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BIOPHYSICALLY BASED COMPUTATIONAL MODELS OF ASTROCYTE ~ NEURON COUPLING AND THEIR FUNCTIONAL SIGNIFICANCE

Topic Editors
John Wade, Scott Kelso, Vincenzo Crunelli, Liam J. McDaid and Jim Harkin

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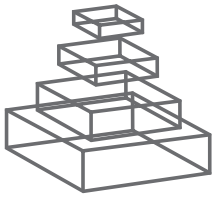

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Neuroscientists are increasingly becoming more interested in modelling brain functions where capturing the biophysical mechanisms underpinning these functions requires plausible models at the level of neuron cells. However, cell level models are still very much in the embryo stage and therefore there is a need to advance the level of biological realism at the level of neurons/synapses. Recent publications have highlighted that astrocytes continually exchange information with multiple synapses; if we are to fully appreciate this dynamic and coordinated interplay between these cells then more research on bidirectional signalling between astrocytes and neurons is required. A better understanding of astrocyte-neuron cell coupling would provide the building block for studying the regulatory capability of astrocytes networks on a large scale. For example, it is believed that local and global signalling via astrocytes underpins brain functions like synchrony, learning, memory and self repair.

This Research Topic aims to report on current research work which focuses on understanding and modelling the interaction between astrocytes and neurons at the cellular level (Bottom up) and at network level (Top down). Understanding astrocytic regulation of neural activity is crucial if we are to capture how information is represented and processed across large neuronal ensembles in humans.

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EDITORIAL
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Biophysically based computational models of astrocyte ~ neuron coupling and their functional significance

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Neuroscientists are becoming increasingly interested in modeling brain functions however, capturing underlying biophysical mechanisms requires plausible, biologically realistic models at the cellular level. Moreover, the (often conflicting) demands of biological realism and computational tractability have to be accommodated. Recent publications highlight the interaction of astrocytes with multiple synapses: if we are to fully appreciate this dynamic and coordinated interplay between cells, then more research on bidirectional communication between astrocytes and neurons is required. For example, a better understanding of astrocyte-neuron coupling may lead to providing building blocks for studying the regulatory capability of astrocytic networks on a larger scale. This Research Topic presents ten papers which cover a range of issues from computational models of astrocyte-neuron interactions to the role of astrocytes in neurological disorders.

In the paper by Gordleeva et al. (2012) the basic physiological functions of tripartite synapses and astrocytic regulation at the level of neural network activity is considered. The paper concludes that astrocytes support homeostatic regulation of network activity by modulating neural networks into a bistable regime of activity with two stable firing levels and spontaneous transitions between them. The comprehensive review article by Volman et al. (2012) deals with the premise that pathways for astrocyte-neuron interaction can enhance the information processing capabilities of brains, yet in other circumstances may lead the brain on the road to pathological ruin. The paper focuses on existing computational models of astrocytic involvement in epileptogenesis and their relevance to existing physiological data. Similarily, Min et al. (2012) review recent experimental literature on astrocyte-neuron interactions and discuss the dynamic effects of astrocytes on neuronal excitability and short- and long-term synaptic plasticity. Their paper also addresses potential computational functions of astrocyte-neuron interactions in the brain, in particular how astrocytes may enhance the computational power of neuronal networks in previously unexpected ways. Moreover, Fellin et al. (2012) provide a review of recent literature which highlights the role of astrocytes as essential modulators of neural network activity. The authors focus mainly on how astrocyte-neural interactions lead to the modulation of fundamental slow cortical oscillations and discuss the astrocyte-neural mechanisms underpinning this modulation. In the paper by Allam et al. (2012) a computational model is extended to show that glial transporters modulate synaptic transmission mediated by ionotropic AMPA and NMDA receptors at glutamatergic synapses. Their results demonstrate that astrocytes may play a crucial role in spike timing dependent processes with important implications for neurological diseases. Le Meur et al. (2012) use whole-cell recordings in rat acute brain slices and electron microscopy to test whether hippocampal astrocytes release the inhibitory transmitter GABA. They observe that slow transient inhibitory currents share characteristics with the slow NMDA receptor-mediated currents shown previously to result from astrocytic glutamate release. Their results provide quantitative characteristics of astrocyte-to-neuron GABAergic signaling and suggest that all principal neurons in the hippocampal network are under a dual, excitatory and inhibitory, influence of astrocytes. The relevance of astrocytic release of GABA and glutamate on the pathophysiology of the hippocampus remains to be established. The paper by De Pittà et al. (2012) considers the plausibility of astrocytes performing information processing by calcium signaling and hence their role in setting the basal tone of synaptic transmission. The work presents a theoretical treatment focusing on the modulation of synaptic release probability by the astrocyte and its implications for synaptic plasticity. The analysis of signaling pathways underlying such modulation refines the tripartite synapse model and has profound implications on our understanding of brain function.

In the paper by Wade et al. (2012) it is shown that retrograde signaling via astrocytes may underpin self-repair in the brain. Their model of self-repair is based on recent research showing that retrograde signaling mediated by astrocytes increases the probability of neurotransmitter release at damaged or low transmission probability synapses. Model simulations demonstrate that astrocytes are capable of bidirectional communication with neurons and that indirect signaling through retrograde messengers, such as endocannabinoids, leads to modulation of synaptic transmission probability. This opens up the possibility of developing highly adaptive, distributed computing systems that can, at fine levels of granularity, fault detect, diagnose, and self-repair autonomously. Reato et al. (2012) present a study of a parsimonious network model of neurons and astrocytes consisting of excitatory and inhibitory neurons described by Izhikevich-like neuron dynamics. Experimentally observed Ca^{2+} change in astrocytes in response to neuronal activity is modeled using linear equations. Their paper suggests that glutamate release from astrocytes above certain intracellular Ca^{2+} concentrations provides a non-linear positive feedback signal to neurons. The threshold of focal ictal discharge (ID) generation is lowered when an excitatory feedback-loop between

astrocytes and neurons is included. Simulations show that astrocytes can contribute to ID generation by directly affecting the excitatory/inhibitory balance of the neuronal network. Witcher and Ellis (2012) highlights the fact that more than one-third of all epilepsy patients have incompletely controlled seizures or debilitating medication side effects in spite of optimal medical management. The cellular mechanisms underlying seizure activity are not completely understood. Their paper describes multiple lines of evidence supporting the likely contribution of astroglia to epilepsy, focusing on individual astrocytes and their network functions. Witcher and Ellis (2012) conclude that astrocytes are likely important targets for the developing field of neuromodulation in the treatment of epilepsy.

In short, while the papers in this Research Topic are representative of current thinking in the field, they also draw attention to the fact that research into astrocytes and their interaction with neurons is still very much in the embryo stage.

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Computational models of neuron-astrocyte interaction in epilepsy

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Astrocytes actively shape the dynamics of neurons and neuronal ensembles by affecting several aspects critical to neuronal function, such as regulating synaptic plasticity, modulating neuronal excitability, and maintaining extracellular ion balance. These pathways for astrocyte-neuron interaction can also enhance the information-processing capabilities of brains, but in other circumstances may lead the brain on the road to pathological ruin. In this article, we review the existing computational models of astrocytic involvement in epileptogenesis, focusing on their relevance to existing physiological data.

Keywords: astrocytes, regulatory signaling, epileptogenesis, trauma, inflammation

INTRODUCTION

There has been an increasing effort to understand the etiology of brain disorders using computational models of the neural circuits that can predict the impact of changes in intrinsic neuronal properties and interneuronal coupling on the dynamics of neuronal networks. The ability of biophysically detailed models to address working hypotheses for brain disorders is especially important given the methodological and ethical concerns associated with the experimental studies on humans.

Models based on the biophysical underpinnings of brain pathologies, focusing on neurons, and neuronal networks, are useful in determining how different intrinsic and network properties can result in pathological activity. For example, in models of cortical networks, an imbalance in the ratio of fast excitation and inhibition can lead to epileptic seizures; however, this approach is primarily based on the relatively fast neuronal dynamics and thus cannot explain how the slow (>days) transition from normal to pathological state occurs. In contrast, some brain disorders (e.g., epilepsy and schizophrenia to provide few examples) may result from aberrations in slow, homeostatic, mechanisms that modulate the function of single neurons and properties of synaptic plasticity/connectivity. Thus, though useful, models of fast neuronal dynamics provide only limited insight into slowly developing pathological states of the brain, and a paradigm shift is needed for computational approaches to succeed in revealing the mechanisms underlying brain pathologies.

Neurons are critical for information processing in the human brain, but they comprise less than half of brain mass, which also includes glial cells. Glial cells are further subdivided into microglia, oligodendrocytes, Schwann cell, and astrocytes. Astrocytes are the most numerous glial cell type and account for up to one third of brain mass (Kandel, 1991). Because of their important role in glucose metabolism and maintenance of extracellular ion homeostasis, astrocytes have been viewed as "support cells," secondary to neurons and having little to do with brain activity *per se*. However, growing experimental evidence [reviewed in Seifert et al. (2006); Giaume et al. (2010); Halassa and Haydon (2010)] points to the possibility that glial cells (and astrocytes in particular) are actively involved in the modulation of synaptic transmission and neural excitability. This has led researchers to propose that astrocytes can dynamically redefine the functional architecture of neuronal networks (Nedergaard et al., 2003).

Unlike the fast millisecond time scale for action potential and fast chemical transmission, astrocytes are regulated mainly through calcium signaling on a much slower time scale of seconds to minutes, consistent with the slow time scale associated with homeostatic regulation of neuronal activity. Furthermore, astrocytes respond to chronic changes in neuronal activity (such as those incurred by trauma) by releasing various pro-inflammatory molecules that offset the perturbation. Thus, astrocyte-neuron signaling appears to be a plausible setting to explain at least part of the etiology of brain diseases linked to the aberrations in homeostatic regulation.

This review attempts to survey existing computational models of astrocytic involvement in disorders of the nervous system, with particular emphasis on models of epileptogenesis. The dynamical signature of epilepsy is hypersynchronization of collective neuronal activity, suggesting a breakdown in homeostasis. We also provide a perspective on what is needed to better understand how these non-neuronal cells contribute to brain pathologies.

MODULATION OF SYNAPTIC TRANSMISSION

Although hyperexcitability of individual neurons can significantly contribute to epileptogenesis, epilepsy is widely considered to be a "network disease" driven by aberrant synaptic interaction between neurons (McCormick and Contreras, 2001). Synaptic plasticity (in particular short-term synaptic depression and facilitation) can critically mold the functional strength of synaptic coupling; thus, it is possible that specific properties of synaptic transmitter release could predispose a network to seizures.

Astrocytes are ideally positioned to modulate synaptic contributions to epileptogenesis. Experimental data [reviewed in Araque et al. (1999)] shows that astrocytic processes are often apposed to synaptic junctions, giving rise to the notion of "tripartite synapse" consisting of presynaptic bouton, the astrocyte, and the postsynaptic density. Stimulation of synaptic boutons and subsequent release of neurotransmitter elicits complex calcium responses in nearby astrocytes, showing that these glial cells can "eavesdrop" on synaptic activity [reviewed in Haydon (2001)]. Elevation of astrocytic calcium culminates in the regulated release of molecules such as glutamate and/or ATP which, by diffusing in the extracellular space (ECS) and binding to dedicated receptors, can modulate the synaptic transmission (Araque et al., 1998) and the excitability of adjacent neurons (Parpura and Haydon, 2000; Reyes and Parpura, 2008) (**Figure 1**). It was shown recently that NMDA-R mediated astrocytic modulation of postsynaptic neuronal excitability can promote epileptogenesis in neuronal networks (Gomez-Gonzalo et al., 2010). This suggests the role for tripartite synapses not only in epilepsy, but perhaps also in other diseases of the nervous system that are based on aberrant synaptic communication (Halassa et al., 2007).

The first, to our knowledge, computational model that explored the potential involvement of astrocytes in synaptic mechanisms of epileptogenesis incorporated the basic notion of astrocytic "eavesdropping" on neuronal activity and assumed that the sole effect of neuronal activity-dependent astrocytic calcium elevations was to promote neuronal depolarization (Nadkarni and Jung, 2003; Appendix). This study concluded that astrocytes promote epileptogenesis through positive feedback. Similar conclusions were reached in another modeling study that showed that glutamate released from astrocytes could be responsible for paroxysmal depolarization shifts, often associated with epileptic activity (Silchenko and Tass, 2008). However, gliotransmitter released from astrocytes can either up- or down-regulate the release of glutamate from synaptic boutons (Araque et al., 1998; Zhang et al., 2003), and computational modeling studies suggested that such negative feedback modulation of synaptic transmission could account for the regulation of spontaneous paroxysmal activity in neural cultures (Volman et al., 2007). Thus, in the context of the tripartite synapse, astrocytic signaling could have either positive or negative impact on neuronal activity, or perhaps a combination of both. Revealing the mechanisms behind this diversity of modulating effects could help in understanding the conditions for seizure suppression or promotion by astrocytes.

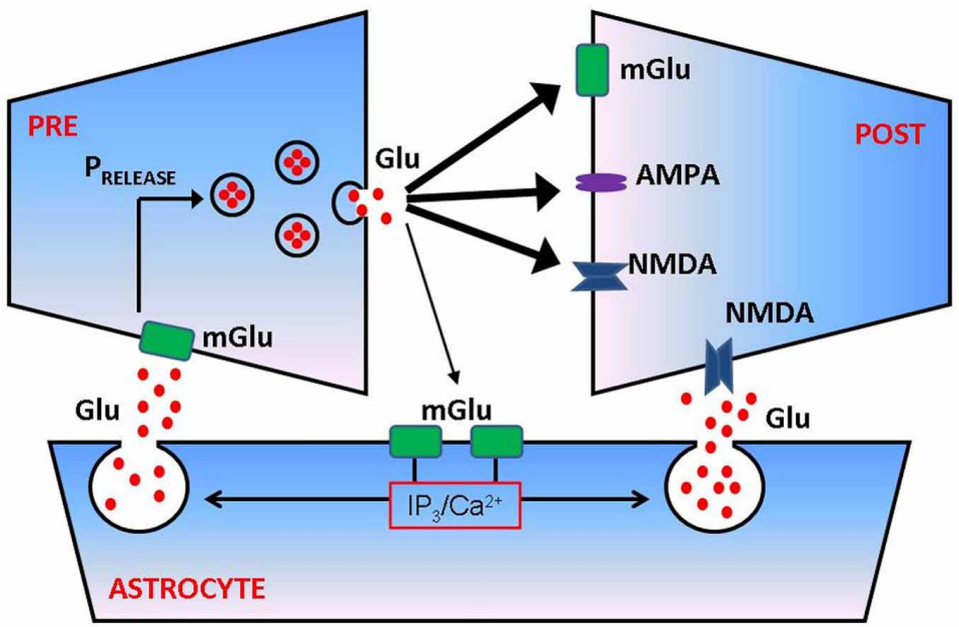

FIGURE 1 | Astrocytes regulate presynaptic transmission and postsynaptic excitability at glutamatergic synapse. Following successful vesicular fusion, a fraction of released glutamate can spill over and reach metabotropic glutamate (mGlu) receptors on the membrane of adjacent astrocyte. Glutamate activation of astrocytic mGlu receptors activates a cascade of biochemical events that culminates in elevation of inositol trisphosphate (IP3) and increased intra-astrocytic free calcium concentration. Calcium causes a release of astrocytic glutamate (a process termed gliotransmission) which can bind mGlu receptors on presynaptic membrane and/or NMDA receptors on postsynaptic side, thus modulating the probability of synaptic transmitter release ($P_{RELEASE}$) and/or postsynaptic excitability. For more quantitative details regarding the process of astrocyte modulation of synaptic transmission, see De Pitta et al. (2011).

Synaptic neurotransmitter release is a calcium-driven process; thus, a change in presynaptic calcium levels through glial modulation (for example by astrocytic activation of presynaptic receptor channels) could affect synaptic transmission. A computational modeling of a tripartite synapse supported the possibility that astrocytes may optimize the synaptic transmission of information by affecting the levels of presynaptic calcium (Nadkarni et al., 2008). In another recent modeling study (De Pitta et al., 2011), astrocytes either depressed or facilitated synaptic transmission, which suggested that the ultimate effect of astrocytes on transmitter release probability was determined by the interplay between the different mGlu-Rs (located presynaptically) and the baseline synaptic vesicle release probability. Incorporating these pathways, the model was successful at explaining several contradictory experimental observations regarding the role of astrocytes in the modulation of synaptic transmission.

REGULATION OF EXTRACELLULAR ION AND WATER LEVELS

Besides eavesdropping on synaptic activity, astrocytes also possess the means to "estimate" the level of neuronal activity in their proximity. The shape of a neuronal action potential is governed by ionic gradients across the membrane, but, in turn, the action potential itself causes changes in local ion concentrations (**Figure 2**). The principal effect is a spike-activity-dependent accumulation of potassium ions in the ECS. Astrocytes maintain potassium homeostasis by employing inward rectifying potassium channels to take up the excess K^+ that accumulates following action potential activity and redistributing K^+ ions across space compartments. Thus, astrocytes can monitor the level of neuronal activity through changes in extracellular potassium concentration.

