

# Gliotransmitters Travel in Time and Space

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The identification of the presence of active signaling between astrocytes and neurons in a process termed gliotransmission has caused a paradigm shift in our thinking about brain function. However, we are still in the early days of the conceptualization of how astrocytes influence synapses, neurons, networks, and ultimately behavior. In this Perspective, our goal is to identify emerging principles governing gliotransmission and consider the specific properties of this process that endow the astrocyte with unique functions in brain signal integration. We develop and present hypotheses aimed at reconciling confounding reports and define open questions to provide a conceptual framework for future studies. We propose that astrocytes mainly signal through high-affinity slowly desensitizing receptors to modulate neurons and perform integration in spatiotemporal domains complementary to those of neurons.

## Introduction

Accumulating evidence supports the presence of a dynamic, bidirectional regulation of neuronal communication by astrocytes. Astrocytes detect synaptic activity through the activation of metabotropic or ionotropic receptors. For instance, synaptically released glutamate from Schaffer collaterals activates G protein-coupled receptors (GPCRs), such as the type 5 of the metabotropic glutamate receptors (mGluRs), localized on hippocampal astrocytes (Porter and McCarthy 1996; Pasti et al., 1997; Perea and Araque, 2005; Panatier et al., 2011). Activation of these receptors in turn causes variations of astrocytic intracellular  $\text{Ca}^{2+}$  that can trigger the release of various active substances, such as glutamate, ATP, and D-serine, the so-called gliotransmitters (Bezzi and Volterra, 2001). Such glia-derived transmitters have been shown to act on neurons in timescales ranging from seconds to minutes and to regulate synaptic transmission and plasticity through a wide variety of mechanisms (Araque et al., 1999b; Bezzi et al., 1998; Brockhaus and Deitmer, 2002; Henneberger et al., 2010; Jourdain et al., 2007; Panatier et al., 2006; Parpura et al., 1994; Pascual et al., 2005; Pasti et al., 1997; Perea and Araque, 2007; Serrano et al., 2006; Shigemitsu et al., 2012; Zhang et al., 2003). These findings have established the concept of the “tripartite synapse,” which represents an integrative functional view of synaptic physiology that considers astrocytes as active protagonists regulating information transfer between neurons (Araque et al., 1999a). Indeed, the term “tripartite synapse” was coined to emphasize the modulation of the extracellular space around synapses by astrocytes, whether this modulation occurs via the clearance of syn-

aptic transmitters or the delivery of signaling compounds to the synaptic, extrasynaptic, or perisynaptic loci, and whether it produces a feedback mechanism, a homosynaptic modulation, or a feedforward, heterosynaptic action that might impact neuronal circuitry.

Although considerable progress has been made, a combination of conceptual and technical challenges needs to be overcome for a comprehensive understanding of how astrocytes impact and shape brain function. Our goal here is to critically evaluate the currently available findings and develop a conceptual framework to guide future work. In particular, we will emphasize that a detailed consideration of spatial and temporal properties and interactions is required to fully understand the reciprocal signaling between neurons and astrocytes and the physiological consequences of gliotransmission.

## $\text{Ca}^{2+}$ Signaling in Astrocytes: Decoding Neuronal Activity

Astrocytes possess  $\text{Ca}^{2+}$  excitability and display intracellular  $\text{Ca}^{2+}$  elevations in response to synaptic activity from physiological sensory and motor stimuli (Bekar et al., 2008; Nimmerjahn et al., 2009; Perea et al., 2009; Petzold et al., 2008; Schummers et al., 2008; Wang et al., 2006; Winship et al., 2007). The astrocyte  $\text{Ca}^{2+}$  signal that arises from synaptically released neurotransmitters is not a stereotyped “on-off” response but rather has multiple and varied patterns and kinetics that depend on the synaptic system involved (Perea and Araque, 2005), the pattern and frequency of afferent input activity (Pasti et al.,

1997; Todd et al., 2010), and include changes in amplitude, frequency, kinetics, and spatial diffusion. Most importantly, since  $\text{Ca}^{2+}$  kinetics shape cell activity and responsiveness, the tight dependency of  $\text{Ca}^{2+}$  responses on the type and properties of neuronal signals indicate that  $\text{Ca}^{2+}$  responses in astrocytes encode neuronal information.

Most of our knowledge derives from monitoring  $\text{Ca}^{2+}$  signals in astrocyte somata as an indicator of astrocytic responsiveness. These slow  $\text{Ca}^{2+}$  events were observed in response to intense neuronal activity and led to the notion that while astrocytes can detect information conveyed by intense firing activity (although at a slower timescale with respect to fast responses at the synaptic sites), they lack sensitivity to low levels of synaptic activity. Recent studies revealed, however, that small, rapid, and localized  $\text{Ca}^{2+}$  responses can be elicited in microdomains of astrocytic processes by minimal synaptic activity (Di Castro et al., 2011; Panatier et al., 2011). These data suggest that astrocytes may integrate the activity of several individual synapses to generate the larger  $\text{Ca}^{2+}$  responses observed upon sustained and intense stimulation. There are a number of observations that support such a possibility, although no direct evidence is yet available. For instance, Beierlein and Regehr (2006) showed that an increased number of stimuli generated  $\text{Ca}^{2+}$  responses that covered a larger area of a Bergmann glial cell process. However, it was not assessed whether the larger  $\text{Ca}^{2+}$  responses were directly the result of a summation of the smaller ones. Also, Di Castro et al. (2011) reported complex spatial-temporal properties of  $\text{Ca}^{2+}$  responses elicited by axonal firing in astrocytic processes, sometimes with multiple initiation points. Moreover, the rise phase of  $\text{Ca}^{2+}$  signals with slower and expanded kinetics appeared to be summative of smaller  $\text{Ca}^{2+}$  events. These observations argue against a simple propagation-dependent alteration of  $\text{Ca}^{2+}$  response.

