesting that astrocytes serve as a bridge for nonsynaptic communication between neurons. In conclusion, astrocytes not only influence the active synapses through short-range signaling but they might also have long-range effects on distant synapses.

Hippocampal slices are a useful experimental model to study synaptic transmission, and consequently they have been also widely used to analyze the astrocyte effects on synaptic transmission. Although a comprehensive characterization of the phenomenon in different brain areas is still lacking, glia-mediated synaptic transmission modulation has also been documented in retina, supraoptic nucleus and cerebellum, as well as at the neuromuscular junction in the peripheral nervous system (for reviews see Refs [58,73]). Finally, the effects of the activity of single astrocytes on single synapses have been investigated recently in the hippocampus by performing paired recordings from pyramidal neurons and single astrocytes while stimulating SC single synapses, that is, by experimentally isolating the tripartite synapse [46]. Astrocyte $Ca^{2+}$ elevations transiently increase the probability of neurotransmitter release from presynaptic terminals, thus enhancing the synaptic efficacy (Figure 5). This effect is mediated by $Ca^{2+}$- and SNARE protein-dependent release of glutamate from astrocytes, which activates group I metabotropic glutamate receptors at the presynaptic terminal [46].

## Astrocytes and synaptic plasticity

Astrocytes operate at lower time scales than synaptic neurotransmission. Whereas fast neurotransmission occurs in milliseconds, astrocytic effects on neuronal physiology last seconds or tens of seconds. In addition, astrocyte regulation of synaptic transmission runs on different time scales, because astrocytes can control transiently the synaptic strength (during seconds), and they can also contribute to long-term synaptic plasticity. Several mechanisms underlying the astrocyte effects on long-term potentiation (LTP) have been described. Some studies indicate a passive or tonic mode of action, in which astrocytes tonically suppress or potentiate synaptic transmission [69,72,74,75]. Astrocytes through ATP/adenosine release control the strength of the basal hippocampal synaptic activity by tonic suppression of neurotransmission, which results in an increase in the dynamic range for LTP [69]. In the hypothalamic supraoptic nucleus, changes in the astrocytic coverage of synapses influence NMDA-receptor-mediated synaptic responses due to changes in the ambient levels of D-serine released by astrocytes [72].

By contrast, astrocytes participate in the generation of LTP through a phasic signaling process, in which the temporal coincidence of the astrocyte $Ca^{2+}$ signal and the postsynaptic neuronal activity induces LTP through the activation of presynaptic type I mGluRs by $Ca^{2+}$-dependent glutamate release from astrocytes [46]. These findings have expanded our traditional vision of the Hebbian LTP (a paradigm of synaptic plasticity based on the coincident activity of pre and postsynaptic neuronal elements) to include astrocytes as new sources of cellular signals involved in synaptic plasticity.

## Astrocytes and animal behavior

The elucidation of the actual impact of astrocyte $Ca^{2+}$ signaling and gliotransmission on animal behavior