Unlike astrocytic sensing of synaptic activity (which can in principle play a regulatory role in the flow of neural information, as mentioned below), astrocytic regulation of extracellular ion concentration is mostly associated with neuropathology. Physiological experiments and theoretical arguments (Frankenhaeuser and Hodgkin, 1956) show that elevated extracellular potassium can significantly increase the reversal potential of potassium channel conductance, thus rendering potassium currents depolarizing. Cumulatively, these observations led to the "high K^+" theory of epileptogenesis (Fisher et al., 1976; Lothman and Somjen, 1976), according to which activity-dependent elevation of extracellular potassium acts as a positive feedback mechanism promoting dynamical instability that culminates in seizure generation (Janigro et al., 1997) [reviewed in Frohlich et al. (2008b)]. Because astrocytes are involved in setting potassium levels (Janigro et al., 1997), it is imperative to understand how astrocytes contribute to "high K^+" epileptogenesis.

Early computational models that explored the role of high K^+ in epileptogenesis (Kager et al., 2000; Bazhenov et al., 2004; Frohlich et al., 2006, 2010) focused on the dynamics of single neurons and neuronal networks and made simplistic assumptions regarding the astrocytic mechanisms for potassium regulation (Appendix). More recent models have incorporated more diverse mechanisms of ion movement in single neurons (Cressman et al., 2009; Somjen et al., 2008; Krishnan and Bazhenov, 2011) and ion exchange between single neurons and astrocytes (Somjen et al., 2008; Oyehaug et al., 2011) (**Figure 2**). The interrelated dynamics of sodium and potassium currents during epileptogenesis were examined in a network model that incorporated both neuronal and glial cells (Ullah et al., 2009). Although,

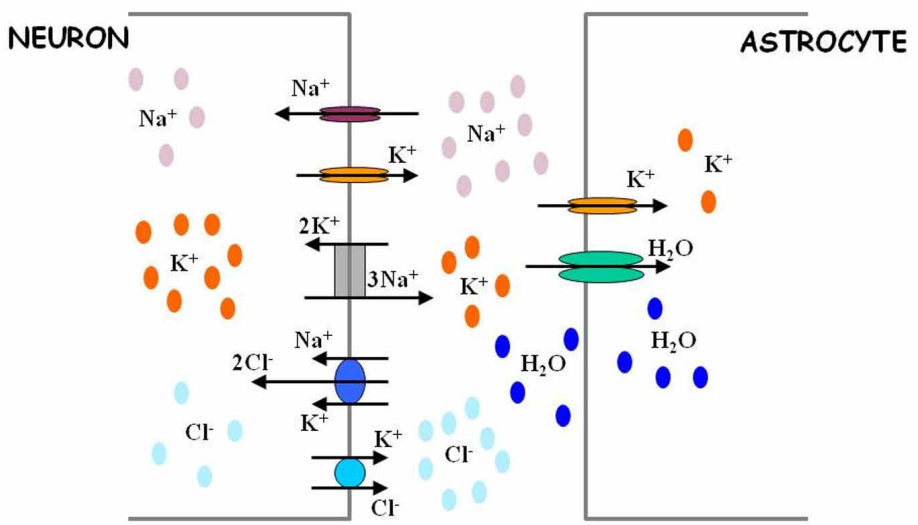

FIGURE 2 | Astrocytes regulate extracellular ion concentrations and water level. Neuronal excitability is defined in part by activity-dependent Nernst potentials of sodium (Na^+), potassium (K^+), and chloride (Cl^-) ions. The intracellular and extracellular concentrations of these ions are critically determined by the action of different ion transport mechanisms (sodium potassium pump and chloride co-transporters). Astrocytes possess potassium channels and thus are in position to regulate the levels of extracellular potassium. Through this regulation, astrocytes can also indirectly affect the levels of intracellular chloride ions in neurons. The efficacy of extracellular potassium regulation by astrocytes may be achieved by water-dependent regulation of extracellular space, as explained in Text. For more quantitative details regarding the process of ion level regulation by astrocytes, follow Ostby et al. (2009).

these studies highlighted different aspects of ion concentration regulation by astrocytes (pumps, transporters, etc.), they all converged to the same conclusion—the failure of astrocytes to maintain proper ionic micro-environment in neuronal surrounding can be critical for initiating epileptogenesis through the "high K^+" mechanism.

Several lines of evidence suggest that astrocytes could also affect potassium-mediated epileptogenesis through regulation of extracellular water levels (Simard and Nedergaard, 2004). Water movement in the brain is likely to involve aquaporin 4 (AQP4) channels, which are widely expressed in astrocytes (Nagelhus et al., 2004). Interestingly, AQP4 channels in astrocytes are co-localized with potassium Kir4.1 channels (Nagelhus et al., 2004). Furthermore, potassium clearance was significantly compromised in AQP4 knockout mice (Binder et al., 2006). In these $AQP4^{-/-}$ mice, the threshold for seizure generation was higher, but seizure duration was longer compared to controls (Amiry-Moghaddam et al., 2003; Binder et al., 2006). This suggests that astrocytic regulation of extracellular water could contribute to epileptogenesis during intense neuronal activity through the depolarization of astrocytes by the accumulation of extracellular K^+ ions. This depolarization would activate the electrogenic sodium bicarbonate cotransporter, favoring the uptake of these ions by the astrocyte. The uptake of sodium and bicarbonate ions by astrocytes would establish an osmolarity gradient (higher intracellular osmolarity) and thus would drive the water into glial cells through AQP4 channels, eventually contributing to astrocytic swelling and shrinkage of ECS. Activity-dependent shrinkage of ECS implies a smaller distribution volume for extracellular potassium, thus lowering the seizure threshold. The implications of activity-dependent, astrocyte-mediated shrinkage of ECS were recently explored in a computational model that incorporated a variety of astrocytic ion exchange mechanisms and water regulation (Ostby et al., 2009).

Epileptogenesis has also been shown to be facilitated by the intracellular accumulation of chloride in neurons (Dzhala et al., 2005). The electrochemical gradient of chloride defines the reversal potential of gamma-aminobutyric acid (GABA) receptor-mediated chloride currents, and thus determines the extent to which GABAergic synaptic signaling (normally assumed to have hyperpolarizing seizure-suppressing influence) is inhibitory. During physiologically normal activity, the intracellular chloride concentration is much lower than its extracellular levels, keeping E_{Cl} low and thus ensuring hyperpolarizing effect of GABAergic synapse. The high levels of activity that occur during seizures results in intracellular chloride accumulation and a higher GABA reversal potential, which can potentially lead to depolarizing GABA currents. In early development, intracellular chloride concentration is so high that GABA has a depolarizing effect on the neuronal membrane potential (Ben-Ari and Holmes, 2005). Increase of intracellular chloride concentration can also directly affect the resting potential and excitability of neurons. Using model incorporating Na^+, K^+, and Cl^- concentration dynamics, it was shown that an increase of intracellular chloride concentration extends seizure duration making possible long-lasting epileptic activity characterized by multiple transitions between tonic and clonic states (Krishnan and Bazhenov, 2011).

The impact of chloride on epileptogenesis primarily thus depends on the homeostasis of *intracellular* chloride concentration. Because astrocytes cannot directly access ion levels in neurons, astrocytes are not expected to be involved in chloride mechanisms in epileptogenesis. However, chloride homeostasis in neurons is affected by the opposing action of cation-chloride co-transporters, NKCC1 (importing two ions of chloride along with one ion of sodium and one ion of potassium) and KCC2 (exporting one ion of chloride along with one ion of potassium) (Payne et al., 2003) (**Figure 2**). The relative expression of these different chloride co-transporters directly affects seizure generation (Glykys et al., 2009; Dzhala et al., 2010). In particular, KCC2 co-transporter can mediate an effective coupling between extracellular potassium concentration and intracellular chloride concentration: a higher extracellular potassium level leads to higher intracellular chloride concentration (Payne et al., 2003). Thus, a dysfunction in extracellular potassium uptake by astrocytes could indirectly facilitate the destabilizing effect of intracellular chloride on a neuron's spiking activity.

ASTROCYTES IN POST-TRAUMATIC EPILEPSY

Traumatic brain injury (TBI) (e.g., as a result of penetrative wound) can increase the predisposition to epileptic seizures after a latent period following a traumatic event (Annegers et al., 1998). Although there is a clear causal link between TBI events and the later emergence of epileptic seizures, the etiology of "post-traumatic epilepsy" remains elusive (Agrawal et al., 2006).

A possible immediate outcome of TBI is the massive death of neuronal cells and damage to synaptic connectivity between neurons, which can create areas with chronic neuronal and synaptic inactivity. Evidence from *in vitro* studies suggests that chronic inactivity can modify several parameters of neuronal circuitry (e.g., synaptic connectivity, synaptic conductances, local neuronal morphology, intrinsic neuronal excitability) to compensate for the loss of activity incurred by trauma. As a general rule, chronic inactivity promotes the upregulation of depolarizing influences and downregulation of hyperpolarizing influences, while overexcitation leads to downregulation of depolarizing influences and upregulation of hyperpolarizing influences. This suggests that homeostatic regulatory pathways may be activated after traumatic event to offset the perturbation in electrical activity (Turrigiano, 1999). Regional homeostatic adjustments of neuronal excitability and synaptic transmission could contribute to breaching the excitation-inhibition balance (Fritschy, 2008), thus promoting seizure generation in traumatized networks (Timofeev et al., 2010). Understanding the mechanisms of trauma-triggered homeostatic plasticity could therefore generate insights into the etiology of post-traumatic epilepsy.

Several computational modeling studies have addressed the role of homeostatic plasticity in post-traumatic epilepsy. In one model of trauma that included cell death, post-traumatic axonal sprouting led to the emergence of seizure-like activity in an otherwise non-seizing network (Santhakumar et al., 2004;

Schneider-Mizell et al., 2010). This is consistent with earlier theories showing that network topology of synaptic connectivity can critically determine its seizing propensity (Netoff et al., 2004). In deafferentation models of cortical trauma (mimicking loss of excitation and chronic reduction of neuronal activity), homeostatic scaling of synaptic conductances could restore the "normal" firing rates, but this came at the expense of paroxysmal bursting (Houweling et al., 2005; Frohlich et al., 2008a; Appendix). Interestingly, the rate of paroxysmal activity depended on both intrinsic neuronal parameters (Houweling et al., 2005) and the spatial pattern of trauma (Volman et al., 2011b,c). Although these studies addressed different aspects of post-traumatic reorganization, all supported the notion that trauma-triggered homeostatic regulation can contribute to post-traumatic epileptogenesis.

Recent *in vitro* studies show that glial cells are critically involved in the homeostatic scaling of synaptic conductances that follows prolonged synaptic inactivity (Beattie et al., 2002; Stellwagen and Malenka, 2006; Steinmetz and Turrigiano, 2010). Following chronic synaptic inactivity, astrocytes release tumor necrosis factor alpha (TNFα) which diffuses and acts on postsynaptic neurons to scale up the number of AMPA/NMDA receptors and scale down the number of GABAa receptors (**Figure 3**). Thus, signaling by TNFα could represent a homeostatic regulatory mechanism compensating for a chronic reduction in neural excitability and could contribute to post-traumatic epileptogenesis. An observation in support of this hypothesis is that TNFα is released by glial cells early (1–2 h) after trauma, as a part of neuroinflammatory cascade. Computational models constructed with variable "glia" to explore this hypothesis concluded that glial release and diffusion of TNFα could affect post-traumatic paroxysmal activity (Savin et al., 2009).

THE NEXT FRONTIER

MODELING THE EFFECTS OF CELL MORPHOLOGY

In the first-generation computational models of astrocyte-neuron interaction, astrocytes and neurons were usually assumed to be point entities, neglecting completely their intracellular organization and dynamics. This simplifying assumption allowed the modulation of basic neural functions by astrocytes to be explored. However, the spatial aspects of cellular organization may also be important to understanding how glial cells affect brain pathologies. For example, the spatial co-localization of potassium and water transport mechanisms in astrocytes is involved in the regulation of traumatic edema and consequent post-traumatic epilepsy, as mentioned above.

Another elegant example of the importance of spatial organization is derived from energy considerations (Hertz et al., 2007). The cell body of a typical astrocyte accounts for only ∼2% of its total volume, with larger, branching organelle-containing processes constituting ∼60% (Hertz et al., 2007). The remaining ∼40% are taken by tiny (∼3 microns length by ∼0.2 microns width) filopodia and lamellipodia which cannot accommodate energy-efficient organelles such as mitochondria. This implies that the terminal thin processes (which regulate synaptic function) and thicker organelle-containing branches use different energy strategies for their function (Hertz et al., 2007). This could have important implications for the ability of astrocytes to modulate synaptic function.

In experimental models of epilepsy, astrocytes undergo significant rapid morphological remodeling following the traumatic event (Oberheim et al., 2008), a process known as reactive gliosis (Sofroniew, 2009). Specifically, astrocytes that are located relatively close (200 microns) to the boundary between intact and injured cortical regions lose their trademark star shape and elongate in the direction perpendicular to the trauma boundary (Oberheim et al., 2008). The implications of this remodeling for network function remain largely unknown, but it was recently proposed that trauma-induced morphological reorganization of astrocytes could reduce the incidence of paroxysmal activity by providing better functional segregation of synaptic input from intact vs. injured neurons (Volman et al., 2011d). Functional segregation of synaptic input could help localize the release of TNFα to the regions of synaptic inactivity and thus prevent pathological over-excitation of relatively intact areas.

Modeling the microphysiology of astrocyte-neuron interactions is a challenging problem without knowing the distribution of different proteins on the surfaces of astrocytes and the movement of ions across membranes. One promising approach is based on Monte Carlo simulations of cellular microphysiology, which can explore the impact of different astrocytic morphologies (Nadkarni et al., 2010).

NEURO-GLIAL NETWORKS

Much of the information exchange between neurons is achieved with fast chemical synapses (although some types of neurons are also coupled by gap junctions). In contrast, astrocytes lack chemical synapses but are extensively coupled by gap junctions, an "astrocytic syncytium" through which various substances are transported (Scemes and Spray, 2004). Given the coupling between astrocytic calcium and modulation of synaptic transmission, and the role of synaptic communication in neuronal network dynamics, gap junction coupling between astrocytes could affect pathological neuronal dynamics. Indeed, several studies found that the levels of astrocytic gap junction protein are compromised in experimental models of epilepsy (Lee et al., 1995; Fonseca et al., 2002), but the exact implications of this aberration are not clear yet.

Experimentally, mice with deficient gap junctional coupling between astrocytes exhibit impaired potassium clearance and higher extracellular potassium accumulation during synchronized neuronal firing (Wallraff et al., 2006); thus, astrocytic gap junctions could aid in potassium buffering. These early observations were re-validated in a recent study that also revealed, in a hippocampal preparation, that gap junctions between astrocytes are important regulators of synaptic transmission (Pannasch et al., 2011). These findings suggest that astrocytic networks might control the scale of activity in neuronal networks (and perhaps vice versa) through regulation of synaptic transmission, ECS volume, and extracellular ion homeostasis.

Computational modeling of neuronal network dynamics suggests that gap junctions between neurons can either promote

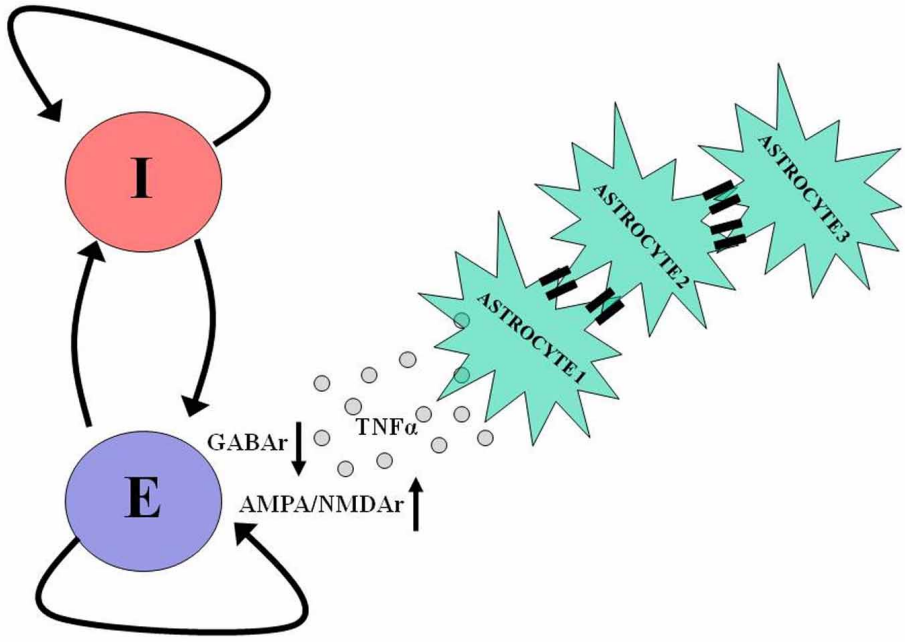

FIGURE 3 | Astrocytes take part in neuronal homeostatic plasticity. In response to prolonged neuronal and synaptic inactivity, as occurs following trauma, astrocytes release tumor necrosis factor alpha (TNFα), which diffuses and binds to its dedicated receptors on pyramidal neurons causing a reduction in the number of postsynaptic GABA receptors and an increase in the number of postsynaptic AMPA and NMDA receptors. Such homeostatic modulation aims to offset the decrease in neuronal activity by shifting the excitation-inhibition balance in favor of excitation. The participation of astrocytes in homeostatic plasticity can be further facilitated by slow (10s of seconds) intercellular communication via gap junctions between these cells. For more details regarding the role of astrocytes in homeostatic plasticity and intercellular communication, see Pannasch et al. (2011).

or suppress epileptic-like activity in a way that depends on the topology and strength of the coupling and the level of neuronal excitation. A surprising prediction of the model was that an increase in gap junctions may reduce membrane input resistance thus making a network of neurons less excitable (Volman et al., 2011a). Whether or not the same principles apply to calcium excitability in astrocytic networks is not clear. Gap junctions are usually modeled as linear electrical coupling between a pair of cells that is constant, but in fact their permeability to different ions can be modulated by several factors, including neural activity itself (Rouach et al., 2000). This adds an additional level of complexity to intercellular signaling between glial cells and implies that the "transfer function" of a gap junction could depend non-linearly on the permeating ion. One modeling study addressed the role of non-linear gap junctional transport in the context of astrocytic calcium waves (Goldberg et al., 2010) but further studies that account for their variable permeability and co-transport of different ions are needed.