Therefore, it appears that astrocyte  $\text{Ca}^{2+}$  signaling is characterized by a complex spatial-temporal profile ranging from small, local fast responses to larger, global but slower responses that result from the integration of signals derived from restricted regions of processes close to synapses. This integration appears to be governed by a nonlinear continuum of astrocyte excitability from which local changes can be incremented to larger and more global responses.

### Synaptic Modulation and Plasticity

Release of gliotransmitters is a consequence of  $\text{Ca}^{2+}$  elevation in astrocytes. Different, but not mutually exclusive,  $\text{Ca}^{2+}$ -dependent and  $\text{Ca}^{2+}$ -independent mechanisms have been identified, including  $\text{Ca}^{2+}$ -dependent release via exocytosis (Bezzi et al., 2004; Crippa et al., 2006; Montana et al., 2004; Zhang et al., 2004) and  $\text{Ca}^{2+}$  flux through plasma membrane ion channels (Woo et al., 2012) but the issue as to how astrocytes release transmitters remains a subject of debate (for reviews, see Hamilton and Attwell, 2010; Parpura and Zorec, 2010; Volterra and Meldolesi, 2005). These gliotransmitters activate neuronal receptors and account for astrocyte-mediated modulation of synaptic transmission and plasticity (Table 1). Our current understanding of astrocyte-mediated synaptic modulation, obtained from in situ and in vivo observations, reveals a high degree of richness in terms of the signaling processes and physiological

consequences of astrocyte neuromodulation. Here, we draw four general conclusions regarding gliotransmission.

First, a single gliotransmitter acts on different targets. For instance, astrocytic glutamate transiently potentiates excitatory transmission in the hippocampal dentate gyrus by acting on presynaptic NMDA receptors (NMDARs) (Jourdain et al., 2007), while at hippocampal CA3-CA1 synapses, it can activate presynaptic mGluRs (Navarrete and Araque, 2010; Navarrete et al., 2012; Perea and Araque, 2007). In the CA1 hippocampal region, astrocytic glutamate has been also reported to potentiate inhibitory transmission by acting on presynaptic kainate receptors (Kang et al., 1998; Liu et al., 2004) and to favor neuronal synchrony by acting on postsynaptic NMDARs (Fellin et al., 2004). Similarly, adenosine, produced via rapid ectonucleotidase-mediated ATP metabolism, can act presynaptically to modulate presynaptic inhibition as well as postsynaptically to regulate NMDAR trafficking (Deng et al., 2011; Martín et al., 2007; Panatier et al., 2011; Pascual et al., 2005; Zhang et al., 2003). Hence, just like neurotransmitters, a single gliotransmitter can have multiple effects depending on the type of circuit and targeted neurons, the pre- or postsynaptic location of neuronal receptors, and the receptor subtype activated.

Second, astrocytes can release multiple gliotransmitters. For example, in addition to glutamate, astrocytes in CA1 can release the NMDA receptor coagonist D-serine (Henneberger et al., 2010; Zhuang et al., 2010) and ATP (Zhang et al., 2003). After its conversion to adenosine, this latter gliotransmitter acts on either A1 or A2A receptors to depress or enhance excitatory synaptic transmission, respectively (Panatier et al., 2011; Pascual et al., 2005; Serrano et al., 2006). Thus, astrocytes immersed in the same circuit can release different types of gliotransmitters that exert diverse modulatory actions to influence synaptic transmission in multiple forms. A major challenge for future research will be to clarify the context specificity of the different regulatory actions. For instance, are several transmitters released from the same astrocyte? If so, are they always coreleased or do the specific features of the  $\text{Ca}^{2+}$  signals (their magnitude and spatial-temporal properties) govern the type of gliotransmitter that is released? Because of limitations in the approaches to studying signaling dynamics, our focus has necessarily been on  $\text{Ca}^{2+}$  as the proximate stimulus for gliotransmission. Are there additional second messengers that could selectively modulate gliotransmission? Are there  $\text{Ca}^{2+}$ -independent gliotransmitter release pathways that operate under physiological conditions?

Third, gliotransmission can coordinate networks of neurons and synapses. Because the astrocytic  $\text{Ca}^{2+}$  signals evoked locally by active synapses can eventually expand intracellularly from their initial source toward different cell locations under different conditions, such as high-frequency synaptic activity or concomitant activity of multiple synapses (see below), this implies that the coding signal travels throughout astrocytic processes and triggers gliotransmitter release at distant sites, affecting other synapses and circuits. Indeed, astrocytes activated by endocannabinoids released from neurons enhance synaptic efficacy at relatively distant synapses (several tens of micrometers away from the endocannabinoid source); stimulation of astrocytic CB1 receptors causes astrocytic glutamate