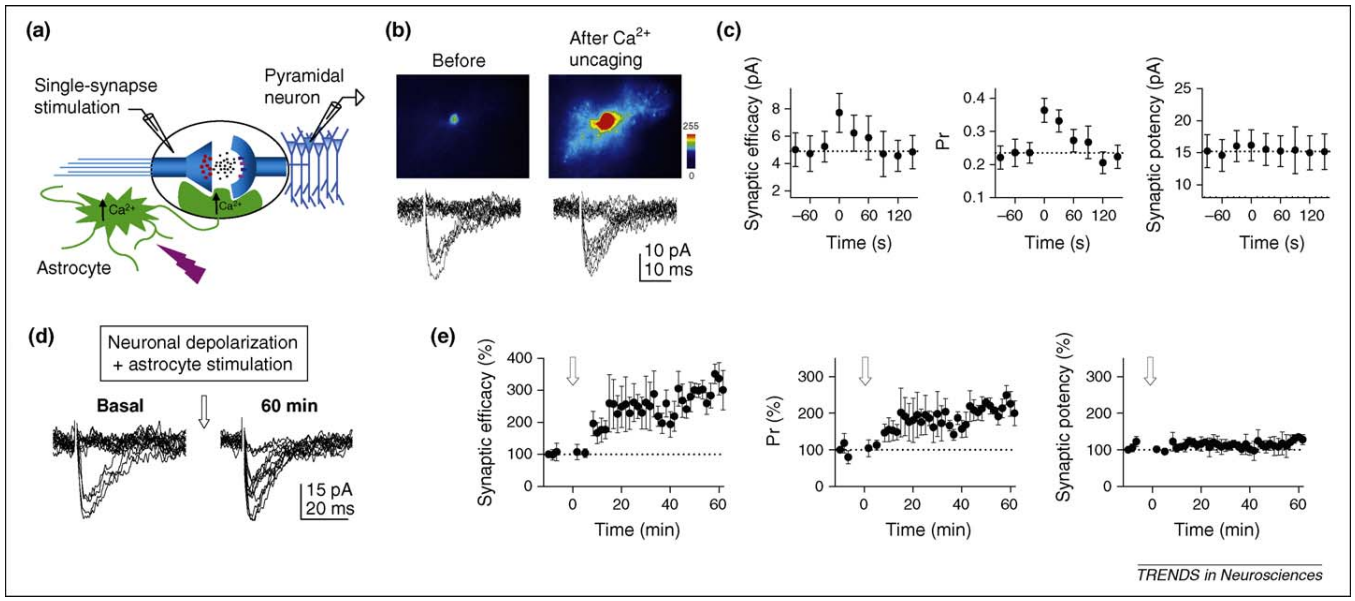


**Figure 5.** Astrocytes control synaptic transmission and plasticity at the tripartite synapse. **(a)** Schematic drawing showing recordings from one pyramidal neuron and one astrocyte, and the stimulation of a single synapse. **(b)** Astrocyte $Ca^{2+}$ levels (top) and synaptic responses (bottom) before and after $Ca^{2+}$ uncaging at a single astrocyte. Note the increase in the proportion of successful synaptic responses after astrocyte $Ca^{2+}$ elevation. **(c)** Astrocytes potentiate synaptic efficacy (i.e. mean amplitude of all responses including failures), increasing the probability of transmitter release (Pr, ratio between the number of successes versus the total number of stimuli) without modulating the amplitude of synaptic responses (i.e. synaptic potency, defined as mean amplitude of the successful responses). Note the transient increase of synaptic efficacy and Pr after elevating astrocyte $Ca^{2+}$ (at time zero). **(d,e)** Temporal coincidence of astrocyte $Ca^{2+}$ signal and postsynaptic neuronal depolarization induces long-term potentiation (LTP) of synaptic transmission. Excitatory synaptic currents before and 60 min after transiently pairing neuronal depolarization and astrocyte $Ca^{2+}$ uncaging. (e) Graphs showing relative changes in synaptic efficacy, Pr and synaptic potency parameters over time. Arrows indicate pairing of astrocyte $Ca^{2+}$ signal and mild neuronal depolarization. Note the persistent potentiation of synaptic efficacy and Pr induced by the transient pairing (for 5 min) of neuronal and astrocyte stimulation (at time zero). Reproduced, with permission, from Ref. [46].

represents the ultimate challenge for the concept of the tripartite synapse. The development of transgenic animal models will be useful for this purpose. However, controversial data on this issue have been reported recently using different transgenic mice. Changes in hippocampal neuronal excitability and synaptic transmission were not detected when astrocyte $Ca^{2+}$ elevations were evoked by selective activation of Mas-related G-protein-coupled receptor member A1 (MrgA1) receptors, a type of receptor coupled to $Ca^{2+}$ release from internal stores that are not expressed endogenously in the brain but were transgenically expressed specifically in astrocytes of a transgenic mice [76]. Inherent problems of transgenic mice derived from the transgene expression under heterologous promoters, such as proper spatiotemporal expression of exogenous receptors as well as appropriate coupling to intracellular signaling cascades and cellular events (such as gliotransmitter release), might account for these negative results (for a discussion, see Ref. [23]). By contrast, a ground-breaking study has recently demonstrated that astrocytes contribute to the control of sleep homeostasis by using transgenic mice in which SNARE-dependent release of gliotransmitters from astrocytes was abolished. This study shows that adenosine metabolized from ATP released by astrocytes participates in the accumulation of sleep pressure and contributes to cognitive deficits associated with sleep loss [77]. Although they are not perfect experimental models, transgenic mice have the potential to reveal currently unknown roles for astrocytes in different brain functions.