Challenges associated with realistic modeling of intercellular astrocytic communication depend on the microphysiology of the cell since calcium waves propagate intracellularly at a speed of ∼15 microns/s but this can vary greatly throughout the cell depending on the topology of endoplasmic reticulum in which most of the cellular calcium is stored (Yagodin et al., 1994); consequently, the cellular morphology and the exact locations of gap junctions are likely to be important determinants of intercellular communication. This issue has been addressed studying the spatial characteristics of calcium waves in networks of reconstructed astrocytes (Kang and Othmer, 2009). This study confirmed that calcium waves in astrocytes are partially regenerative; however, the model used 2-dimensional projections of reconstructed astrocytes, and whether or not the same conclusions hold in realistic 3-dimensional models remains to be shown.

Activation of astrocytic regulatory pathways, such as the release of gliotransmitters, is triggered by changes in neuronal and synaptic activity. This in turn implies that the spatial distribution of neurons and the structure of synaptic connectivity influence glial signaling. Indeed, neuronal activity can itself shape the coupling properties of astrocytic syncytium (Rouach et al., 2000). Existing network models of neuronal glial interaction, such as the ring network model (Ullah et al., 2009), have circumvented this issue by assuming simplistic network topologies and connectivity. The implications of complex neuro-glial network topologies for normal and pathological dynamics remain to be studied.

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APPENDIX

MODELING TOOLBOX 1—GLIAL MEDIATED REGULATION OF SYNAPTIC NEUROTRANSMITTER RELEASE AND NEURAL EXCITABILITY

In response to mechanical stimulation or stimulation of G-protein coupled receptors, astrocytes can release a variety of factors, the most documented ones being ATP and glutamate (Araque et al., 1999; Zhang et al., 2003). Depending on the localization of its glial release site, the released glutamate can activate receptors on postsynaptic membrane (thus modulating neural excitability) or bind to its dedicated receptors on presynaptic membrane (and thus modulate presynaptic neurotransmitter release) (see also **Figure 1**).

Modulation of neural excitability by glial glutamate is a relatively well understood process, as it involves activation of postsynaptic NMDA receptors (Parpura and Haydon, 2000). Thus, glial-generated NMDA current can be modeled as:

$$I_{\mathrm{ASTRO}}^{\mathrm{NMDA}}(t) = \frac{g_F(t) - g_S(t)}{1 + 0.33\left[\mathrm{Mg}^{2+}\right]\exp(-0.06\,V_m)}(V_m - E_{\mathrm{NMDA}}) \tag{1}$$

$$\frac{dg_{F,S}}{dt} = \frac{-g_{F,S}}{\tau_{F,S}} + G_{\mathrm{ASTRO}}^{\mathrm{GLU}}(t) \tag{2}$$

where $G_{\mathrm{ASTRO}}^{\mathrm{GLU}}(t)$ represents the glutamate pulse due to glial glutamate release, the τ_F and the τ_S are the fast and slow NMDA time scales, $\left[\mathrm{Mg}^{2+}\right]$ is the extracellular level of magnesium, and V_m is neuronal membrane potential. However, this detailed modeling requires knowledge of $G_{\mathrm{ASTRO}}^{\mathrm{GLU}}(t)$ dependence on astrocytic calcium, information that is not fully available at the moment.

An alternative, phenomenological, way to model the effect of astrocytes on neuronal excitability was proposed by Nadkarni and Jung (2003):

$$I_{\mathrm{ASTRO}} = 2.11\Theta\left(\log(y)\right)\log(y) \tag{3}$$

$$y = \left[\mathrm{Ca}^{2+}\right]_{\mathrm{ASTRO}} - 196.69 \tag{4}$$

In equations (3)–(4), $\Theta(x)$ is the Heaviside function (taking on value of 1 if the argument is greater than zero and value of 0 otherwise), $\left[\mathrm{Ca}^{2+}\right]_{\mathrm{ASTRO}}$ is the concentration of free calcium (measured in nM) in astrocyte, and current is given in pA. Fit parameters were derived from experimental data [details in Nadkarni and Jung (2003)].

Astrocytic modulation of presynaptic function is more varied. A basic assumption, supported by experimental data, is that astrocytes modulate the properties of short-term synaptic plasticity (both short-term depression and short-term facilitation). On semi-phenomenological level, short-term synaptic plasticity is well captured with Tsodyks–Markram use model which assumes that at any given time, "synaptic resource" (proportional to the number of neurotransmitter vesicles) per synaptic bouton is limited and only a fraction $x(t)$ of it is available for release at time t (Tsodyks and Markram, 1997):

$$\frac{dx}{dt} = \Omega_R(1 - x) - \sum_i ux\delta(t - t_i) \tag{5}$$

$$\frac{du}{dt} = -\Omega_F u + U_0(\Gamma)\sum_i (1 - u)\delta(t - t_i) \tag{6}$$

In Equations (5)–(6), Ω_R is the rate of recovery from short-term synaptic depression, Ω_F is the rate of recovery from "astrocytic effects", and u is proportional to the probability of vesicular release. The modulating effect of astrocytes on synaptic transmission is captured with the "gating function," $U_0(\Gamma)$. The exact form of this gating function depends on the specifics of synapse under study [i.e., different physiological and ultra-structural parameters of a synapse will result in different functional forms of $U_0(\Gamma)$]. A detailed discussion of astrocytic modulation of presynaptic function is provided in the article by De Pitta et al. in this issue, and in De Pitta et al. (2011).

MODELING TOOLBOX 2—GLIAL MEDIATED REGULATION OF ION CONCENTRATIONS

In physiological conditions, potassium and chloride currents generate hyperpolarizing influences on neuronal membrane potential and thus can be considered to be seizure-suppressing; however, in pathological situations these currents become depolarizing due to the change in their reversal potentials. Reversal potentials of potassium and chloride currents are determined by the electrochemical gradients of their constituent ions:

$$E_{\mathrm{K}} = 26.64 \cdot \log\left(\frac{\left[\mathrm{K}^+\right]_{\mathrm{OUT}}}{\left[\mathrm{K}^+\right]_{\mathrm{IN}}}\right)\ [\mathrm{mV}];$$

$$E_{\mathrm{Cl}} = 26.64 \cdot \log\left(\frac{\left[\mathrm{Cl}^-\right]_{\mathrm{IN}}}{\left[\mathrm{Cl}^-\right]_{\mathrm{OUT}}}\right)\ [\mathrm{mV}] \tag{7}$$

so the high extracellular (intracellular) concentration of potassium (chloride) shifts the reversal potential toward more positive values.

The dynamics of extracellular potassium concentration can be captured with the following equation:

$$\frac{d\left[\mathrm{K}^+\right]_{\mathrm{OUT}}}{dt} = \sum I_{\mathrm{K}} + \left(\Delta\left[\mathrm{K}^+\right]\right)_{\mathrm{DIFF}} - G \tag{8}$$

in which $\sum I_{\mathrm{K}}$ is the sum over all neuronal potassium currents (from ion channels and/or pumps), $\left(\Delta\left[\mathrm{K}^+\right]\right)_{\mathrm{DIFF}}$ is the contribution from potassium diffusion in extracellular space, and G is the term that represents buffering of extracellular potassium by astrocytes. Relatively detailed biophysical models exist to describe glial buffering of potassium, based on activation of glial potassium channels (Oyehaug et al., 2011); however, in many cases it is convenient to describe glial buffering in a semi-phenomenological way by assuming the existence of glial buffer, [B], that binds free potassium ions. Glial contribution

can then be written as:

$$G = \frac{-d[B]}{dt} = k_{\mathrm{ON}}\left[\mathrm{K}^+\right]_{\mathrm{OUT}}[B] - k_{\mathrm{OFF}}([B]_T - [B]) \quad (9)$$

$$k_{\mathrm{ON}} = \frac{k_{\mathrm{OFF}}}{1 + \exp\left(\frac{[\mathrm{K}^+]_{\mathrm{TH}} - [\mathrm{K}^+]_{\mathrm{OUT}}}{\alpha_{\mathrm{KG}}}\right)} \quad (10)$$

where k_{ON} and k_{OFF} are forward (binding) and backward (unbinding) rates, correspondingly, $[\mathrm{B}]_{\mathrm{T}}$ is the total buffer concentration (usually assumed to be very high), and $[\mathrm{K}^+]_{\mathrm{TH}}$ is the threshold concentration of extracellular potassium above which glial buffer is activated (∼10 mM) (Kager et al., 2000; Krishnan and Bazhenov, 2011).

Because of the electrogenic nature of $\mathrm{Na}^+/\mathrm{K}^+$ exchange pump bringing in 2 K^+ ions in exchange for 3 Na^+ ions, increase of the extracellular K^+ (or intracellular Na^+) concentration leads to the outward current that may hyperpolarize the cell. This current can be modeled as (Kager et al., 2000; Krishnan and Bazhenov, 2011):

$$I^{\mathrm{pump}} = I_{\mathrm{K}}^{\mathrm{pump}} + I_{\mathrm{Na}}^{\mathrm{pump}}, I_{\mathrm{K}}^{\mathrm{pump}} = -2I_{\max}A, \quad I_{\mathrm{Na}}^{\mathrm{pump}} = 3I_{\max}A \quad (11)$$

$$A = (1/(1 + (K_{o\alpha}/[K_o])))^2(1/(1 + (Na_{i\alpha}/[Na_i]))) \quad (12)$$

where $\mathrm{K}_{o\alpha}$ and $\mathrm{Na}_{i\alpha}$are baseline ionic concentrations.

The dynamics of intracellular chloride concentration can be modeled with an equation similar to equation (8):

$$\frac{d\left[\mathrm{Cl}^-\right]_{\mathrm{IN}}}{dt} = \sum I_{\mathrm{Cl}} + \frac{\left(\left[\mathrm{Cl}^-\right]_{\mathrm{IN}} - \left[\mathrm{Cl}^-\right]_{I\infty}\right)}{\tau_{\mathrm{Cl}}} \quad (13)$$

where the characteristic time τ_{Cl} is the inverse of rate of chloride extrusion by KCC2 exporter. The KCC2 mediated effective coupling between extracellular potassium concentration and intracellular chloride concentration can be modeled as (Krishnan and Bazhenov, 2011):

$$\tau_{\mathrm{Cl}} = \tau_{\mathrm{BASE}} + \frac{\tau_{\mathrm{Cl}\infty}}{1 + \exp\left(\frac{[\mathrm{Cl}^-]_{I\infty} - [\mathrm{K}^+]_{\mathrm{OUT}}}{\alpha_{\mathrm{KCl}}}\right)} \quad (14)$$

MODELING TOOLBOX 3—GLIAL MEDIATED HOMEOSTATIC SYNAPTIC PLASTICITY

Homeostatic synaptic plasticity (HSP) allows neurons and networks to adjust synaptic conductance in order to offset the effect of long-term perturbation on their firing rates (Turrigiano, 1999). Astrocytes contribute to neuronal HSP by releasing tumor necrosis factor alpha (TNFα) that binds to neuronal TNFα receptors and causes insertion and removal of AMPA and GABA receptors, correspondingly (Stellwagen and Malenka, 2006). Astrocytic release of TNFα was observed to occur following prolonged neuronal inactivity (Stellwagen and Malenka, 2006). Given that astrocytes "sense" neuronal activity through glutamate levels, it is plausible to assume that astrocytic involvement in HSP is triggered by prolonged inactivity of pyramidal (PY) neurons. In the most general form, this information can be captured with the following equations:

$$g_{\mathrm{AMPA}} = g_{\mathrm{AMPA}} + \alpha_{\mathrm{HSP}}^{\mathrm{GLU}} \cdot \left(f_0 - \langle f \rangle_{T,\mathrm{PY}}\right) \quad (15)$$

$$g_{\mathrm{GABA}} = g_{\mathrm{GABA}} - \alpha_{\mathrm{HSP}}^{\mathrm{GABA}} \cdot \left(f_0 - \langle f \rangle_{T,\mathrm{PY}}\right) \quad (16)$$

In Equations (15)–(16), f_0 is the preset target rate of PY neurons, $\langle f \rangle_{T,\mathrm{PY}}$ is the averaged (over time and PY neurons) firing rate of PY neurons in "astrocytic domain" (Volman et al., 2011d), and $\alpha_{\mathrm{HSP}}^{\mathrm{GLU}}$, $\alpha_{\mathrm{HSP}}^{\mathrm{GABA}}$ are the rates of HSP convergence for specific type of synaptic receptor. Because astrocytic TNFα can only up regulate synaptic conductance, Equations (15)–(16) can only be applied in the case when $\langle f \rangle_{T,\mathrm{PY}} \leq f_0$ (i.e., recovery from prolonged inactivity). Homeostatic down regulation of neuronal excitability is likely achieved through other neuronal mechanisms (Turrigiano, 1999). This "division of labor" between astrocytes and neurons may result in non-trivial collective activity following perturbations such as, e.g., brain trauma (Volman et al., 2011d).

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GABA release by hippocampal astrocytes

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Astrocytes can directly influence neuronal activity through the release of various transmitters acting on membrane receptors expressed by neurons. However, in contrast to glutamate and ATP for instance, the release of GABA (γ-amino-butyric acid) by astrocytes is still poorly documented. Here, we used whole-cell recordings in rat acute brain slices and electron microscopy to test whether hippocampal astrocytes release the inhibitory transmitter GABA. We observed that slow transient inhibitory currents due to the activation of $GABA_A$ receptors occur spontaneously in principal neurons of the three main hippocampal fields (CA1, CA3, and dentate gyrus). These currents share characteristics with the slow NMDA receptor-mediated currents previously shown to result from astrocytic glutamate release: they occur in the absence of synaptic transmission and have variable kinetics and amplitudes as well as low frequencies. Osmotic pressure reduction, known to enhance transmitter release from astrocytes, similarly increased the frequency of non-synaptic GABA and glutamate currents. Simultaneous occurrence of slow inhibitory and excitatory currents was extremely rare. Yet, electron microscopy examination of immunostained hippocampal sections shows that about 80% of hippocampal astrocytes [positive for glial fibrillary acidic protein (GFAP)] were immunostained for GABA. Our results provide quantitative characteristics of the astrocyte-to-neuron GABAergic signaling. They also suggest that all principal neurons of the hippocampal network are under a dual, excitatory and inhibitory, influence of astrocytes. The relevance of the astrocytic release of GABA, and glutamate, on the physiopathology of the hippocampus remains to be established.

Keywords: gliotransmission, glia, pyramidal cells, granule cells, glutamate, GABA, taurine

INTRODUCTION

In the central nervous system, astrocytes are in close apposition with neurons and synapses (Schikorski and Stevens, 1997; Grosche et al., 1999; Ventura and Harris, 1999) with which they establish reciprocal functional interactions (Araque et al., 2001; Auld and Robitaille, 2003; Perea et al., 2009). Indeed, beside their ability to sense neuronal activity through the activation of receptors and transporters for neurotransmitters (Marcaggi and Attwell, 2004; Schipke and Kettenmann, 2004), astrocytes can also release chemical transmitters that modulate neuronal excitability and synaptic transmission (Volterra and Meldolesi, 2005; Hamilton and Attwell, 2010; Perea and Araque, 2010; Zorec et al., 2012).

Within the growing list of transmitters released by glia (or gliotransmitters), glutamate, ATP, and more recently D-Serine have received much interest (Volterra and Meldolesi, 2005; Fiacco and McCarthy, 2006; Haydon and Carmignoto, 2006; Oliet and Mothet, 2006). However, other classical transmitters are also released by glial cells and modulate neuronal activity. This has been clearly demonstrated for taurine in the supraoptic nucleus of the hypothalamus where, in response to a decrease of plasma osmolarity, astrocytes release this inhibitory amino acid which then inhibits vasopressin neurons through the activation of glycine receptors (Hussy, 2002 for review). Despite early reports showing the accumulation of γ-amino-butyric acid (GABA) by glial cells (Barres et al., 1990; Gallo et al., 1991; Ochi et al., 1993), the idea that this major inhibitory transmitter of the mammal central nervous system could also be a gliotransmitter has not been so far widely held (Angulo et al., 2008). Recently, however, studies performed in primary cultures (Liu et al., 2000) and in acute brain slices (Barakat and Bordey, 2002; Kozlov et al., 2006; Lee et al., 2010; Jimenez-Gonzalez et al., 2011) demonstrated a release of GABA from glial cells. In particular, astrocytes in the olfactory bulb and in the thalamus inhibit neighboring neurons through the release of GABA which induces slow transient currents mediated by the activation of GABA-A receptors (Kozlov et al., 2006; Jimenez-Gonzalez et al., 2011). These membrane currents have on average slower and more variable kinetics than GABA-A receptor-mediated inhibitory postsynaptic currents (IPSCs). Despite their inhibitory nature, several characteristics of these slow GABAergic currents resemble those of NMDA receptor-mediated currents due to the astrocytic release of glutamate described in other brain areas (Parri et al., 2001; Angulo et al., 2004; Fellin et al., 2004; Perea and Araque, 2005;

D'Ascenzo et al., 2007). In particular, slow GABAergic currents can occur simultaneously in neighboring neurons of the olfactory bulb (Kozlov et al., 2006) as this is the case for slow glutamatergic currents of glial origin in the hippocampus (Angulo et al., 2004; Fellin et al., 2004). Interestingly, both excitatory and inhibitory slow currents of glial origin were recorded in granule cells of the olfactory bulb (Kozlov et al., 2006).