**Table 1. Gliotransmitter Actions in Different Brain Regions**

Brain Area	Neuromodulation	References
<b>Glutamate</b>		
Hippocampus	Depression of evoked EPSCs and IPSCs	Araque et al., 1998a; Liu et al., 2004
Hippocampus	Frequency increase of miniature PSCs	Araque et al., 1998b; Santello et al., 2011
Hippocampus	Frequency increase of miniature IPSCs	Kang et al., 1998
Hippocampus	Frequency increase of spontaneous EPSCs	Jourdain et al., 2007; Fiacco et al., 2007
Hippocampus	Frequency increase of spontaneous IPSCs	Liu et al., 2004
Hippocampus	Postsynaptic SIC	Araque et al., 1998a; Pasti et al., 2001; Sanzgiri et al., 1999; Angulo et al., 2004; Fellin et al., 2004; Cavelier and Attwell, 2005; Kang et al., 2005; Perea and Araque, 2005; Tian et al., 2005; Fellin et al., 2006; Nestor et al., 2007; Navarrete and Araque, 2010; Shigetomi et al., 2008; Sasaki et al., 2011; Navarrete et al., 2012
Hippocampus	Increase of neuronal excitability	Bezzi et al., 1998
Hippocampus	Heterosynaptic depression	Andersson et al., 2007
Hippocampus	Modulation of LTD	Han et al., 2012
Hippocampus	Modulation of LTP	Navarrete et al., 2012
Hippocampus	Synaptic potentiation	Perea and Araque, 2007; Navarrete and Araque, 2010; Navarrete et al., 2012
Hippocampus	Modulation of action potential	Sasaki et al., 2011
Hippocampus	Modulation of basal synaptic transmission	Bonansco et al., 2011
Hippocampus	Regulation of mEPSC kinetics	Han et al., 2012
Cortex	Postsynaptic SIC	Ding et al., 2007; Gómez-Gonzalo et al., 2010; Navarrete et al., 2012; Chen et al., 2012
Cortex	Modulation of LTD	Min and Nevian, 2012
Ventro basal thalamus	Postsynaptic SIC	Parri et al., 2001; Pirttimaki et al., 2011
Spinal cord dorsal horn	Postsynaptic SIC	Bardoni et al., 2010; Nie et al., 2010
Medial nucleus of the trapezoid body	Postsynaptic SIC	Reyes-Haro et al., 2010
<b>ATP/Adenosine</b>		
Hippocampus	Heterosynaptic depression of EPSCs	Serrano et al., 2006; Zhang et al., 2003; Chen et al., 2012; Pascual et al., 2005
Hippocampus	Modulation of LTP	Pascual et al., 2005; Schmitt et al., 2012; Lee et al., 2010
Hippocampus	Basal synaptic depression	Pascual et al., 2005
Hippocampus	Regulation of basal neurotransmission	Di Castro et al., 2011; Panatier et al., 2011
Hippocampus	Depression of evoked EPSCs	Martín et al., 2007
Cortex	Regulation of basal synaptic transmission	Halassa et al., 2009
Cortex	Regulation of cortical slow oscillations	Fellin et al., 2009
Cerebellum	Depression of spontaneous EPSCs	Brockhaus and Deitmer, 2002
Retina	Light-evoked neuronal activity	Newman and Zahs, 1998
Retina	Depression of light-evoked EPSCs	Newman, 2003
Nucleus accumbens	Postsynaptic SIC	D'Ascenzo et al., 2007
Hypothalamic paraventricular nucleus	Increase of EPSC amplitude	Gordon et al., 2005, 2009
Medulla oblongata	Activation of chemoreceptor neurons	Gourine et al., 2010
<b>D-Serine</b>		
Hippocampus	Modulation of LTP	Yang et al., 2003; Henneberger et al., 2010; Zhang et al., 2008
Cortex	Modulation of LTP/LTD	Takata et al., 2011; Fossat et al., 2012
Retina	Potentiation of NMDA receptor transmission	Stevens et al., 2003
Hypothalamic supraoptic nucleus	Modulation of LTP/LTD	Panatier et al., 2006

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**Table 1. Continued**

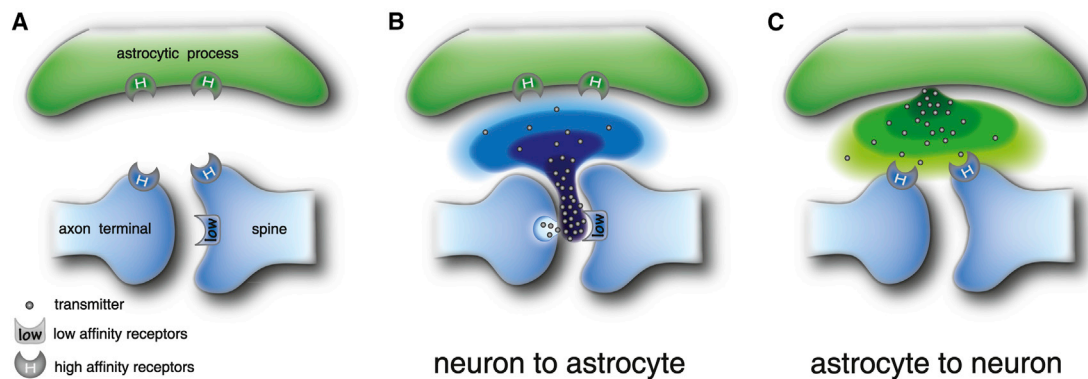
Brain Area	Neuromodulation	References
Amygdala	Modulation of NMDA receptors	Li et al., 2013
Amygdala	Increase of synaptic scaling	Stellwagen and Malenka, 2006
TNF		
Hippocampus	Insertion of AMPA receptors	Beattie et al., 2002
Hippocampus	Increase of synaptic scaling	Stellwagen and Malenka, 2006
GABA		
Hippocampus	Postsynaptic SOC	Le Meur et al., 2012
Cerebellum	Tonic current	Lee et al., 2010
Olfactory bulb	Postsynaptic SOC	Kozlov et al., 2006
Undefined		
Cortex	Regulation of cortical up states	Poskanzer and Yuste, 2011
Neuromuscular junction	Synaptic depression	Robitaille, 1998; Perez-Gonzalez et al., 2008
Neuromuscular junction	Synaptic potentiation	Castonguay et al., 2001

release and neuronal mGluR activation, a different effect than the direct activation of presynaptic receptors by endocannabinoids that causes homosynaptic depression of neurotransmission (Navarrete and Araque, 2010). In hippocampal CA1, astrocytes stimulated by highly active synapses release ATP that after conversion to adenosine depresses other synapses through A1 receptor activation, leading to heterosynaptic depression (Pascual et al., 2005; Serrano et al., 2006). Hence, as a whole, these observations suggest that astrocytes operate as bridges for intersynaptic communication.