## Are all synapses tripartite?

Experiments designed to observe the effects of the astrocyte $Ca^{2+}$ signal on single hippocampal synapses showed that not all recorded synapses displayed modulation of the synaptic efficacy after astrocyte stimulation, but only a subset of synapses (around 40%) underwent astrocyte-induced potentiation [46]. Experimental conditions might account for some ineffective cases because, owing to the limits of optical resolution, it could not be excluded that the stimulated astrocyte was not in sufficient close proximity to the recorded synapse. Alternatively, it is feasible that, in some cases, the stimulated astrocyte and the recorded synapse were not functionally connected. Whether this absence of connectivity is due to functional or structural bases is unknown, but it is interesting to note that ultrastructural data shows that only a subset of hippocampal excitatory synapses (again around 40%) are covered by astrocytic processes [78], which is consistent with the hypothesis that not all synapses are functionally tripartite. The fact that $Ca^{2+}$ elevations evoked in a large population of astrocytes by ATP application potentiated neurotransmission in only ~40% of the recorded synapses further supports this hypothesis [46]. If this is the case, it would be interesting to test whether tripartite synapses are stable or dynamic functional units. The latter idea seems to be favored by the observation that coordinated structural changes in astrocytic processes and synaptic spines occur in hippocampal synapses [79] and in the somatosensory cortex where whiskers stimulation evoke morphological changes on astrocytic processes that cover synapses [80].

**Box 2. Future questions**

Regarding the properties and physiological consequences of the astrocyte–neuron communication, important issues remain largely unknown.

Among the general issues, key topics need to be further investigated:

(i) The molecular and cellular events underlying astrocyte–neuron signalling *in vivo.*

(ii) Role of astrocyte–neuron communication in brain function and animal behavior. While this communication is largely characterized at cellular and subcellular levels, what are its actual roles in neural network activity, brain function and animal behavior?

(iii) Role of astrocyte–neuron communication in brain pathology. Might the disruption of astrocyte–neuron signaling mechanisms result in brain diseases? Might this signaling lead to brain pathology and under what certain circumstances? Are astrocytes the appropriate cellular targets to direct therapeutic approaches for the treatment of some brain diseases?

To investigate these issues, new transgenic mice such as those designed to silence the molecular mechanisms involved in synaptically evoked astrocytic responses or in gliotransmitter release might be useful. Likewise, great help might be provided by transgenic mice that enable the selective stimulation of astrocytes *in vivo,* which is the strongest challenge that must be overcome to reveal the actual role of astrocytes in brain function and animal behavior.

Examples of particular questions are:

(i) What is the involvement of other neurotransmitter systems, such as dopamine or serotonin, on astrocyte excitability?

(ii) What are the specific properties of synaptic information processing by astrocytes in different brain areas? How are these properties regulated by different neurotransmitters?

(iii) Are different gliotransmitters co-released by single astrocytes? Are different gliotransmitters released by different astrocytes or by different astrocytic processes or domains?

(iv) What are the specific incoming inputs, the molecular mechanisms and the physiological conditions that govern the precise release of each gliotransmitter?

(v) Are tripartite synapses plastic elements? If so, what are the cellular signaling events and the molecular mechanisms that control the structural and functional plasticity?

The plasticity in the establishment of tripartite synapses might have strong impact on the function of the neuron–glia network. In any case, the fact that only a subset of synapses were effectively modulated by single astrocytes indicates that neuromodulation does not result from a wide spillover of the gliotransmitter but, instead, suggests the existence of specific signaling pathways between astrocytes and neurons, probably as a point-to-point form of communication.

## Concluding remarks

Since the beginning of the 'glia revolution' in the 1990s, compelling evidence has been accumulated by many laboratories to firmly establish the concept of the tripartite synapse, in which astrocytes have functionally relevant roles in synaptic physiology. We know now that astrocytes are cellular processors of synaptic information and that they regulate synaptic transmission and plasticity. Consequently, astrocytes are involved in the processing, transfer and storage of information by the nervous system and, therefore, in addition to neurons, they must be considered as cellular elements involved in brain function. During recent years, a great advance has been produced in our knowledge of events underlying astrocyte–neuron interactions at cellular level, but it is apparent that we have only begun to appreciate the actual role of astrocytes in brain function and animal behavior (Box 2). A more comprehensive characterization of these cellular events and the actual impact of astrocytes on the activity of the neuron–glia network is still required. Finally, considering current evidence, it will not be surprising if future studies, in which the development of transgenic animal models will be fundamental, reveal important roles of astrocytes in different brain tasks and animal behavior.

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