The purpose of the present study was to investigate whether inhibitory astrocyte-to-neuron signaling operates in the hippocampus and whether it co-exists with excitatory gliotransmission. Our results show that neurons of all hippocampal fields are under a dual GABAergic and glutamatergic astrocytic influence and that GABA is present in a large proportion, if not all, hippocampal astrocytes.

MATERIALS AND METHODS

SLICE PREPARATION

All experiments followed European Union and institutional guidelines for the care and use of laboratory animals (Council directive 86/609EEC). Fourteen- to 29-day-old Wistar rats were either anaesthetized with an intraperitoneal (i.p.) injection of a mixture of ketamine (65 mg/kg)/xylazine (14 mg/kg) or humanely killed by cervical dislocation and decapitated. Transverse hippocampal slices (400 µm) were cut in an oxygenated ice-cold solution containing (in mM): 235 Sucrose, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 7 $MgCl_2$, 20 Glucose, 26 $NaHCO_3$, and 5 Pyruvate. The slices were incubated at 34°C for 30 min and then maintained at room temperature for 0.5–4 h in an oxygenated physiological solution containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 20 Glucose, 26 $NaHCO_3$, and 5 Pyruvate. Finally the slices were transferred into a recording chamber perfused at 2.5 $ml.min^{-1}$ with the same solution. Recordings were performed at room temperature. Since lowering osmolarity of the extracellular solution could favor the generation of transient currents (see "Results") a hypotonic solution containing (in mM): 96 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 2 $CaCl_2$, 1 $MgCl_2$, 20 Glucose, 26 $NaHCO_3$, and 5 Pyruvate has been used in some experiments. In preliminary experiments we substituted chloride for choline to verify that the effect of the hypotonic solution was not due to chloride concentration reduction ($n = 3$, data not shown).

For experiments aiming at inhibiting the vacuolar H^+-ATPase the slices were incubated for 2.5 h at 34°C in the physiological solution containing 4 µM of the inhibitor bafilomycin A1; the control slices were from the same rat and were incubated in the same conditions but without bafilomycin A1. In all treated slices we verified that no synaptic currents occurred spontaneously or when adding 10 mM potassium chloride to the standard extracellular solution (Angulo et al., 2004; Le Meur et al., 2007).

ELECTROPHYSIOLOGY

Pyramidal neurons from the subiculum, the CA1 and CA3 regions, and granule cells from the dentate gyrus were visually identified by means of infrared videomicroscopy. Whole-cell recordings were performed with an intracellular solution containing (in mM): 104 CsGluconate, 10 TEACl, 1 $MgCl_2$, 10 HEPES, 10 BAPTA, 5 phosphocreatine, 2 ATP, and 0.3 GTP, the pH was adjusted to 7.3 with CsOH. With this intracellular solution patch pipettes had a resistance of 3–5 MΩ. All potentials were corrected for a junction potential of −10 mV. Most recordings were performed at a holding potential of –30 mV to distinguish excitatory and inhibitory currents on the basis of their polarity. The reversal potential for chloride (E_{Cl}) calculated with the Nernst equation was −62 mV.

DATA COLLECTION AND ANALYSIS

Membrane currents were recorded using an Axopatch 200B (Molecular Devices, Sunnyvale, CA, USA) amplifier. They were filtered at 2–5 kH, digitized at 5–20 kHz with a 1322A Digidata (Molecular Devices, Sunnyvale, CA, USA). Series resistance was not compensated but was regularly monitored throughout the experiment using a −1 mV step and recordings showing unstable (>20% increase) series resistance were rejected. Acquisitions and off-line analysis were performed using pClamp9 softwares (Molecular Devices, Sunnyvale, CA, USA). For evaluating statistical differences between two samples Student's *t*-test was performed; when more than two samples were compared an ANOVA test was used, followed by Dunnett's or Tukey tests. Differences were considered to be significant if $p < 0.05$. Values are given as mean ± standard error to the mean (SEM), and n refers to the number of cells unless otherwise stated.

To calculate the probabilities for the occurrence by chance of double events (biphasic currents) resulting from simultaneous slow inward currents (SICs) and slow outward currents (SOCs), we used a Monte Carlo sampling method. We used the average frequencies of SICs and SOCs observed during the 3 min of hypotonic solution applications in 21 DG neurons to estimate the lambda parameters of Poisson laws from which we generated the simulated distributions of SICs and SOCs in 10,000 sample cells, in time windows of 500 ms (corresponding to upper limit of SOC duration), during a period of 3 min. For each sample cell the SICs and SOCs distributions were digitized into 0 (no event) or 1 (at least 1 event) to create a SIC and a SOC vector. Then the SIC and SOC vectors were multiplied (so that the value 1 corresponded to a double event in the resulting vector), and the number of double events was counted for each sample cell. Finally, the sample cells were categorized according to their number of double events, and the frequencies for the observations of different numbers of double events were calculated.

DRUGS

Tetrodotoxin (TTX) was purchased from Latoxan (Valence, France). Strychnine, Gabazine, ATP 2,3-Dioxo-6-nitro-1,2,3,4-tetrahydrobenzo[F]quinoxaline-7-sulfonamine disodium salt (NBQX disodium salt), D-(-)-2-Amino-5-phosphonopentanoic acid (D-AP5), Bafilomycin A1 were purchased from Tocris Cookson (Bristol, UK).

DOUBLE GABA–GFAP (GLIAL FIBRILLARY ACIDIC PROTEIN) IMMUNOCYTOCHEMISTRY FOR ELECTRON MICROSCOPY

Three Wistar rats were deeply anesthetized by i.p. injection of a mixture of Nembutal (5 mg/100 g body weight; Abbott Laboratories Inc., IL, USA) and urethane (130 mg/100 g body weight; Sigma-Aldrich, St. Louis, MO, USA). They were transcardially perfused with PBS (0.1 M, pH 7.4) and then fixed

with 500 ml of 2% glutaraldehyde and 1% formaldehyde in PBS. Perfusates were used at 4°C. Tissue blocks were extensively rinsed in 0.1 M PBS (pH 7.4).

Coronal hippocampal vibrosections were cut at 50 μm and collected in 0.1 M PBS (pH 7.4) at RT. Sections were pre-incubated in a blocking solution of 10% bovine serum albumin (BSA), 0.1% sodium azide and 0.02% saponin prepared in Tris-HCl buffered saline (TBS, pH 7.4) for 30 min at RT. A pre-embedding silver-intensified immunogold method and an immunoperoxidase method were used for the co-localization of GABA and GFAP in hippocampal sections. They were incubated in primary rabbit anti-GABA (1:1000; Somogyi and Hodgson, 1985) and mouse anti-GFAP (1:1000; Sigma Chemical Company St. Louis, MO, USA) antibodies both in 10% BSA/TBS containing 0.1% sodium azide and 0.004% saponin on a shaker for 2 days at 4°C. After several washes in 1% BSA/TBS, tissue sections were incubated in a secondary 1.4 nm gold-labeled anti-rabbit IgG (Fab' fragment, 1:100, Nanoprobes Inc., Yaphank, NY, USA) for the detection of GABA, and in a biotinylated anti-mouse IgG (1:200, Vector Laboratories Burlingame, CA, USA) for the detection of GFAP, both in 1% BSA/TBS with 0.004% saponin on a shaker for 4 h at RT. Tissue was washed in 1% BSA/TBS and processed by a conventional avidin–biotin horseradish peroxidase complex method (ABC; Elite, Vector Laboratories Burlingame, CA, USA). Thereafter, tissue was washed again in 1% BSA/TBS overnight at 4°C and postfixed in 1% glutaraldehyde in TBS for 10 min at RT. Following washes in double-distilled water, gold particles were silver-intensified with a HQ Silver kit (Nanoprobes Inc., Yaphank, NY, USA) for about 12 min in the dark and then washed in 0.1 M PB (pH 7.4). Sections were incubated subsequently with 0.05% DAB in 0.1 M PB and 0.01% hydrogen peroxide for 5 min and washed in 0.1 M PB for 2 h at RT.

Stained sections were osmicated (1% OsO4 in 0.1 M PB, pH 7.4, 20 min), dehydrated in graded alcohols to propylene oxide and plastic embedded flat in Epon 812. Eighty nanometer ultrathin sections were collected on mesh nickel grids, stained with uranyl acetate and lead citrate, and examined in a PHILIPS EM2008S electron microscope. Tissue preparations were photographed by using a digital camera coupled to the electron microscope. Figure compositions were scanned at 500 dots per inch (dpi). Labeling and minor adjustments in contrast and brightness were made using Adobe Photoshop (CS, Adobe Systems, San Jose, CA, USA).

ANALYSIS OF THE PROPORTION OF GABA-IMMUNOLABELED GFAP+ PROFILES IN HIPPOCAMPAL CA1 AND CA3

Rat hippocampal sections processed for the co-localization of GABA and GFAP with pre-embedding immunocytochemistry were used for semiquantitative analysis. Tissue showing good and reproducible DAB immunoreaction and silver-intensified gold particles were cut at 80 nm. Electron micrographs (10,000–25,000X) were taken from grids (132 μm side) containing DAB immunodeposits; all of them showed a similar DAB labeling intensity indicating that selected areas were at the same depth. Furthermore, to avoid false negatives, only ultrathin sections in the first 1.5 μm from the surface of the tissue block were examined. Positive labeling was considered if at least one immunoparticle was within the cellular profile. Percentages of GABA+ and GABA– astrocytic elements with GAFP were analyzed using a statistical software package (GraphPad Prism 4, GraphPad Software Inc, San Diego, USA).

RESULTS

SLOW INWARD AND OUTWARD CURRENTS IN PRINCIPAL NEURONS OF THE HIPPOCAMPUS

We recorded membrane currents from principal neurons of the hippocampus held at a holding potential of −30 mV to distinguish excitatory and inhibitory currents on the basis of their polarity (see "Materials and Methods"). As previously described (Angulo et al., 2004), in the presence of TTX SICs of glial origin occurred spontaneously at a low frequency in pyramidal neurons of the subiculum and the CA1 regions (**Figures 1A,B**). A 10–90% rise time >10 ms was used to distinguish SICs from synaptic currents (Angulo et al., 2004). Similar currents were also observed at slightly higher frequencies in CA3 and DG principal neurons (**Figures 1A,B**). The SIC average frequencies were 0.35 ± 0.03 and 0.29 ± 0.07 events/min in CA3 and in DG, respectively. The 10–90% rise time of SICs was 85.87 ± 10.68 ms and 37.18 ± 4.56 ms in CA3 ($n = 6$ cells, 114 SICs) and in the DG ($n = 10$, 29 SICs), respectively. The amplitude of SICs did not differ significantly in the different regions of the hippocampus and had a mean value of 101.26 ± 10.70 pA ($n = 259$) at a holding potential of −30 mV. Furthermore, bath application of D-AP5 (50–100 μM) or MK801 (50 μM) abolished SICs recorded in CA3 and in DG neurons ($n = 5$; data not shown). Thus, SICs in CA3 and DG principal neurons were generated by the activation of NMDA receptors and were most probably due to the release of glutamate from astrocytes as this is the case for SICs observed in other central neurons (Angulo et al., 2004; Fellin et al., 2004; Perea and Araque, 2005; Kozlov et al., 2006; D'Ascenzo et al., 2007; Pirttimaki and Parri, 2012). It should be noted, however, that values of SIC frequency and amplitude observed here are smaller than those reported in the above mentioned studies. This is most likely due to our recording conditions adjusted to record simultaneously SICs and SOCs, setting smaller driving force for non-selective cationic currents (and for chloride currents) and thus leading to a less favorable signal to noise ratio for the detection of these events.

In addition to SICs, SOCs were also recorded from neurons of all hippocampal regions (**Figures 1A,B**). A 10–90% rise time >7.5 ms was used to distinguish these slow currents from synaptic currents (see below). Frequency of SOCs was close to that of SICs except in CA1 pyramidal cells in which SOC frequency was about 3 times lower than SIC frequency (0.04 ± 0.02 events/min for SOCs and 0.14 ± 0.02 events/min for SICs; **Figure 1B**). SOC frequencies in DG and CA3 neurons were 0.15 ± 0.04 events/min and 0.34 ± 0.04 events/min, respectively (**Figure 1B**). In the olfactory bulb, we showed that SOCs recorded in mitral cells and in granule cells were due to the release of GABA from astrocytes activating neuronal GABA-A receptors (Kozlov et al., 2006). Thus, the occurrence of SOCs in hippocampal neurons suggests that an inhibitory gliotransmitter is also released in the hippocampus.

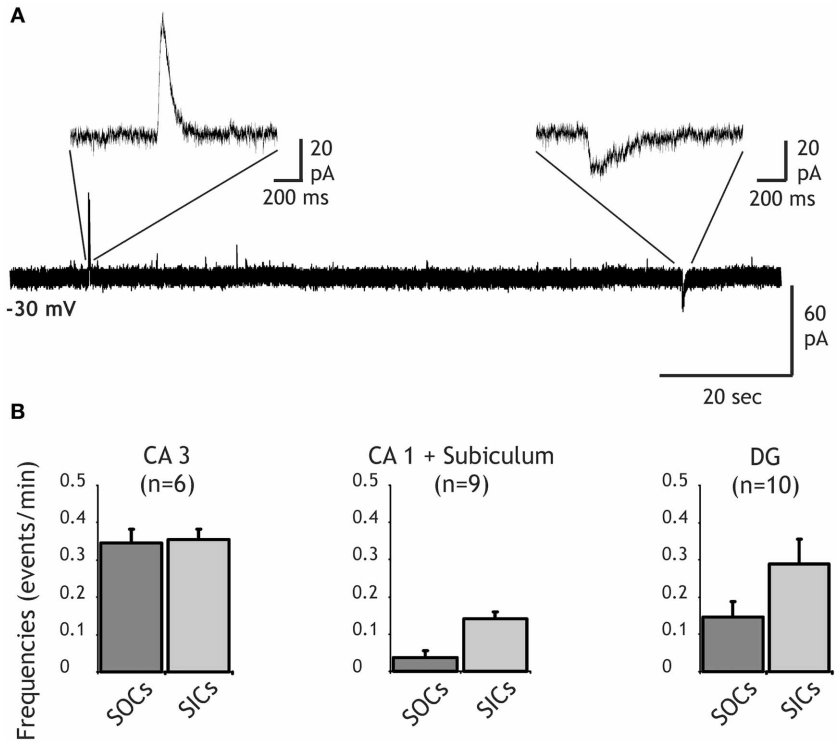

FIGURE 1 | Distribution of SIC and SOC frequencies in principal neurons of the hippocampus. (A) Recording from a dentate gyrus granule cell held at a membrane of −30 mV in the presence of TTX (1 μM) and NBQX (10 μM). A slow outward current (SOC) and a slow inward current (SIC) are shown with an expanded time scale. **(B)** Frequency histograms of SOCs (dark bars) and SICS (light bars) recorded in pyramidal cells of CA3 (left), CA1 and the subiculum (middle) and in granule cells of the dentate gyrus (right).

CHARACTERISTICS OF SOCs IN THE HIPPOCAMPUS

In a first step to characterize further SOCs in the hippocampus we tested whether they could occur independently of synaptic transmission. We therefore abolished vesicular release by incubating slices for several hours with bafilomycin A1 (4 μM), a blocker of vesicular H^+-ATPases (Drose and Altendorf, 1997). The abolition of the H^+ gradient prevents the vesicular accumulation of transmitters and thus synaptic transmission (Zhou et al., 2000; Rossi et al., 2003). In slices treated with bafilomycin A1 and for which we verified that synaptic release was actually abolished (**Figures 2A,B**; see also "Materials and Methods") SOCs were still observed in neurons from all hippocampal fields. We concentrated our analysis, however, on CA3 and DG neurons in which SOCs were more frequent than in CA1 cells (see **Figure 1**). The SOC frequencies in slices treated with bafilomycin A1 were 0.15 ± 0.07 and 0.41 ± 0.28 events/min in the DG ($n = 6$ cells, 64 SOCs) and CA3 ($n = 9$ cells, 57 SOCs), respectively. The SOC amplitude was 71.05 ± 14.99 pA and 46.93 ± 6.93 pA in the DG ($n = 64$ SOCs) and in CA3 ($n = 57$ SOCs), respectively. The 10–90% rise time of SOCs recorded in DG neurons after abolishing vesicular release spanned a large range (3.8–546.5 ms) with a mean of 26.19 ± 8.41 ms ($n = 64$ SOCs) and a distribution such that 83% had a rise time >7.5 ms (**Figure 2**). The distribution of the 10–90% rise time of SOCs in CA3 was similar, ranging from 5.6 to 162 ms with a mean of 35.70 ± 4.85 ($n = 57$ SOCs). Because some SOCs had a rise time compatible with synaptic currents (**Figure 2C**), we used a value of 7.5 ms as a threshold to separate SOCs from synaptic currents in all recordings performed in the absence of bafilomycin A1 (e.g., in **Figures 1** and **3**). Using this criterion, we found that the frequency of SOCs in the DG and CA3 regions was not significantly different in slices treated or not with bafilomycin A1 (see values given above). The distributions of the SOC 10–90% rise times and amplitudes recorded in control slices or in bafilomycin A1 treated slices were not significantly different (not shown). All together these results indicated that SOCs occur spontaneously in the hippocampus and independently of neuronal vesicular release.