Fourth, as a consequence of the diversity of gliotransmitters and their targets, there is also diversity in the forms of consequent modulation observed. In the CA1 region of the hippocampus in situ, a form of long-term potentiation (LTP) can be triggered by the coincidence of postsynaptic activity and astrocyte  $Ca^{2+}$  elevation that stimulates glutamate release. This form of LTP is independent of postsynaptic NMDAR-mediated signaling and requires presynaptic mGluR activation (Perea and Araque, 2007). In the same hippocampal CA1 region, astrocytes release the gliotransmitter D-serine that acts as the endogenous coagonist of postsynaptic NMDARs necessary for the induction of NMDAR-mediated LTP at synapses located within the morphological territory of the D-serine-releasing astrocyte (Henneberger et al., 2010). Basal levels of adenosine, derived from astrocytic ATP, regulate the dynamic range for LTP generation (Pascual et al., 2005). In contrast, glutamate released from stimulated astrocytes mediates the spike-timing-dependent long-term depression (LTD) of excitatory transmission in the neocortex through activation of presynaptic NMDARs (Min and Nevejan, 2012), again supporting the idea that the same gliotransmitter can have specific effects, depending on the circuit and the type and location of the targeted receptors. The involvement of astrocyte signaling in synaptic plasticity has been recently observed in vivo, whereby cholinergic activity evoked during sensory stimulation induced LTP that required muscarinic receptor-dependent astrocyte  $Ca^{2+}$  elevations and gliotransmitter release (Chen et al., 2012; Navarrete et al., 2012; Takata et al., 2011). Astrocytes activated by nucleus basalis cholinergic afferents to the visual cortex have been also revealed to play a critical

role in the selective potentiation of the neuronal response to specific visual stimuli (Chen et al., 2012).

Does the above evidence imply that induction of synaptic plasticity requires astrocyte signaling? There is no simple yes or no answer to this question. Indeed, synaptic plasticity encompasses multiple phenomena. Whereas some forms of activity-dependent plasticity depend on NMDA receptors and are expressed postsynaptically through insertion or removal of AMPA receptors from synapses, others do not depend on NMDARs and/or are expressed presynaptically through changes in the probability of transmitter release. Likewise, whereas NMDAR-dependent plasticity is homosynaptic, other forms coexist, such as heterosynaptic plasticity, which affects neighboring inputs, or homeostatic plasticity, which impacts synapses on a given neuron in a global manner. Indeed, synaptic plasticity is diverse, and factors such as brain region, age, history of synaptic activity, afferent input stimulation, and circadian rhythm can influence the plasticity mechanisms observed. This has led to conflicting reports regarding the role of astrocytes in modulating synaptic plasticity and given rise to apparently paradoxical scenarios such as those concerning hippocampal LTP in  $IP_3R2^{-/-}$  mice that lack GPCR-mediated  $Ca^{2+}$  signaling in astrocytes (Petraevicz et al., 2008). This deficiency does not prevent the induction of NMDAR-dependent LTP (Aguilhon et al., 2010), but it abolishes cholinergic-induced presynaptic LTP in both hippocampus (Navarrete et al., 2012) and cortex (Takata et al., 2011) as well as nucleus basalis-induced stimulus-specific plasticity in visual cortical neurons (Chen et al., 2012). These observations suggest that  $IP_3R2$  expression in astrocytes is essential for some forms of LTP but, at the same time, that its genetic ablation does not eliminate all forms of LTP. Thus, some forms of synaptic plasticity may rely on purely neuronal mechanisms, while others may require or involve the contribution of signals from astrocytes. In addition, astrocytic signals may not always require  $IP_3R2$ -dependent  $Ca^{2+}$  elevations. For instance,  $Ca^{2+}$  increases mediated through TRPA1 channels have been recently reported to occur in hippocampal astrocytes and to promote D-serine release thereby regulating NMDA receptor activation (Shigetomi et al., 2013). Given the complexity, it is not possible to draw



**Figure 1. Bidirectional Neuron-Astrocyte Communication Granted by High-Affinity, Slowly Desensitizing Receptors**

(A) Schematic drawing of the tripartite synapse illustrating the location of low- and high-affinity ligand receptors.

(B) Neurotransmitters rapidly activate low-affinity receptors at the postsynaptic neuronal membrane and diffuse outside the synaptic cleft to activate high-affinity receptors at the astrocytic membrane.

(C) Gliotransmitters activate high-affinity receptors at perisynaptic locations in the neuronal membrane. Decreasing neurotransmitter (B) or gliotransmitter (C) concentrations over distance from release sites is illustrated by different color intensity.

broad conclusions based on the analysis of data from an individual phenomenon. It is clear that additional work is needed to clarify the relevance of astrocytic signaling to the diverse plasticity mechanisms operating in the brain.

### Emerging Hypotheses and Perspectives

Understanding glial regulation of neuronal function is both a conceptual and a technical challenge. Even though the body of evidence discussed above supports the existence of a dynamic, bidirectional regulation of neuronal communication by glial cells, the complexity and diversity of the mechanisms involved and the heterogeneity of astroglial cells make understanding and interpreting these phenomena a daunting task. Below we will present and discuss a number of hypotheses that need to be analyzed, providing a framework for future studies aimed at more effectively integrating astrocytes into our current view of brain function.

### Bidirectional Neuron-Astrocyte Communication Granted by High-Affinity, Slowly Desensitizing Receptors

Even though astrocytic processes are in close proximity to pre- and postsynaptic neuronal elements (Auld and Robitaille, 2003; Ventura and Harris, 1999), their relative distance to the synaptic cleft and the presence of very efficient neurotransmitter recapture systems might represent structural and functional limitations to effective astrocyte-to-neuron signaling. Neurotransmitters released at the synaptic cleft rapidly reach postsynaptic receptors, but they need to travel much longer distances to reach receptor targets on astrocytes (Figures 1A and 1B). Consequently, neurotransmitter concentration drops rapidly away from the synaptic cleft, reaching very low levels in the vicinity of the astrocytic membrane. A detailed analysis of the mechanisms by which astrocytes detect neuronal activity reveals a common strategy in different synaptic contexts, finely tuned to allow astrocytes to overcome the problem of neurotransmitter concentration decay over distance (Rusakov and Kullmann, 1998). Indeed, many receptors involved in the astrocytic detection of synaptic activity,