We and others have shown that a decrease of the extracellular osmolarity favors the release of transmitter from astrocytes (Pasantes-Morales et al., 1994; Deleuze et al., 1998; Mongin et al., 1999; Takano et al., 2005; Kozlov et al., 2006; Jimenez-Gonzalez et al., 2011) probably through the activation of volume-regulated anion channels(Kimelberg et al., 2006). Accordingly, we observed that the frequency of SOCs recorded in granule cells of the DG was modulated by hypotonic extracellular solutions. In bafilomycin A1 treated slices recorded in the presence of NBQX, lowering the extracellular osmolarity by 15–20% for 1.5–3 min induced an increase of SOC frequency from 0.15 ± 0.07 events/min in control solution to 1.77 ± 0.23 events/min

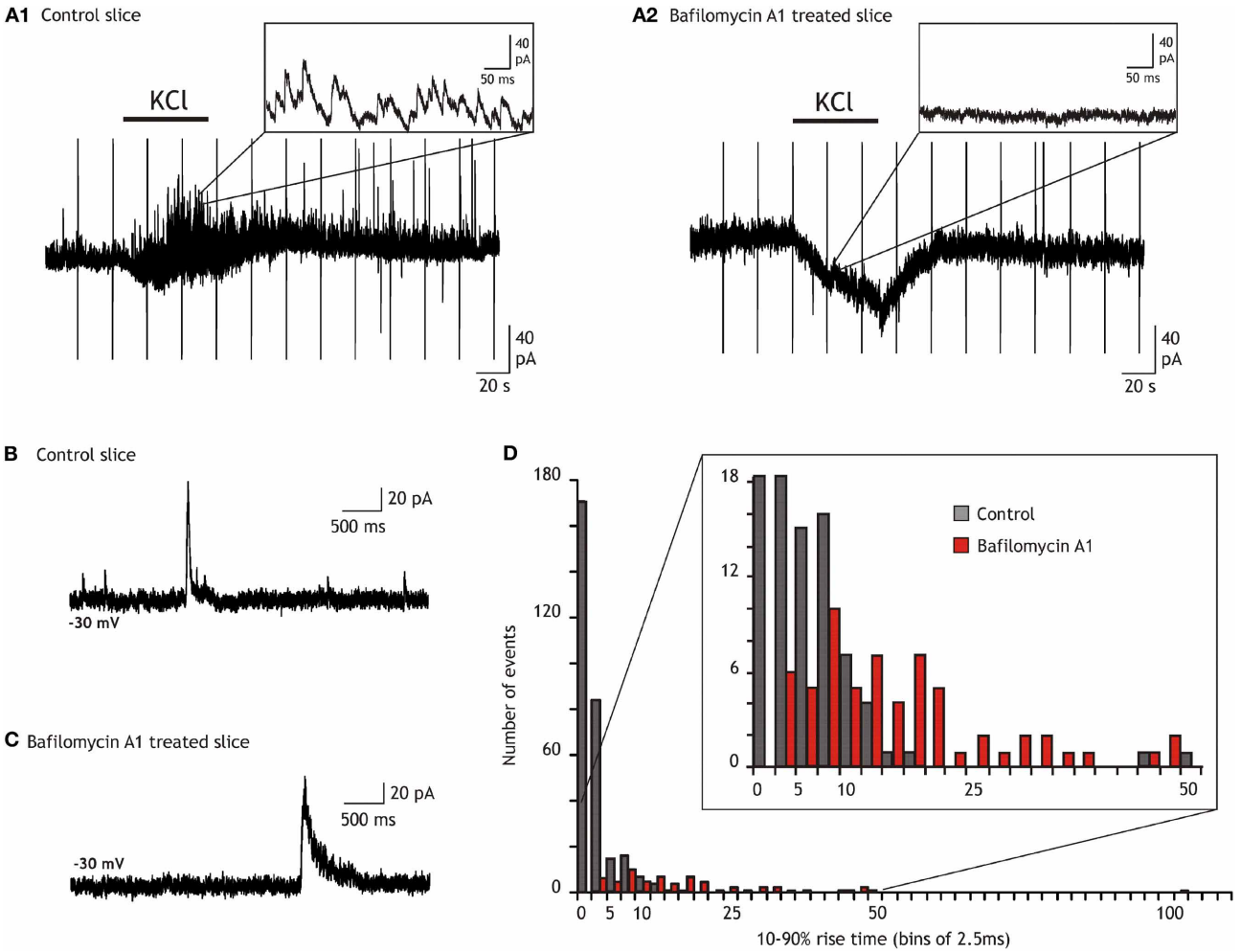

FIGURE 2 | Comparison of the outward current kinetics recorded in the dentate gyrus of control and bafilomycin A1 treated slices. **(A)** Comparison of the synaptic activity and of the effect of bath applied 10 mM KCl on granule cells recorded in a control **(A1)** and in a bafilomycin A1 treated slice **(A2)**. **(B)** Recording from a control slice in the presence of TTX (1 μM), NBQX (20 μM), and AP5 (50 μM). **(C)** Example from a slice treated during 2 h with bafilomycin A1 and recorded in the presence of TTX (1 μM), NBQX (20 μM), and AP5 (50 μM). **(D)** Ten to ninety percentage rise time histograms of the outward currents recorded in control (black) and in bafilomycin A1 treated (red) slices. All currents with amplitude exceeding twice the standard deviation of the noise were considered. Note that the number of currents with rise times faster than 5 ms greatly diminishes in slices treated with bafilomycin A1 while the majority (80%) of the remaining ones, i.e., the SOCs, have rise times slower than 7.5 ms.

in hypotonic and this effect reverted upon re-introduction of the isotonic extracellular solution in the recording chamber ($n = 6$; **Figure 3A**). A similar effect of the hypotonic solution was observed on SIC frequency which increased from 0.38 ± 0.13 to 3.55 ± 1.27 events/min ($n = 6$; **Figure 3A**). SOCs recorded in isotonic or in hypotonic solutions had similar mean amplitude (38.85 ± 12.39 pA, $n = 18$, and 47.02 ± 21.17 pA, $n = 22$, respectively; $p = 0.7260$) and 10–90% rise time (39.53 ± 11.33 ms and 37.17 ± 9.90 ms, respectively; $p = 0.8825$). We also compared the effects of hypotonic solutions on SOCs and inhibitory synaptic activities in control slices that had not been pre-incubated with bafilomycin A1 but were recorded in the presence of TTX and NBQX. In these conditions, hypotonic solutions still increased the SOC frequency but did not changed the frequency of miniature inhibitory synaptic currents (**Figure 3B**), stressing further the differences between synaptic transmission and the generation of SOCs and SICs.

SOCs in the olfactory bulb and in the thalamus are due to the activation of GABA-A receptors by GABA released from astrocytes. However, Taurine is another inhibitory aminoacid which can be released by astrocytes (Deleuze et al., 1998; Takano et al., 2005) and could therefore contribute to SOCs by activating glycine receptors expressed by hippocampal neurons (Mori et al., 2002). In granule cells of the DG recorded in slices treated with bafilomycin A1, SOCs (and SICs) were still observed in the presence of the glycine receptor antagonist strychnine (10 μM; **Figure 4A**). Strychnine did not reduce significantly the frequency of spontaneous SOCs (**Figure 4A**). Moreover, in the presence of strychnine the application of hypotonic extracellular solutions still increased the SOC frequency (**Figure 4A**). SOCs recorded in

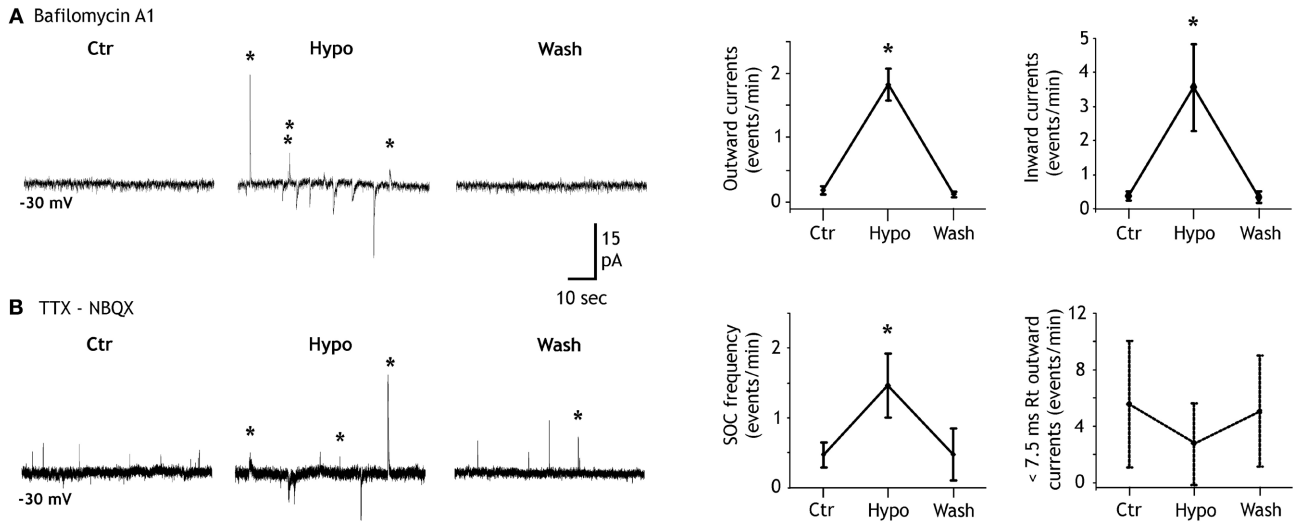

FIGURE 3 | Hypotonicity increases SIC and SOC frequencies. (A) Left, example of a granule cell recorded in a bafilomycin A1 treated slice and illustrating the increase of the SOC (labeled with stars) frequency induced by a 20% reduction of the extracellular solution osmoloraty. Note that hypotonicity also increases the frequency of the slow inward currents (SICs). Right, quantification of the effect induced by hypotonic solutions for six granule cells. **(B)** Same as in **A** but in a control slice. Note that the hypotonic solution increases the frequency of SOCs (stars) but not that of synaptic currents (unlabeled outward currents). Right, quantification of the effect induced by hypotonic solutions on the frequency of SOCs (10–90% rise time >7.5 ms; $n = 8$ granule cells; left graph) and on that of miniature IPSCs (10–90% rise time <7.5 ms; same granule cells; right graph).

the presence of strychnine had similar mean amplitude (34.07 ± 6.17 pA; $n = 16$ SOCs, $p = 0.25$) and 10–90% rise time (24.98 ± 4.10 ms; $n = 14$ SOCs, $p = 0.23$) than those of SOCs recorded in control conditions. In contrast, bath application of the GABA-A receptor antagonist SR95531 (10 μM) almost abolished all SOCS occurring spontaneously and the application of hypotonic solutions did not induce a significant increase in SOC frequency (**Figure 4B**). The few remaining SOCs observed in the presence of 10 μM gabazine had smaller amplitude and slower rise times (17.76 ± 6.8 pA and 111.56 ± 19.67 ms, $n = 5$ SOCs) than those observed in control conditions. Moreover, in 4 cells recorded in the presence of 20 μM gabazine, we did not observe any SOCs either in isotonic or hypotonic solutions (not shown).

These results indicate that SOCS in the hippocampus are due to the activation of GABA-A receptors. Yet, they do not exclude that taurine released by astrocytes activates GABA-A receptors. Indeed, Jia and collaborators reported that relatively low concentrations of taurine (10–500 μM) activate extra-synaptic GABA-A receptors of thalamic neurons (Jia et al., 2008). However, in the presence of strychnine (10 μM) to block glycine receptors, bath application of taurine (0.2–1 mM) onto granule cells ($n = 7$) and CA3 pyramidal neurons ($n = 4$) hold at −30 mV failed to induce detectable outward currents while 10–50 μM muscimol readily activated GABA-A receptors in the same recorded cells (**Figure 4C**). These observations thus strongly suggest that SOCs in hippocampal neurons are due to the release of GABA from astrocytes.

RELATION BETWEEN SICs AND SOCs IN THE HIPPOCAMPUS

Our results suggest that SICs and SOCs are due to an astrocytic release of glutamate and GABA, respectively. In few cases we observed double currents in which a SIC and a SOC occurred simultaneously (i.e., the second event started before the end of the first one). Over 21 tested neurons, three double events occurred out of 98 SICs and 39 SOCs recorded during the 3 min of hypotonic solution application in these 21 neurons (thus, the average observed frequency of these double events was 0.048 event/min). Using the average frequencies of individual SICs and SOCs in these 21 cells, we performed a Monte Carlo simulation to generate the distributions of SICs and SOCs in 10,000 sample cells and test whether these three double events occurred by chance (see "Materials and Methods"). We obtained only 158 samples with three double events over the 10,000 samples, i.e., the *p*-value for the observation of three double events by chance is less than 0.016, i.e., 3 times lower than the observed experimental value (see above). These results suggest that occurrences of SICs and SOCs are not statistically independent. If true, this would indicate that SICs and SOCs are due to the release of glutamate and GABA, respectively, from the same population of astrocytes or from a heterogeneous population of hippocampal astrocytes having a coordinated activity. Additional experimental evidence is needed to support further this hypothesis.

GABA IMMUNOREACTIVITY IN ASTROCYTES OF THE HIPPOCAMPUS

In order to analyze the presence of GABA of hippocampal astrocytes, we used pre-embedding immunohistochemistry and electron microscopy (see "Materials and Methods"). Astrocytic profiles identified by the presence of GAFP contained GABA immunoparticles in the stratum radiatum of both CA1 and CA3 hippocampal regions (**Figure 5**) as well as in the DG.

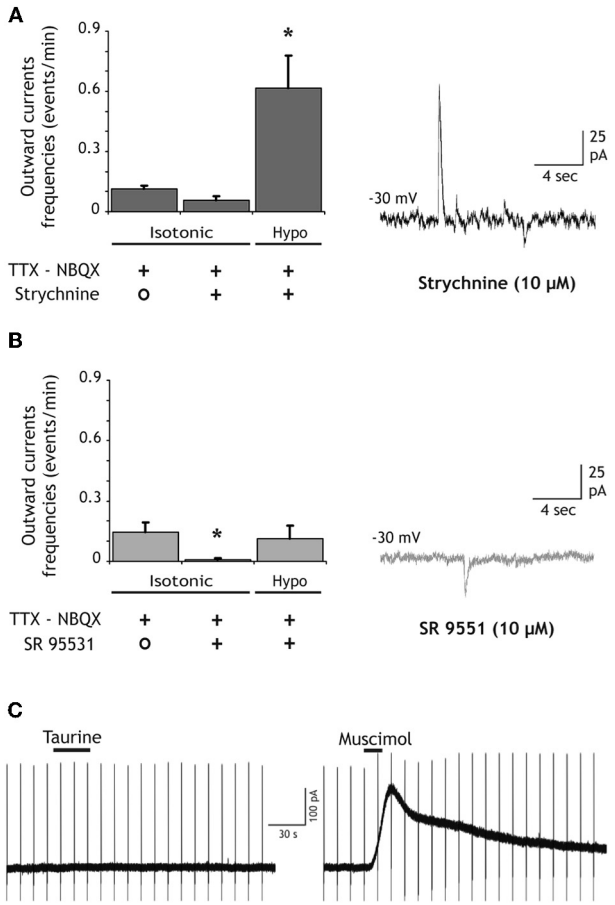

FIGURE 4 | SOCs are due to the activation of GABA-A receptors. **(A)** The competitive antagonist of glycine receptors strychnine (10 µM) does not inhibit SOCs nor does it prevent the increase of SOC frequency induced by hypotonic extracellular solutions ($n = 9$ granule cells). The trace on the right illustrates SOCs and SICs recorded in the presence of strychnine during the application of the hypotonic solution. **(B)** The competitive antagonist of GABA-A receptors SR95531 (GABAzine, 10 µM) strongly reduces the SOC frequency and prevents its increase during the application of hypotonic extracellular solutions ($n = 11$). The trace on the right shows a granule cell recording in the presence of GABAzine during the application of the hypotonic solution. All recordings were performed at −30 mV in the presence of NBQX (10 µM) and TTX (1 µM). **(C)** Taurine does not activate GABA-A receptors of DG granule cells. The left trace shows the absence of response to bath applied taurine (300 µM, 30 s) in a neuron held at −30 mV in the presence of strychnine (10 µM), TTX (1 µM), d-AP5 (40 µM) and NBQX (10 µM). An outward current was induced in the same neuron by muscimol (50 µM, 15 s). Membrane current during applications of 0.2–1 mM taurine varied of average by 2 ± 0.5 pA in DG granule cells ($n = 7$) and by 1.6 ± 2.5 pA in CA3 pyramidal cells ($n = 4$). Vertical deflections in panel **C** correspond to capacitive currents during voltage step commands used to measure membrane and pipette parameters.

Furthermore, the large majority of the analyzed astrocytic elements showed GABA immunolabeling in CA1 (79%, $n = 53$ GFAP positive elements), CA3 (88%, $n = 34$ GFAP positive elements) and in the DG (83%, $n = 23$ GFAP positive elements). These percentages were not statistically different ($p > 0.05$, Pearson's chi-squared test). In all hippocampal regions, small synaptic terminals with pleomorphic synaptic vesicles and forming symmetric synapses with dendritic elements were GABA immunopositive (**Figure 5**), as expected for the localization of GABA. These results indicate that a large proportion of hippocampal astrocytes contain the inhibitory transmitter GABA.

DISCUSSION

We and others previously showed that astrocyte release of glutamate triggers SICs in neurons of the thalamus (Parri et al., 2001), in CA1 neurons of the hippocampus (Angulo et al., 2004; Fellin et al., 2004; Perea and Araque, 2005; Nestor et al., 2007), in neurons of the striatum (D'Ascenzo et al., 2007) and of the olfactory bulb (Kozlov et al., 2006). Moreover, astrocytes in the olfactory bulb and in the thalamus also release GABA which activates GABA-A receptors and thereby induces SOCs in neighboring neurons (Kozlov et al., 2006; Jimenez-Gonzalez et al., 2011). Our present results extent these previous observations by showing that SICs and SOCs occur in all principal neurons of the hippocampus, including CA1 and CA3 pyramidal cells and granule cells of the dentate gyrus.

Beside their sensitivity to GABA-A receptor antagonists (see below), SOCs in the hippocampus share several other properties with SOCs previously observed in the olfactory bulb (Kozlov et al., 2006) and in the thalamus (Jimenez-Gonzalez et al., 2011) which distinguish them from inhibitory synaptic currents. In particular, the rise and decay times of SOCs are much slower than those of inhibitory synaptic currents. Moreover, SOCs persist after having inhibited the vesicular H^+-ATPase, a treatment which readily abolishes synaptic transmission. Finally, lowering extracellular solution osmolarity increases SOC frequency but does not change the frequency of synaptic currents. These observations suggest that a non-vesicular rather than a vesicular mechanism is responsible for the release of transmitter generating SOCs. The dependency of SOC and SIC frequency upon extracellular osmolarity observed in the hippocampus (see **Figure 3**), in the olfactory bulb (Kozlov et al., 2006), and in the thalamus (Jimenez-Gonzalez et al., 2011) suggests the involvement of volume sensitive chloride channels through which astrocytes can release various amino acids, including glutamate, taurine, and aspartate (Mongin and Kimelberg, 2002, 2005; Takano et al., 2005; Kimelberg et al., 2006). Recently, bestrophyn anion channels (Best 1) have been shown to be responsible for the release of GABA from cerebellar glial cells which generate GABA-A receptor-mediated tonic currents in granule cells (Lee et al., 2010). Unfortunately, blockers of volume-regulated and Best 1 anion channels have non-specific effects on GABA-A receptors mediating SOCs (Kozlov et al., 2006). Thus, further experiments are needed to identify the actual molecular mechanism of release generating SOCs.

Whereas there is little doubt that glutamate is the excitatory gliotransmitter responsible for SICs, both taurine and GABA are potential inhibitory gliotransmitters that could mediate SOCs (Hussy, 2002; Takano et al., 2005; Kozlov et al., 2006). In particular, hippocampal neurons express glycine receptors that can be activated by taurine (Mori et al., 2002). Despite the relatively poor specificity of GABA-A receptor antagonists which can also inhibit glycine receptors (Li and Slaughter, 2007), our results

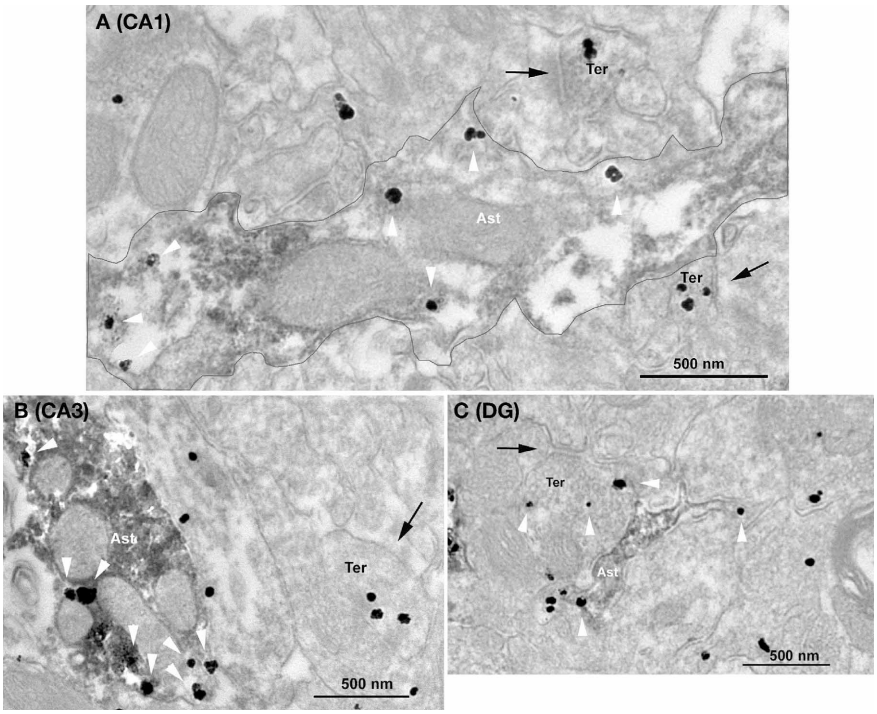

FIGURE 5 | Ultrastructural immunolocalization of GABA in astrocytes of hippocampal CA1, CA3, and dentate gyrus regions. Combined pre-embedding immunogold and immunoperoxidase methods for electron microscopy. Astrocytes (GFAP+) and GABA profiles are labeled by DAB and silver-intensified gold particles, respectively. GABA immunoparticles are localized in synaptic terminals (Ter) with symmetric synapses (arrows) as well as in astrocytes (Ast, white arrowheads) in CA1 **(A)** and CA3 **(B)** stratum radiatum and in the dentate gyrus **(C)**. In A, a GAFP+ astrocytic portion containing GABA is delineated.

rather support the involvement of GABA-A receptors in mediating SOCs: SOCs were abolished by gabazine at a concentration of 10–20 μM which is 5–10 times lower than the concentration needed to block 50% of the glycine receptor-mediated current produced by 60 μM glycine (Li and Slaughter, 2007). Furthermore, a high concentration of strychnine (i.e., 10 μM) failed to inhibit SOCs. Yet, this observation does not rule out the possibility that SOCs are mediated by an astrocyte release of taurine that would activate extrasynaptic GABA-A receptors (Jia et al., 2008). However, our observation that taurine does not activate GABA-A receptors in DG and CA3 neurons strongly support the idea that GABA is the gliotransmitter responsible for triggering SOCs in these neurons. These results are in keeping with those obtained in the olfactory bulb where the kinetics of SOCs is slower in the presence of nipecotic acid, a blocker of GABA transporters (Kozlov et al., 2006) and where SOCs are also observed in granule cells (Kozlov et al., 2006) that do not respond to taurine application (Belluzzi et al., 2004). Therefore, GABA rather than taurine is probably the inhibitory gliotransmitter mediating SOCs in the hippocampus.

The above conclusion is further supported by the electron microscopy examination of hippocampal tissue immunostained with a specific antibody against GABA (Somogyi and Hodgson, 1985) which demonstrates the presence of GABA in the vast majority of hippocampal GFAP positive glial cells. The existence of a positive correlation between the amount of astrocytic GABA and the amplitude of tonic inhibition has been recently proposed (Yoon et al., 2011). We did not observed major differences in the number of astrocytic processes labeled with GABA antibodies in different hippocampal fields and this contrasts markedly with the fact that the frequency of SOCs was significantly lower in CA1 than in CA3 and in the dentate gyrus. Because SOCs have otherwise similar properties in neurons of the different hippocampal fields, this difference in SOC frequency is thus most likely related to differences in the functional state of astrocytes or in the control of astrocytic GABA release in the different fields rather than due to differences in astrocytic GABA content. A tight regulation of astrocytic release of gliotransmitters is also supported by the extreme low occurrence of simultaneous SICs and SOCs. If these events were merely the result of an uncontrolled leak of transmitters from a cytosolic compartment through anionic channels, one would expect to see mostly double events. Yet, the probability of co-occurrences indicates the existence of some statistical dependency between SICs and SOCs in hypotonic conditions which could be a consequence of an enhanced coordinated activity of the astrocytic network in these conditions.

The physiological roles of GABA and glutamate astrocytic release remain largely unknown. It is noteworthy that both SICs and SOCs can synchronize the activity of neighboring neurons within a given astrocytic domain (Angulo et al., 2004; Fellin et al., 2004; Kozlov et al., 2006; Halassa et al., 2007). Moreover, in the thalamus the incidence of SICs is modulated on the long

term by the activity of neuronal glutamate afferents (Pirttimaki et al., 2011). It is thus likely that the impact of SOCs and SICs on neuronal activity, and in particular on the synchronization of neuronal activity, varies according to the overall activity of neuronal networks and according to the physiological and pathological conditions. Along this line, the involvement of SICs in epileptic discharges has been debated (for review Carmignoto and Haydon, 2012) and recently they have been shown to lower the threshold for the generation of ictal discharges in an *in vitro* model of temporal lobe epilepsy (Gomez-Gonzalo et al., 2010). The roles of SOCs in pathological conditions have not yet been evaluated but the fact that they seem to rely on the activation of extrasynaptic receptors (Jimenez-Gonzalez et al., 2011) may also suggests that they play important functions in pathological conditions in which extrasynaptic GABA-A receptors have been implicated (Maguire et al., 2005; Cope et al., 2009). Furthermore, it would be particularly interesting to study the contribution of SOCs in temporal lobe epilepsy in which a shift of chloride equilibrium toward depolarizing potentials contributes to the generation of interictal discharges in the hippocampus (Cohen et al., 2002; Huberfeld et al., 2007). In such conditions, a hyperpolarizing or depolarizing effect of SOCs, inducing small ensemble of neurons to be synchronously inhibited or excited would depend on local regulation of chloride equilibrium at extrasynaptic site.

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Astrocyte regulation of sleep circuits: experimental and modeling perspectives

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Integrated within neural circuits, astrocytes have recently been shown to modulate brain rhythms thought to mediate sleep function. Experimental evidence suggests that local impact of astrocytes on single synapses translates into global modulation of neuronal networks and behavior. We discuss these findings in the context of current conceptual models of sleep generation and function, each of which have historically focused on neural mechanisms. We highlight the implications and the challenges introduced by these results from a conceptual and computational perspective. We further provide modeling directions on how these data might extend our knowledge of astrocytic properties and sleep function. Given our evolving understanding of how local cellular activities during sleep lead to functional outcomes for the brain, further mechanistic and theoretical understanding of astrocytic contribution to these dynamics will undoubtedly be of great basic and translational benefit.

Keywords: glia, astrocytes, sleep, ATP, adenosine, neuronal networks, slow oscillations, computational models

INTRODUCTION

Astrocytes are characterized by a highly ramified structure of cellular processes that occupy non-overlapping domains (see **Figure 1**). Each astrocyte can contact a few neuronal cell bodies, hundreds of dendrites, and tens of thousands of synapses (Bushong et al., 2002; Halassa et al., 2007). Moreover, astrocytes can be coupled by gap junctions (Cotrina et al., 1998; Giaume et al., 2010; Verkhratsky, 2011) to form a cellular network called "astrocytic syncytium." This elaborate morphology underlies the complexity of astrocyte function in the brain. Indeed, the impact of astrocytes on synaptic physiology is multifaceted, ranging from structural support to the regulation of the composition of the extracellular space and neuromodulation (for reviews see Haydon, 2001; Fellin and Carmignoto, 2004; Allen and Barres, 2005; Volterra and Meldolesi, 2005; Barres, 2008; Fellin, 2009).

While astrocytes lack active membrane properties (Zhou et al., 2009), their passive properties are crucial to regulating extracellular potassium (Higashi et al., 2001; Kucheryavykh et al., 2007). Astrocytes also clear extracellular excitatory and inhibitory neurotransmitters via specific transporters, a process that ensures high fidelity synaptic transmission (Kimelberg et al., 1986; Dabir et al., 2006).

One of the most exciting discoveries in modern neuroscience has been the demonstration that astrocytes release chemical transmitters (**Figure 2**) among which D-serine and ATP are two of the most studied. D-serine increases the current flowing through NMDA receptors by acting as a co-agonist and providing an important regulatory feedback that controls synaptic transmission and plasticity (Panatier et al., 2006) (**Figure 2C**). In contrast, ATP binds to purinergic receptors (Gordon et al., 2005), or activates adenosine A_1 and A_2 receptors (through its metabolite, Adenosine) to modulate neuronal excitability and information transfer at the synapse (Pascual et al., 2005; Panatier et al., 2011) (**Figure 2B**). Two studies extended these initial findings, demonstrating that astrocytes provide powerful modulation of sleep dynamics and behavior (Fellin et al., 2009; Halassa et al.,

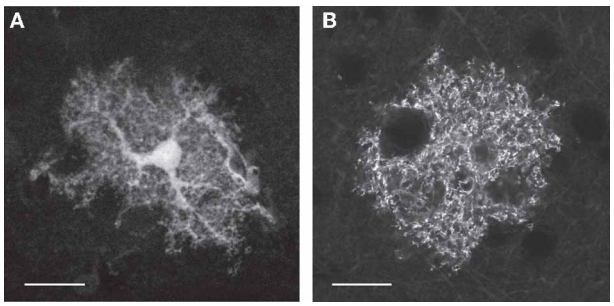

FIGURE 1 | Astrocytes are characterized by complex morphology. (A,B) Single-plane fluorescence images of an astrocyte expressing soluble GFP **(A)** or a membrane-bound YFP **(B)**. In **A** the cell body and the principal cellular processes are clearly visible. When the fluorophore is bound to the membrane **(B)**, small distal processes are more easily identified because of the increased surface-to-volume ratio of these thin cellular compartments. Scale bar 20 μm. Courtesy of A. M. De Stasi and T. Fellin.

2009). Taken together, these observations strongly support the notion that local astrocytic modulation at the synaptic level translates into an active control of network activities, such that higher brain functions are served by the integrated circuits of neurons and glia (Halassa and Haydon, 2010).

This review aims to frame such recent findings on the active role of astrocytes in sleep and behavior in light of our evolving understanding of sleep phenomenology and function, focusing on sleep's expression in forebrain circuits. Moreover, we pay particular attention to the computational implications of such findings in the attempt to build a theoretical framework for future experiments. This endeavor will allow us and others to conceptualize what is currently known about astrocytic modulation of neuronal network function and test new computationally inspired hypotheses. We hope that a community-wide effort of similar motivation will bridge the gap between experiments and models, in order to promote better understanding of the function of integrated circuit function in the brain.

SLEEP: BACKGROUND AND FUNCTION

Although details can differ among animals, a number of common characteristics have been used to define sleep as a universal behavior (Cirelli and Tononi, 2008). Among them are changes in posture, reduced responsiveness to environmental stimuli, and changes in global brain activity (Bjorness and Greene, 2009). Such characteristics are based on mammalian physiology, which traditionally classifies sleep into categories of rapid eye movement (REM) and non-rapid eye movement (NREM) stages using electroencephalographic (EEG) and electromyographic (EMG) criteria. While in REM sleep the EEG is characterized by high frequency, low amplitude activity; NREM sleep, by contrast, has an abundance of low frequency, high amplitude oscillations. These rhythmic activities are oscillations of varying frequency, amplitude and duration, including "slow oscillations," "slow waves," and "sleep spindles" (see **Box 1** for a description of the neuronal circuits thought to generate these activity patterns).

Among the many functions attributed to sleep, one of the key ones is its role in memory. Two main hypotheses have been proposed to explain the finding that sleep appears to enhance memory. The *synaptic scaling hypothesis* suggests that the neocortex undergoes net synaptic potentiation during waking experiences, due to sensory-driven synaptic activity and increased release of neuromodulators (Tononi and Cirelli, 2003). According to this hypothesis, sleep evolved as a mechanism to downscale synapses while preserving their relative weights, balancing energy and space demands of the brain. This model is supported by several biochemical and electrophysiological observations. For example, recordings from layer II/III neocortical pyramidal neurons have shown that both the amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) increase as a function of wakefulness, and decrease as a function of sleep (Liu et al., 2010). Phosphorylation at Ser831 of the AMPA glutamate receptor 1 subunit (GluR1) in cortical synaptoneurosomes—a change that increases single AMPA channel conductance and is known to be induced by synaptic potentiation—is enhanced by wakefulness, and reversed by sleep (Vyazovskiy et al., 2008). *In vivo* recordings of the neocortex in the freely behaving preparation have shown that neural firing rates (Vyazovskiy et al., 2009) and trans-callosal synaptic transmission (Vyazovskiy et al., 2008) exhibit a similar bidirectional modulation by wake and sleep.

By contrast to the synaptic scaling hypothesis, the *memory trace reactivation hypothesis* suggests that sleep is a state in which memories are consolidated by offline reactivation of the neurons involved in memory encoding during recent wakefulness (Lee and Wilson, 2002). This hypothesis is supported by recordings of neuronal ensembles from the hippocampus, where sequences of pyramidal neuronal firing encoding spatial trajectories during active behavior are replayed during subsequent sleep (Lee and Wilson, 2002). Replay events occur during 200–300 Hz oscillations of the hippocampal local field potential (LFP) known as ripples, whose electrical disruption has been shown to attenuate spatial memory (Girardeau et al., 2009).