including metabotropic glutamate, muscarinic, CB1, P2Y, and GABA<sub>B</sub> receptors, have been described as high-affinity, slowly desensitizing receptors. This implies that low perisynaptic neurotransmitter concentrations may be sufficient to activate astrocytes. For instance, the receptor that senses the synaptic release of glutamate at astrocytic processes is the mGluR5 (Panatier et al., 2011) and not the rapidly desensitizing low-affinity AMPA receptor (AMPA) (Dingledine et al., 1999; Traynelis et al., 2010). Likewise, activation of astrocytes by synaptically released ACh is mediated by slowly desensitizing muscarinic receptors and not by rapidly inactivating nicotinic receptors (Araque et al., 2002; Giniatullin et al., 2005; Quick and Lester, 2002). A similar scenario seems to apply also to the GABAergic and purinergic signaling systems mediated by the activation of the slowly desensitizing P2Y and GABA<sub>B</sub> receptors (Bowser and Khakh, 2004; Guthrie et al., 1999; Venance et al., 1997; Waldo and Harden, 2004). All these receptors are characterized by high-affinity ligand binding ( $K_D$  in the nanomolar/low micromolar range) that allows astrocytes to be activated by low concentrations of neurotransmitters. An alternative strategy is ectopic neurotransmitter release from sites located in axon terminals outside the synaptic cleft, as seen with Bergmann glia at climbing fiber-Purkinje cell synapses in the cerebellum, where the activation of lower-affinity AMPA receptors appears to be mediated by glutamate released directly in the face of the Bergmann glia processes (Matsui et al., 2005). The properties of the receptors mediating the astrocyte response to neurons are thus finely tuned to sense the low amounts of neurotransmitters and to avoid desensitization caused by a slow increase in neurotransmitter concentrations.

The situation is similar when considering the possible actions of gliotransmitters on neurons (Figure 1C). Indeed, owing to the same constraints, receptors within the synaptic cleft may hardly be sensitive to gliotransmitter released by astrocytes. In contrast, receptors located at perisynaptic axon terminals (e.g., presynaptic NMDA and mGluRs) and extrasynaptically at the postsynaptic membrane (e.g., NR2B subunit-containing NMDARs) are likely to be more easily accessed by astrocytic glutamate. Most importantly, all these receptors have high

binding affinities and slow deactivation and desensitization kinetics and could thus be activated even by slowly increasing concentrations of gliotransmitters (Figure 1C). A useful example of this is the unmasking of pure AMPAR-mediated responses triggered by astrocytic glutamate in CA1 pyramidal neurons when the desensitization of AMPARs is inhibited and NMDARs are blocked (Fellin et al., 2004).

Exceptionally, the gliotransmitter D-serine has a high affinity for the coagonist binding sites of synaptic NMDARs, which are almost fully occupied under basal conditions (Henneberger et al., 2010). This suggests the presence of ambient D-serine within the cleft possibly due to tonic release and/or inefficient clearance, as no known uptake system controls the spatial diffusion of this gliotransmitter.

Hence, the *first unifying hypothesis* is that the presence of slowly desensitizing high-affinity receptors determines both the selectivity and the sensitivity of astrocyte activation and dictates the regulation of synaptic transmission and plasticity by astrocytes.

Besides receptor characteristics, the precise spatiotemporal properties of gliotransmitter release are currently poorly defined. For example, it is not clear whether there is a colocalization of hot spots of intracellular  $\text{Ca}^{2+}$  elevation with release sites that trigger gliotransmission. In addition, gliotransmitter release may be influenced by the different  $\text{Ca}^{2+}$  signaling properties and/or location of different astrocytic receptors. For example, stimulation of both PAR-1 and P2Y1 receptors evokes  $\text{Ca}^{2+}$ -dependent glutamate release, but only PAR-1 receptor-evoked release enhances postsynaptic excitability (Shigetomi et al., 2008), while P2Y1 receptor stimulation has mainly presynaptic effects (Jourdain et al., 2007; Pascual et al., 2012; Santello et al., 2011). It is also known that changes in the spatiotemporal properties of glutamate release can alter astrocyte-induced synaptic regulation, particularly because of the dynamic competition with the uptake mechanism that shapes extracellular glutamate levels (Santello et al., 2011).

In summary, spatiotemporal properties of astrocyte  $\text{Ca}^{2+}$  signals and gliotransmitter release combined with actions on high-affinity, slowly desensitizing neuronal receptors located at perisynaptic sites allow gliotransmission to influence synaptic transmission.

### Astrocytes as Spatial and Temporal Integrators

Astrocytes have been proposed to be involved in a large array of synaptic events, from the regulation of basal synaptic transmission to various types of synaptic plasticity. At first glance, the extent of all these astrocytic actions coupled with a large array of modulatory mechanisms may appear counterintuitive and confusing. For instance, how could astrocytes regulate local synaptic events and heterosynaptic, network-based phenomena? How could astrocytes contribute to antagonistic plasticity events such as heterosynaptic depression (Pascual et al., 2005; Serrano et al., 2006) and long-term potentiation (Henneberger et al., 2010; Navarrete et al., 2012; Perea and Araque, 2007; Takata et al., 2011)? How can one reconcile the variety of gliotransmitters and mechanisms involved in the regulation of the different synaptic events?

To reconcile the available information, we propose as a *second unifying hypothesis* that astrocytes act as time and

space integrators, decoding neuronal information occurring in a large array of neuronal activity. This time and space integration encompasses faster and more local changes based on the rapid activation of small compartments along the astrocytic processes (Di Castro et al., 2011; Grosche et al., 1999; Panatier et al., 2011; Pasti et al., 1997) up to complex multiastrocytic and neuronal interactions that are induced by sustained, intense, and extended activity resulting in long-term changes in the synaptic network properties. There are a number of common properties that emerge from the multiplexing capabilities of astrocytes.