Recent observations that astrocytes actively modulate brain rhythms thought to mediate these two processes, raise the need for computational studies of astrocytic function in order to fully understand the outcome of these two possible sleep functions.

LOCAL EXPRESSION OF SLEEP DYNAMICS

While EEG tends to provide excellent information about the precise timing of brain events (i.e., excellent temporal resolution), the location in the brain where these events are taking place are relatively poor (i.e., limited spatial resolution). However, the development of high-resolution monitoring of neural activity had major impact on the study of sleep. High-density EEG recordings have shown that slow oscillations occurring during NREM sleep are traveling wave originating from frontal cortical areas (Massimini et al., 2004). Intracranial recordings in patients undergoing epilepsy surgery demonstrated that both slow waves and sleep spindles are expressed in a spatially confined manner, rather than simultaneously occurring across multiple cortical regions (Nir et al., 2011). Asynchronous spindle generation has also been corroborated by magnetoencephalography (MEG), revealing distinct spindles that may be obscured in EEG recordings (Dehghani et al., 2010). Moreover, several studies have shown

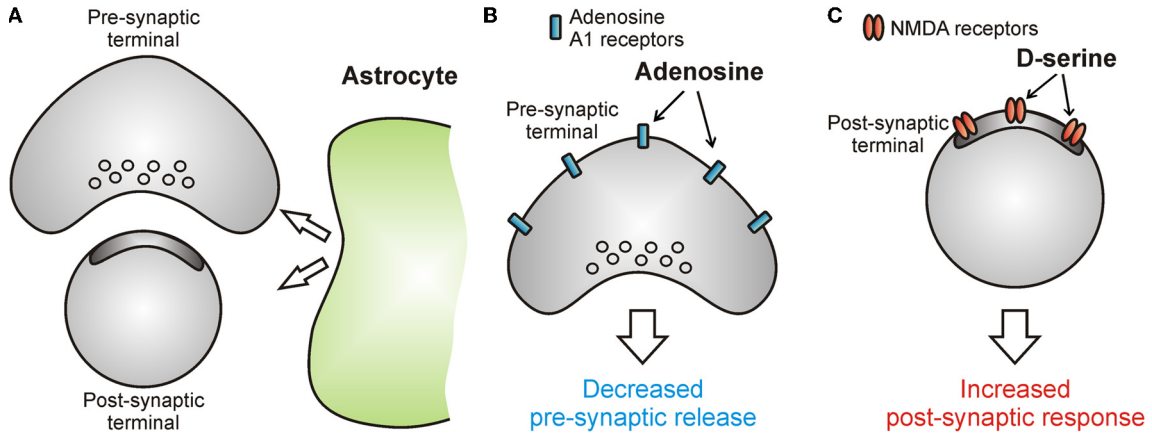

FIGURE 2 | Astrocytes release chemical transmitters to modulate information transfer at the synapse. (A–C) Schematic drawings showing the effect of astrocytic neuromodulation on synaptic function. Astrocytes release ATP which, after rapid extracellular degradation to adenosine, activates adenosine A_1 receptors located at pre-synaptic sites and leads to a decrease in the release of neurotransmitter **(B)**. Astrocytes can also release D-serine which potentiates the current flowing through post-synaptic NMDA receptors thus leading to increased post-synaptic responses **(C)**.

Box 1 | Neural circuits underlying sleep dynamics.

The mammalian NREM sleep EEG is characterized by the expression of slow waves that include the cortical slow oscillations (<1 Hz) and delta oscillations (1–4 Hz) (Buzsáki, 2006). While earlier reports have proposed that these two dynamics are distinct, recent experiments have suggested that they may be expression of the same cellular process: cortical neuronal UP and DOWN states (Timofeev and Chauvette, 2011). During slow oscillations the membrane potential of neocortical neurons is characterized by rhythmic fluctuations between two main states, the UP and the DOWN states at frequency <1 Hz (Contreras and Steriade, 1995). While DOWN-state transitions are characterized by the absence of synaptic inputs and membrane potential stability close to a value corresponding to the membrane resting potential, the UP state is a 0.200–1.5 s-long 15–20 mV depolarization during which action potential firing occurs. UP states are generated by bursts of synaptic inputs and are blocked by the voltage-gated sodium channel blocker tetrodotoxin (TTX). Moreover, UP- and DOWN-state transitions can be generated in cortical slices (Sanchez-Vives and McCormick, 2000) or surgically isolated cortical slabs *in vivo* (Timofeev et al., 2000). Although these initial observations suggested that cortical circuits are sufficient to generate this type of network oscillation, it must be considered that afferent fibers from other brain regions modulate UP- and DOWN-state generation in the cortex. In fact, incoming sensory inputs from the thalamus have been shown to trigger cortical UP-state transitions *in vivo* (Steriade et al., 1993) while electrical stimulation of thalamocortical axons reliably generates cortical UP-states in thalamocortical slice preparation (MacLean et al., 2005). Further, while much of the earlier work characterizing these cortical states had been performed under anesthesia, recent work in the head-fixed preparation has corroborated the cellular basis of the cortical slow oscillation (Chauvette et al., 2011).

In addition to slow waves, sleep spindles are characteristic features that are present in NREM sleep. These phasic 11–15 Hz oscillations, of approximately 0.5–3 s duration, appear in the EEG at a frequency of 0.1–0.2 Hz (Steriade, 2000). Spindles are thought to be generated by the thalamic reticular nucleus (TRN), a shell of GABAergic neurons that surround the dorsal thalamus and exert powerful synaptic inhibition on thalamocortical relay neurons (Bazhenov et al., 2000; Halassa et al., 2011). TRN and relay neurons express T-type Ca^{2+} channels, allowing them two modes of firing: tonic firing and bursting. At a depolarized membrane potential above the resting potential of −65 mV, these cells fire single Na^{+} spikes (tonic firing). But when hyperpolarized below the resting potential, they exhibit T-type Ca^{2+} channel-mediated spikes each characterized by a large Ca^{2+} spike crowned by high frequency bursts of Na^{+} spikes at a frequency of ∼300 Hz (McCormick and Bal, 1997). It is thought that rhythmic bursting of the TRN at the spindle frequency entrains the thalamus by rhythmically de-inactivating T-type Ca^{2+} channels, causing thalamic relay neural populations to oscillate at the spindle frequency and thereby drive the neocortex (Timofeev and Chauvette, 2011). Recent evidence also underlies the importance of the corticothalamic interactions in the regulation of spindles (Bonjean et al., 2011).

the occurrence of sleep dynamics in localized brain regions during waking behavior (Vyazovskiy et al., 2011) and the occurrence of waking dynamics in localized brain areas during sleep (Nobili et al., 2011). These discoveries paint a new picture of brain state regulation adding further complexity than previously thought (Rector et al., 2009; Krueger and Tononi, 2011). Sleep may be better described as a continuous variable along a state-spectrum. In addition, graded activation of state-controlling microcircuits may be utilized to achieve diverse outcomes pertinent to processes such as working memory and selective attention (Harris and Thiele, 2011).

An intriguing question is the mechanism by which this local regulation of brain dynamics occurs. It is known that local increases in slow waves are dependent on prior behavior (Huber et al., 2004, 2006). For example, training on a motor task that requires hand movement has been shown to result in disproportionate increase of slow waves in the contralateral sensory-motor cortex (Huber et al., 2004). This suggests that

the expression of slow waves could either be driven by local circuitry, or be modified by it. Further, the amplitude of slow waves might be dependent on the underlying local synaptic weights (Esser et al., 2007). Therefore, local modulators of synaptic transmission could serve as ideal candidates for mediating local generation of sleep dynamics. Below, we discuss anatomical and functional evidence suggesting that astrocytes are ideal candidates for providing local modulation of sleep dynamics. Among the different functions that astrocytes play in the brain, we will focus our attention on the process of neuroactive molecule release.

ASTROCYTE REGULATION OF SYNAPSES

In the last 20 years, astrocytes have been shown to directly release chemical transmitters in a process termed "gliotransmission" (Zhang and Haydon, 2005). In this process, astrocytes release neurotransmitters, cytokines, peptides, and neuromodulators that can provide an important regulatory feedback to neurons (Halassa and Haydon, 2010).

Astrocytes express the enzyme serine racemase (Wolosker et al., 1999a,b), a protein that converts the amino acid L-serine to D-serine. D-serine is known to bind the glycine-site of the N-methyl-D-Aspartate receptor (NMDAR) with four times the affinity of glycine (Wolosker et al., 1999a,b) (**Figure 2C**). The impact of astrocytic D-serine on synaptic transmission and plasticity has been mostly studied in the hypothalamus and the hippocampus, where the proximity of astrocytic processes to neurons determines D-serine availability to the synapse, and thus the ability of the synapse to express NMDAR-dependent plasticity (Panatier et al., 2006; Henneberger et al., 2010). More recently, studies in the mouse neocortex *in vivo* have shown that astrocytic Ca^{2+} signaling and associated D-serine release are important for translating cholinergic modulatory signals to NMDAR-dependent cortical plasticity (Takata et al., 2011).

ATP released from astrocytes can modulate neurons directly through the activation of P_2 receptors (Gordon et al., 2009; Gourine et al., 2010). Alternatively, after rapid degradation to adenosine by ectonucleotidases (Dunwiddie et al., 1997; Dunwiddie and Masino, 2001), it can modulate synaptic transmission through the activation of adenosine receptors (Pascual et al., 2005; Serrano et al., 2006; Panatier et al., 2011). Astrocytic ATP can be stored in secretory compartments that fuse in a SNARE-dependent fashion upon an increase of the intracellular Ca^{2+} concentration (Coco et al., 2003; Zhang et al., 2007). Using a mouse model in which SNARE-dependent gliotransmission was impaired (dnSNARE mouse), Pascual et al. showed that astrocytes release ATP to control the strength of hippocampal synapses through its metabolite adenosine (Pascual et al., 2005) (**Figure 2B**). Using extracellular field recordings and brain slice preparation, these authors found that basal synaptic transmission was increased in transgenic mice, compared to wild type (WT) mice, a phenotype that was mimicked by the application of the A_1-receptor antagonist, DPCPX (or CPT) in WT animals. In contrast, application of the A_1 receptor agonist, CCPA, partially recovered the phenotype in transgenic mice, causing the reduction of fEPSP. Evidence that adenosine is generated by degradation of ATP comes from the observation that in slices from WT animals, the application of the ectonucleotidase inhibitor ARL67156 caused an inhibition of synaptic transmission that was blocked by the P_2 receptor antagonist RB-2. This effect was absent in transgenic mice. Moreover, using a luciferine-luciferase assay, extracellular ATP levels were found to be reduced in transgenic mice, compared to controls.

The same study (Pascual et al., 2005) provided evidence that astrocytes release ATP in two modes: *tonic* release, which leads to a persistent synaptic suppression, and *phasic* release, which modulates synaptic plasticity when activity-dependent recruitment of astrocytes occurs. More specifically, it was demonstrated that glia-derived adenosine is responsible for activity-dependent heterosynaptic depression at excitatory synapses through A_1 receptors (Pascual et al., 2005). Thus, astrocytes operate as sensors of neuronal activity and provide an activity-dependent phasic and tonic modulatory feedback to synapses at a local scale. On a longer timescale, adenosine acting through adenosine A_1 receptors has been shown to control the surface expression of postsynaptic NMDA receptors (Deng et al., 2011), suggesting that astrocytic modulation of synaptic physiology can span multiple timescales. How this modulation relates to neural network function and behavior has just become possible to study (Fellin et al., 2009; Halassa et al., 2009), given the advances in molecular genetics and astrocyte-specific manipulations. Below, we review the first few studies that have started addressing this topic.

ASTROCYTIC REGULATION OF NEURONAL CIRCUITS AND SLEEP

The first study to demonstrate an impact of astrocytes and gliotransmission on brain dynamics used the dnSNARE mouse (discussed above) showing that impairment of vesicular gliotransmission attenuates cortical slow oscillations (Fellin et al., 2009). Intracellular patch-clamp recordings *in vivo* revealed that the reduction of the slow oscillations was due to decreased UP-state probability of cortical neurons. (See **Box 1** for definition of UP and DOWN states). UP-state transitions were found to be shorter and DOWN-state transitions longer in transgenic mice compared to controls (**Figure 3**), leading also to a reduction in the frequency of UP-state transitions. In contrast, the maximal amplitude of the UP-state was unaffected by transgene expression. Importantly, the reduction in slow oscillations was confirmed by chronic EEG recordings in freely behaving mice during natural sleep (Fellin et al., 2009). Following sleep deprivation, the power of slow oscillations in NREM sleep was selectively reduced in these animals. These network effects were the consequence of astrocytic modulation of intracortical synaptic transmission at two sites: a hypofunction of postsynaptic NMDA receptors, and by reducing extracellular adenosine, a loss of tonic A_1 receptor-mediated inhibition. Indeed, AMPA/NMDA current ratio was found to be increased in transgenic mice compared to controls. This was due to selective decrease in NMDA receptor current due to impaired D-serine release and reduction in the surface expression of NMDA receptors. Moreover, application of the adenosine receptor antagonist CPT caused a significant increase in synaptic transmission in slices derived from WT—but not transgenic animals—demonstrating that the tonic level of adenosine was dependent on astrocytic gliotransmission. This dual astrocytic

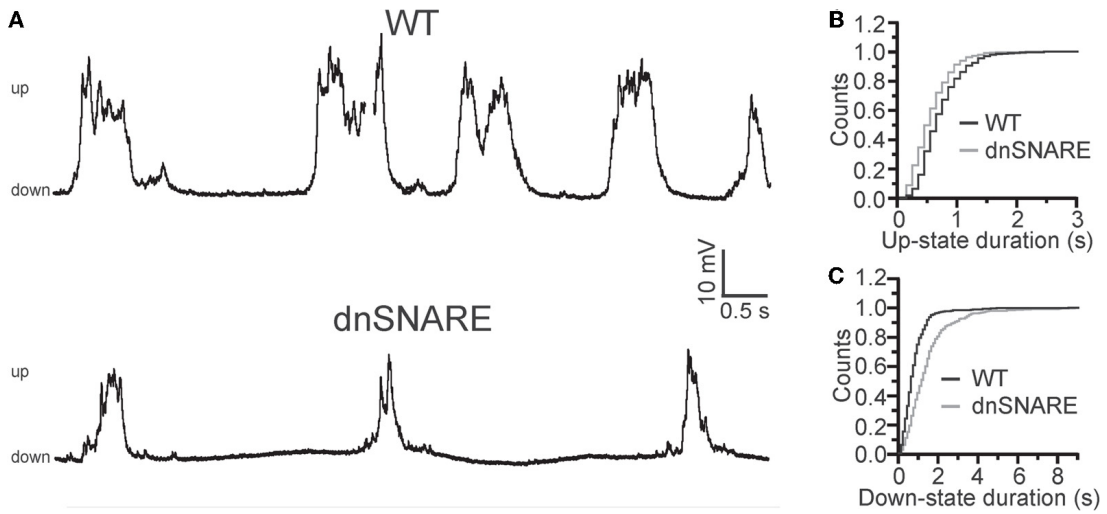

FIGURE 3 | Astrocytes modulate slow oscillations. (A) Representative patch-clamp recordings from WT (*top*) and dnSNARE (*bottom*) anesthetized animals *in vivo*, showing reduced slow oscillations in transgenic mice with impaired gliotransmission (dnSNARE). **(B,C)** Cumulative distribution showing shorter UP **(B)** and longer DOWN- **(C)** state durations in dnSNARE mice (gray line) compared to WT controls (black line). Modifed from Fellin et al. (2009).

regulation at synaptic level correlated with the observed changes in network activity recorded in living animals. *In vivo* application of CPT caused an increase in slow oscillations in WT animals, but not in transgenic animals, while application of the NMDA receptor antagonist D-AP5 caused a significant decrease in slow oscillation power in WT but not in transgenic mice. Based on these results, it was concluded that astrocytic regulation at the synaptic level translates into active feedback at the neuronal-network level (Fellin et al., 2009). It is important to note that astrocytic regulation of network UP-states has also been confirmed by a recent *in situ* study (Poskanzer and Yuste, 2011), and that a role of adenosine in the regulation of low frequency rhythmogenesis has been recently described in the thalamus (Lorincz et al., 2009). In this latter study, infra-slow oscillations were observed to be strongly regulated by ATP and by its metabolite adenosine. Although not tested directly in that study, thalamic astrocytes could be a potential cellular source of this nucleoside.

To address the impact of slow wave reduction on sleep behavior, chronic polysomnography was implemented in the dnSNARE mice and their WT littermates. The major finding was that slow-wave homeostasis was reduced in the dnSNARE mice under baseline conditions and was accentuated after sleep deprivation (Halassa et al., 2009). This electrophysiological phenotype was accompanied by altered sleep homeostasis, where dnSNARE mice failed to exhibit an increased in sleep time following 6 h of sleep deprivation. Consistent with other studies (Pascual et al., 2005; Fellin et al., 2009), the effect of transgene expression was mimicked by intraperitoneal injection of CPT in WT animals. Moreover, attenuation of gliotransmission resulted in the alterations of the cognitive impairments following sleep deprivation assessed with a novel object recognition test, an effect that was phenocopied in WT animals by injection of CPT.