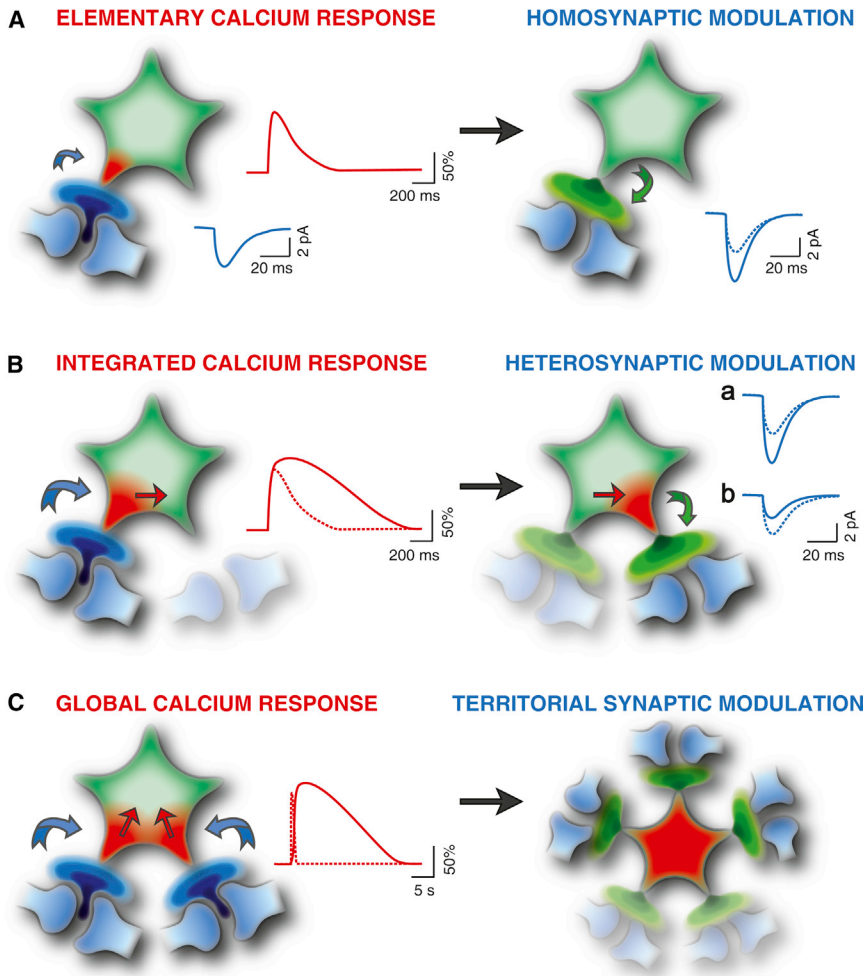
### Spatial Threshold Mechanisms

The spatial and temporal properties of the  $\text{Ca}^{2+}$  dynamics triggered by the neuronal activation of the astrocyte may lead to different modulatory effects on neuronal and synaptic activity. For instance, astrocytic regulation may be confined to a small functional compartment if synaptic transmission remains below a certain level (Figure 2A) (Di Castro et al., 2011; Panatier et al., 2011; Pasti et al., 1997). Upon an increase in the frequency of synaptic activity (or the recruitment of multiple synapses), the intracellular astrocytic  $\text{Ca}^{2+}$  activation, initially restricted to a microdomain, expands beyond the local subcompartment into another process (Figure 2B) and eventually the whole cell (Figure 2C) (Castonguay et al., 2001; Di Castro et al., 2011; Panatier et al., 2011; Pasti et al., 1997; Zonta et al., 2003). The spatial extension of the astrocyte  $\text{Ca}^{2+}$  signal may also be regulated by the spatial and temporal integration of the synaptic inputs from different neurotransmitter signaling pathways, which may control the spatial extension of the regulatory consequences on specific synapses (Fellin and Carmignoto, 2004; Perea and Araque, 2005), revealing synaptic information processing by astrocytes (De Pittà et al., 2012; Perea and Araque, 2005).

### Astrocyte Domains and Spatial Extent of Neuromodulation

It is well established that each astrocyte occupies a determined volume that defines an exclusive astrocytic territory (Bushong et al., 2002; Halassa et al., 2007). As a result, a given astrocyte will be the only one to interact with a determined set of several thousands of synapses and dendrites (Figure 2C). The large diversity of astrocytic receptors, their spatial location, and the spatiotemporal properties of the synaptic-dependent  $\text{Ca}^{2+}$  signals provide the necessary properties that allow a single astrocyte to detect, process, and decode the activity of a variety of synapses upon which it can provide distinct feedback and feedforward modulations.

Since astrocytic  $\text{Ca}^{2+}$  can stimulate gliotransmission, the spatially dynamic nature of the  $\text{Ca}^{2+}$  signal necessarily provides a spatially diverse potential for gliotransmission. For example, low-frequency synaptic activity that leads to local astrocytic  $\text{Ca}^{2+}$  signals is likely to lead to localized gliotransmission (Figure 2A) that will be restricted to exerting feedback modulation of the active synapse. However, with an increased frequency of synaptic activity (Figures 2B and 2C), the capability of the astrocytic  $\text{Ca}^{2+}$  signal to spread through the processes and to even fill the entire astrocyte, now imparts the potential for the resulting gliotransmission to exert feedforward actions on other synapses at distant locations. According to this notion, the spatial extent of the astrocytic activation is conditional on the activity of associated synapses: under some conditions,



**Figure 2. Synaptic Modulatory Actions of Gliotransmitters Depend on Integration by Astrocytes of the  $Ca^{2+}$  Changes Evoked by Different Levels of Neuronal Activity**

(A) Low levels of synaptic activity (blue arrow, left) evoke rapid, spatially restricted  $Ca^{2+}$  elevations at an astrocytic process (red trace), resulting in a gliotransmitter release that locally modulates synaptic transmission (green arrow, right) (Jourdain et al., 2007; Perea and Araque, 2007; Pascual et al., 2012; Santello et al., 2011; Panatier et al., 2011). The change in synaptic efficacy due to gliotransmitter-mediated regulation of the probability of release is illustrated as an increase in the mean amplitude of excitatory postsynaptic events (dashed and solid blue line).

(B)  $Ca^{2+}$  elevations evoked at an astrocytic process by an intense activity of an individual synapse diffuse to a nearby process (red arrow) to trigger gliotransmitter release that affects nearby synapses (right). The red superimposed traces are the integrated  $Ca^{2+}$  response (solid line) and the elementary  $Ca^{2+}$  response (dashed line, same as solid line in A). As a result of this astrocyte modulatory action, synaptic transmission (solid blue line) can be either potentiated (Navarrete and Araque, 2010) or depressed (Zhang et al., 2003; Pascual et al., 2005; Andersson et al., 2007; Serrano et al., 2006) (dashed blue line in a and b, respectively). As in (C), the focus of the phenomenon being described is indicated in color, while the elements that are not the focus are grayed out (but are not necessarily inactive).

(C) Multiple  $Ca^{2+}$  events at different processes evoked by simultaneously active synapses are spatially and temporally integrated (left) resulting in a global, long lasting  $Ca^{2+}$  elevation that can affect synaptic transmission in the territory of individual astrocytes (right) (Henneberger et al., 2010). The global  $Ca^{2+}$  response (solid line) and the integrated  $Ca^{2+}$  response (dashed line, same as solid line in B) are reported. Note the different timescale of  $Ca^{2+}$  traces in (A), (B), and (C).

gliotransmitters act in a highly localized manner, while under others they act on larger neuronal domains. Therefore, they can exert qualitatively different effects, such as the modulation of neighboring synapses (Figure 2B) (Navarrete and Araque, 2010; Pascual et al., 2005; Serrano et al., 2006) and synaptic domains defined by the morphological territory of individual astrocytes (Figure 2C) (Henneberger et al., 2010). This set of observations would argue that neuronal activity-dependent  $Ca^{2+}$  changes in astrocytes could convey specific informative signals to neurons.

#### A Need to Understand Receptor Coupling to $Ca^{2+}$

Based on this analysis and on the evidence of multiple receptors and gliotransmitters, there are a number of fundamental questions to be answered. First, we need to determine the distribution of receptors along the astrocytic processes and the mechanisms that govern this distribution. Second, there is an urgent need to understand better the molecular mechanisms underlying the different modes of  $Ca^{2+}$ -dependent (and possibly also  $Ca^{2+}$ -independent) activation of astrocytes by different types of receptors. Third, it is equally important to determine the association between a set of astrocytic receptors and the selective mecha-

nisms regulating the release of a gliotransmitter. Each of these questions represents a major technological and conceptual challenge that must be tackled in order to provide a solid basis for our understanding of the astrocytic regulation of neuronal communication.

A clearer understanding of these aspects would also allow us to more critically examine the conclusions of studies that have argued against a role for astrocytes in modulating neuronal activity. For example, overexpression and pharmacological activation of the foreign receptor Mas-related gene A1 (MrgA1) receptor in astrocytes produced long-lasting (minutes) and cell ubiquitous  $Ca^{2+}$  elevations that had no impact on synaptic functions (Aguilhon et al., 2010; Fiacco et al., 2007). Intriguingly, prolonged stimulations of endogenous GPCRs producing long-lasting and widespread  $Ca^{2+}$  elevations similar to those evoked via MrgA1 stimulation were also synaptically ineffective (Aguilhon et al., 2010; Fiacco et al., 2007). These long-lasting and widespread  $Ca^{2+}$  elevations are not observed in astrocytes during physiological activity in the brain and may represent an abnormal and possibly pathological response in these cells that does not necessarily reflect the effects that would be observed after activation of astrocytes by physiological stimuli. Consistent with this

hypothesis, a long-lasting  $\text{Ca}^{2+}$  increase evoked in cultured astrocytes by GPCR overstimulation was observed to trigger a solitary episode of glutamate release at the onset of the  $\text{Ca}^{2+}$  change only (Pasti et al., 2001). In contrast, short-lasting  $\text{Ca}^{2+}$  transients, which mimic the typical oscillatory behavior of astrocyte  $\text{Ca}^{2+}$  signals both at rest (Nett et al., 2002) and in response to neuronal activity (Di Castro et al., 2011; Pasti et al., 1997), resulted in multiple glutamate release episodes and, in turn, repetitive activation of neuronal receptors.

If we consider that the type of receptor, its location, and its mode of activation influence the properties of the downstream signaling, we can reasonably expect that all these other parameters, in addition to the duration of the astrocytes response, also profoundly affect gliotransmitter release and its functional consequences. Accordingly, the lack of effects reported in the above studies must be considered in their specific context and carefully weighed.

### Glial and Neuronal Modulation: Convergence of Two Different Time and Functional Domains

Since gliotransmitters are similar to known neurotransmitters and target receptors similar to those targeted by neurotransmitters, one is left wondering whether it is possible for gliotransmission to provide unique encoding in the brain. As a *third unifying hypothesis*, we propose that astrocytes represent an additional neuromodulatory system that acts in complement to the neuronal ones, but with its own time and space domains based upon the particular intrinsic properties of  $\text{Ca}^{2+}$  signaling that encode and integrate incoming inputs from neurons and other environmental sources.

The glial regulation provides an intermediate regulation between the direct neuronal modulation and the very slow and chronic “hormonal-like” regulation carried out by general brain homeostasis. Indeed, owing to their proximity to neurons and synapses, as well as the kinetics of the  $\text{Ca}^{2+}$ -dependent decoding of neuronal activity and glial elaboration, astrocytes can provide a balanced and easily tunable feedback or feedforward response that regulates neuronal communication in a different time domain. Moreover, astrocytes are in contact with thousands of synaptic inputs targeting many dendrites of several neurons. It has been estimated that an individual astrocyte contacts 300–600 neuronal dendrites in the cortex (Halassa et al., 2007) and oversees ~140,000 hippocampal synapses in the hippocampus (Bushong et al., 2002). This allows the astrocyte to integrate and filter a unique volume of synaptic activity. Hence, astrocytic integration and modulation encompasses neuron and synapse types to provide an analysis and output reflecting a unique complex neuronal and glial network.

Finally, in addition to synaptic inputs, astrocytes receive multiple signals and homeostatic information from different cellular sources, including neurons, vascular cells, other astrocytes, and even different types of glial cells. They process this diverse information to produce output signals that convey integrated information reflecting the complex microenvironment. Indeed, astrocytes play fundamental roles linking neuronal metabolic requirements and supply, sensing neuronal activity and providing energy support to neurons through the glucose/glycogen pathways (Magistretti et al., 1999; Pellerin and Magistretti, 1994)

and the regulation of blood flow for oxygen consumption and nutrients (Attwell et al., 2010; Gordon et al., 2008; Haydon and Carmignoto, 2006; Mulligan and MacVicar, 2004; Takano et al., 2006; Zonta et al., 2003). Similarly, astrocytes can exchange information concerning immune state with microglia and detect local pH and osmolality changes to control breathing (Gourine et al., 2010) and water homeostasis, respectively (Haj-Yasein et al., 2011). As a whole, astrocytes act as multiplexer integrators of metabolic, neuronal, and other cell signals.

In fact, the different time and space domains of neuronal encoding coupled with the diversity of their interactions, would allow astrocytes to perform complex and diversified modulation of neuronal functions that would contribute to the enrichment of information processing in the brain. For instance, this integration could feed back in a nonspecific, more homeostatic manner tuned with metabolic regulation. This would be quite powerful in setting a balanced tone of neuronal activity across large areas (Rouach et al., 2008). However, on the other hand of this spectrum, the multiplexing integrations in space and time by astrocytes would allow them also to perform very fine and selective regulation of neuronal activity generating various gradients of plasticity depending on location and properties of the glial and neuronal elements. Hence, astrocytes act as multiplexer integrators of multiple complex cell signals that would influence information processing in a wide array of time and space domains possibly complementing the neuronal processing.

### Conclusions

The field of neuron-glia interactions has grown enormously over the past two decades. In addition, the breath of techniques and approaches has also exploded, revealing the involvement of astrocytes from local synaptic circuitries (Di Castro et al., 2011; Fellin and Carmignoto, 2004; Henneberger et al., 2010; Navarrete et al., 2012; Panatier et al., 2011; Perea and Araque, 2007; Serrano et al., 2006; Takata et al., 2011) up to behavior (Halassa et al., 2009; Han et al., 2012; Saab et al., 2012; Tanaka et al., 2013). It is quite clear that astrocytes play a very large array of roles in multiple brain regions, utilizing a multitude of functional membrane receptors and signaling molecules. Yet, given the complexity and diversity at play, it is no surprise that the literature also reports some discrepant results regarding the roles played by astrocytes in the regulation of synaptic functions. These data highlight our limited understanding of the true nature of astrocytes and their interactions with neurons and point to future directions for research on neuron-glia interactions.

The complexity of such interactions is increasingly appreciated, with functional specificities possibly determined by the type of transmitter, synaptic circuit, or brain region involved, as well as by diversities ascribable to the physiological context of the studies and the age of the animals. For example, consider a recent study (Sun et al., 2013) reporting that expression of the astrocyte mGluR5 receptor decreases with age and that its pharmacological stimulation fails to produce somatic  $\text{Ca}^{2+}$  responses in mature brain astrocytes. From these observations, the authors concluded that glutamatergic tripartite synapses operate only during development. However, other data have demonstrated that  $\text{Ca}^{2+}$  elevations evoked in mature astrocytes by whisker stimulation are predominantly mediated by the



synaptic release of glutamate and activation of astrocytic mGluR5 (Wang et al., 2006) and that the mGluR5 is expressed in the perisynaptic processes of mature brain astrocytes (Di Castro et al., 2011; Lavielle et al., 2011). These latter data are not inconsistent with those of Sun et al., who did not study  $\text{Ca}^{2+}$  responses in astrocytic processes, and they suggest a different interpretation for the decrease in astrocyte mGluR5 expression in the mature brain, i.e., that the refinement of the synaptic circuitry leads to a restriction in the expression of the receptor to perisynaptic processes where it is needed for tripartite modulation (Arizono et al., 2012). This example highlights the current difficulties in correctly understanding the synaptic roles of astrocytes, and we must take a holistic approach to interpreting the literature, aiming to better understand the technical, physiological, and even interpretational reasons behind such discrepant results.

Following this reasoning, in this Perspective we have attempted a conceptual synthesis by proposing that astrocytes contribute to information processing by linking neuronal activities (as well as activities in other cell types) that occur on different spatial and temporal dimensions to achieve a higher level of integration of brain function. We offer three unifying concepts that we hope will provide a useful framework for the studies to come. First, astrocytes participate in synaptic integration differently from neurons. Their activation and output modulatory responses occur on temporal and spatial scales distinct from those of synaptic transmission and rely on the perisynaptic expression of high-affinity slow-desensitizing receptors in both astrocytes and neurons. Second, as integrative and regulatory entities, astrocytes offer a flexible system that can process information on multiple scales and cover spatial territories and temporal frames different from those offered by purely neuronal circuits. Third, during this function, astrocytes can enrich the integration by incorporating information coming from outside the synaptic world (e.g., from vascular, immune, and other cells), to fine tune the synaptic circuitry according to the environmental state.

With the present Perspective, we have tried to outline the complex choreography that exists between neurons and astrocytes, focusing on specific characteristics of the latter that render them central actors in brain function. Indeed, in our view, astrocyte signaling and gliotransmission represent the highly evolved integrative interface in brain communication that couples slow modulatory signaling from multiple sources with fast synaptic transmission.

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