Taken together, these data show that astrocytic purines regulate synaptic transmission. By doing so, they also regulate network dynamics, cortical low-frequency rhythmogenesis and sleep itself. As described previously, although astrocytes are organized in a cellular syncytium, single cells occupy separate domains and lack long-range cellular projections. Thus, by releasing chemical transmitters, astrocytes may function as local modulators of neurons and networks and these processes might have important implication in sleep. For example, local increase in extracellular adenosine following ATP release in the thalamus and cortex may profoundly impact the generation of thalamocortical dynamics. Although adenosine generated by astrocyte-released ATP was shown to act mainly on synaptic transmission (but see Panatier et al., 2011), astrocyte-derived adenosine could also directly impact the intrinsic properties of thalamic cells by activating somatic A_1 receptors leading to cell hyperpolarization. The ability to manipulate neuronal firing and intracellular signaling of neurons and astrocytes by optogenetic technologies (Boyden et al., 2005) will undoubtedly open the door to answering these and other questions on the role of neuron-glial control of brain activity.

ASTROCYTIC MODULATION OF NETWORKS: IMPLICATION FOR MODELING STUDIES

The experimental findings discussed above open new venues in computational modeling of network activity, and suggest that obtaining an accurate description of synaptically connected networks requires the inclusion of local astrocytic neuromodulation. In particular, previous computational studies showed that the strength of synaptic connections is crucial to regulate network synchronization during slow oscillation activity (Bazhenov et al., 2002; Compte et al., 2003; Esser et al., 2007). This is well predicted by the theory that synchronization of coupled oscillators is heavily influenced by the strength of the coupling (Pikovsky et al., 2001; Wang, 2010). Therefore, given the importance of the astrocyte in regulating synaptic physiology, it is obvious that realistic

modeling of slow oscillation activity needs to include astrocytic neuromodulation.

In the remainder of this review, we will focus on two aspects of gliotransmission that we believe critical for future development of modeling studies: temporal and spatial complexity of astrocytic neuromodulatory feedback to networks. While the question of how to define connectivity in neuron-glia networks remains open, arguably the path to the proper answer rests in the distinction and appreciation of the different temporal and spatial scales underlying astrocytic neuromodulation. Precise understanding of these two aspects of astrocyte-to-neuron communication will be essential for the establishment of realistic and constrained models.

TEMPORAL ASPECTS OF GLIOTRANSMISSION

Evidence that gliotransmission operates on different time scales adds levels of complexity to our understanding of brain networks, and provides a challenging framework for experimentation and theory alike. For example, phasic ATP release from astrocytes provides an adenosinergic, activity-dependent heterosynaptic depression. Concurrently, tonic astrocytic adenosine mediates a constant suppression of presynaptic terminals through the activation of A_1 receptors (Pascual et al., 2005; Halassa et al., 2009). Thus, the time scales of activity of gliotransmitters may be different and the phasic or tonic action must be included in a model as a crucial parameter.

In the context of slow oscillations, phasic release of ATP and D-serine by astrocytes (Panatier et al., 2006; Zhang et al., 2007) may modulate cortical UP and DOWN states. In the model originally developed by Hill and Tononi (2005), the depolarizing influence of NMDA currents plays an important role in maintaining the UP state as well as its synchrony among different neurons. This is likely due to the broadening of dendritic integration time constants by NMDA currents (Gasparini and Magee, 2006). The slow decay of NMDA currents could indeed account for integration of the highly heterogeneous synaptic inputs observed during UP states (McCormick et al., 2003), providing sufficient tonic drive to maintain neuronal firing persistence (Wang, 1999). Thus, a transient increase of NMDA currents due to the phasic release of D-serine from the astrocyte could promote neuronal firing, favoring synchrony and prolonging the duration of the UP state. On the other hand, this effect could be contrasted by transient weakening of synaptic transmission by phasic release of ATP and the subsequent accumulation of adenosine (Pascual et al., 2005; Serrano et al., 2006; Panatier et al., 2011). Another intriguing possibility is that astrocytic adenosine modifies synaptic filtering properties affecting the switch between short-term depression or facilitation (Abbott and Regehr, 2004). This scenario was recently addressed in a theoretical study by De Pittà et al. (2011): astrocytic control of the mode of synaptic plasticity was shown to depend on astrocyte-mediated modulation of synaptic filtering. One prediction of this study is that astrocyte-derived adenosine could favor the induction of short-term synaptic facilitation in response to incoming patterned activity, in agreement with experimental results (Pascual et al., 2005). In **Figure 4**, we modeled the firing activity of a cortical neuron triggered by a sample train of synaptic inputs reminiscent of UP and DOWN states (Hill and Tononi, 2005; Destexhe, 2009) under low (Control) and high

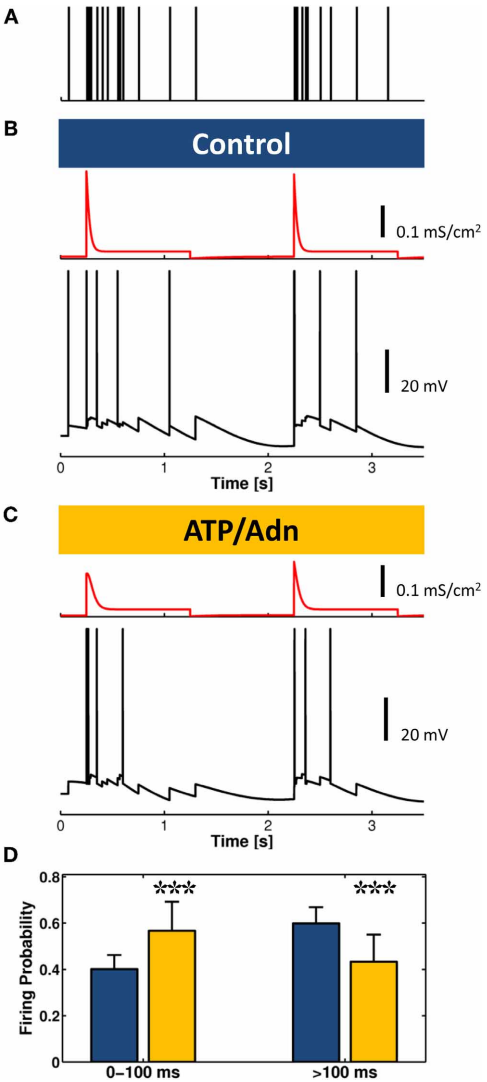

FIGURE 4 | Weakening of synaptic transmission by astrocyte-derived ATP and adenosine could promote bursting at the onset of the UP state. (A) A stereotypical synaptic input alternating a phase of intense presynaptic firing to a relative quiescent phase, reminiscent of UP and DOWN states, respectively, is fed into a model of cortical neuron. **(B)** In control conditions, for low extracellular levels of ATP/adenosine (Adn), synaptic release probability is high, and the average synaptic conductance (*red traces*) is shaped by short-term depression while neuron fires (*black trace*) at sustained rate during the UP state. **(C)** For increased levels of ATP/Adn, the firing rate dramatically decreases due to the upstream reduction of these purines of synaptic release probability, but as shown in the histogram in **(D)**, due to the modulation of the synaptic filtering characteristics by increased extracellular ATP/Adn concentration, the neuron fires a burst of actions potentials at the onset of UP states at higher frequency than in **(B)** in control conditions ($n = 100$; Bar + Error bar: Mean + STD; χ^2 test, $p < 0.001$). Synaptic release and ATP/Adn modulation of it were modeled as in panel **4A** in De Pittà et al. (2011). Postsynaptic currents were computed as the product of postsynaptic conductance and membrane voltage. Each input spike contributed to a change of postsynaptic conductance proportional to the amount of synaptically-released resources by a α-function such as $\alpha(t) = g_{max} \cdot \exp(1 - t/\tau) \cdot t/\tau$ (Ermentrout and Terman, 2010) with $g_{max} = 500\,mS/cm^2$ and $\tau = 20\,ms$. The postsynaptic neuron was modeled as a regular spiking (RS) neuron according to (Destexhe, 2009).

(ATP/Adn) concentration of extracellular purines, which mimics increased extracellular adenosine concentration mediated by astrocytes during wakefulness (Schmitt et al., 2012). In the latter case, neuronal firing is reduced because of the upstream decrease of synaptic release probability by the purines which results in a weakened averaged synaptic conductance (*red traces*). However, under these same circumstances, the neuron mostly fires at the transitions from DOWN to UP states and the firing rate is higher at these transitions due to the modified synaptic filtering characteristics. On a network level, this process may promote synchrony, providing a potential local mechanism to trigger UP states at every cycle of the slow oscillations (Crunelli and Hughes, 2009). *In vitro*, for example, the ensuing inhibiting action of gliotransmission on synaptic release could account for the occurrence of synchronized bursting events and the observed neuronal firing statistics (Volman et al., 2007).

The interplay of tonic vs. phasic and activity-dependent regulation of synaptic transmission by the number of gliotransmitters that can be released from astrocytes is an important aspect to consider when studying how astrocytes shape neuronal network activity (Volman et al., 2007; Nadkarni et al., 2008; Silchenko and Tass, 2008; De Pittà et al., 2011). It is intriguing to consider how a single gliotransmitter can have contrasting effects on synaptic transmission. On one hand, astrocytic adenosine reduces synaptic transmission by binding to presynaptic A_1 receptors (Pascual et al., 2005). On the other hand, activation of A_1 receptors increases surface expression of postsynaptic NMDARs and synaptic strength (Deng et al., 2011). What is the computational value of a tonic reduction of presynaptic release probability while slowly increasing postsynaptic NMDA receptor currents? An intriguing possibility could be *synaptic redistribution* (Markram and Tsodyks, 1996). According to the synaptic-scaling hypothesis of sleep (Tononi and Cirelli, 2006), plastic processes occurring during wakefulness result in a net increase of synaptic strength in forebrain circuits. At individual synapses, such potentiation is likely regulated by spike-timing dependent plasticity (STDP). But at the network level, additional mechanisms might be required to dynamically regulate the progressive increase of synaptic strength and counteract its tendency to destabilize postsynaptic firing rates, either reducing them to zero or increasing them excessively (Abbott and Nelson, 2000). Redistribution of synaptic weights by presynaptic reduction of release probability in parallel to postsynaptic increase of NMDA receptors could be essential. Through this mechanism, synaptic potentiation could occur without increasing the firing rates of postsynaptic neurons or the steady-state excitability of recurrent networks, and at the same time optimizing STDP-mediated potentiation (Abbott and Nelson, 2000). Moreover this mechanism would allow to prevent energy consumption by otherwise undesired network firing (Buzsáki et al., 2002; Tononi and Cirelli, 2006). As such, synaptic redistribution could play a role during wakefulness similar to that hypothesized for synaptic scaling during sleep (Tononi and Cirelli, 2006). On the other hand, it is likely that the two processes are linked. Although the underlying signaling pathways remain to be elucidated, this possibility is corroborated by the recognition of a role of astrocytes in homeostatic brain functions mediated by TNFα (Turrigiano, 2006). TNFα released from astrocytes (Bezzi et al., 2001; Beattie et al., 2002) has been implicated in synaptic scaling (Beattie et al., 2002; Stellwagen and Malenka, 2006). Moreover, TNFα has also been reported to control gliotransmission at granule cell synapses in the dentate gyrus (Santello et al., 2011). Computational models that include regulation of ambient TNFα by astrocytes could provide predictions on the role of gliotransmission during the whole sleep cycle (Krueger, 2008).

SPATIAL ASPECTS OF GLIOTRANSMISSION

Astrocytes are functionally organized cells, and different subcellular regions of astrocytic processes could locally provide different modulatory feedback on neighboring synapses (Fellin et al., 2004; Volterra and Meldolesi, 2005; Marchaland et al., 2008; Di Castro et al., 2011; Panatier et al., 2011). From a modeling viewpoint, this scenario subtends the notion that the "synaptic island" represented by a single astrocyte is intrinsically non-linear in its function and modulates neuronal networks on different time scales and also on distinct spatial scales (Goldberg et al., 2010; Bernardinelli et al., 2011).

Transient Ca^{2+} elevations at astrocytic processes can occur either spontaneously (Hirase et al., 2004; Sasaki et al., 2011) or be triggered by local synaptic activity (Wang et al., 2006), and in turn can locally release ATP (Di Castro et al., 2011; Panatier et al., 2011). Under proper conditions, however, Ca^{2+} signals may propagate along astrocytic processes to other processes of the cell and eventually to the whole cell, thus potentially allowing purinergic gliotransmission to occur on a much wider spatial scale (Panatier et al., 2006). Moreover, Ca^{2+} signals can propagate to other neighboring astrocytes triggering ATP release from other astrocytic cells (Volterra and Meldolesi, 2005). Despite numerous modeling studies put forth to account for the rich spatial dynamics of astrocyte Ca^{2+} signaling (Bennett et al., 2008; De Pittà et al., 2009; Goldberg et al., 2010; Dupont et al., 2011), a comprehensive theoretical framework that attempts to link local, functionally organized Ca^{2+} signals to global, whole-cell Ca^{2+} signals and intercellular Ca^{2+} propagation is still missing. Also, the origin of Ca^{2+} signals (i.e., intracellular vs. extracellular) and how it links to different types of gliotransmission remains to be fully elucidated.

CONCLUSIONS

While much of the research on astrocyte-neuron interactions in the last 15 years has focused on the regulation of synaptic transmission by the astrocyte, recent experiments show that these cells are essential modulators of network activity. Initial reports have shown astrocytic modulation of the cortical slow oscillation, a fundamental brain dynamic observed in sleep and thought to be important for sleep's functions. The involvement of astrocytic modulation in sleep circuits makes mechanistic sense: astrocytes are slower signaling cells compared to neurons and may mediate activity-dependent changes of network function over extended periods of time. These discoveries come at an exciting time in astrocyte research, where our understanding of astrocytes function in the brain is expanding exponentially. Moreover, these new findings call for new and more elaborate *in silico* models of astrocyte-to-neuron communication that will move from the astrocytic modulation of single synapses to that

of networks of synaptically connected neurons. In addition to providing fundamental insight into how the brain operates, further development of the experimental and theoretical knowledge of astrocyte role in the regulation of neuronal circuits will be of tremendous translational impact.

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Astroglial networks and implications for therapeutic neuromodulation of epilepsy

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Epilepsy is a common chronic neurologic disorder affecting approximately 1% of the world population. More than one-third of all epilepsy patients have incompletely controlled seizures or debilitating medication side effects in spite of optimal medical management. Medically refractory epilepsy is associated with excess injury and mortality, psychosocial dysfunction, and significant cognitive impairment. Effective treatment options for these patients can be limited. The cellular mechanisms underlying seizure activity are incompletely understood, though we here describe multiple lines of evidence supporting the likely contribution of astroglia to epilepsy, with focus on individual astrocytes and their network functions. Of the emerging therapeutic modalities for epilepsy, one of the most intriguing is the field of neuromodulation. Neuromodulatory treatment, which consists of administering electrical pulses to neural tissue to modulate its activity leading to a beneficial effect, may be an option for these patients. Current modalities consist of vagal nerve stimulation, open and closed-loop stimulation, and transcranial magnetic stimulation. Due to their unique properties, we here present astrocytes as likely important targets for the developing field of neuromodulation in the treatment of epilepsy.

Keywords: astrocyte, tripartite synapse, neuromodulation, epilepsy, deep brain stimulation, vagal nerve stimulation

INTRODUCTION

Epilepsy is a common chronic neurologic disorder affecting 0.5–1% of the population with an estimated lifetime risk greater than 4% (Hauser et al., 1993; Hesdorffer et al., 2011). More than one-third of all epilepsy patients have incompletely controlled seizures or debilitating medication side effects in spite of optimal medical management (Sander, 1993; Kwan and Brodie, 2000; Sillanpaa and Schmidt, 2006). Medically refractory epilepsy is associated with excess injury and mortality, psychosocial dysfunction, and significant cognitive impairment (Brodie and Dichter, 1996). Treatment options for these patients include new antiepileptic drugs (AEDs), which may lead to seizure freedom in a small percentage of patients (Fisher, 1993; Engel, 2012) and resective surgery which is associated with long term seizure freedom in 60–80% of patients (Engel et al., 2003; Lee et al., 2005b; Engel, 2012). The cellular mechanisms underlying seizure activity are incompletely understood, though multiple lines of evidence support the contribution of astrocytic cells, both individually and in networks. Many properties of astrocytes also make them important targets for the developing field of neuromodulation in the treatment of epilepsy.

ASTROCYTES IN EPILEPSY

Historically, astroglia were thought to provide only metabolic and physical support for neurons. They serve as the primary source of energy for neurons (Brown et al., 2004) and serve to control ionic homeostasis and neuronal excitability by buffering potassium (Kofuji and Newman, 2004). It is now clear, however, that astroglia are directly involved in neuronal signaling, even locally at synapses (Barres, 1991; Bergles et al., 1997; Volterra et al., 2002; Hatton and Parpura, 2004; Lin and Bergles, 2004; Allen and Barres, 2005; Volterra and Meldolesi, 2005). Astroglia synthesize and recycle glutamate (Hertz and Zielke, 2004) and respond to synaptic release of neurotransmitters with both calcium waves and release of gliotransmitters that can further influence synaptic activity (Cornell-Bell et al., 1990a; Grosche et al., 1999; Schipke and Kettenmann, 2004; Pascual et al., 2005; Perea and Araque, 2005; Zorec et al., 2012), with important implications in the epileptic brain (Carmignoto and Haydon, 2012). Perisynaptic astroglial processes may detect spill out of glutamate and other substances from active synapses (Rusakov and Kullmann, 1998; Diamond, 2005), and respond structurally by extending and modifying their processes (Cornell-Bell et al., 1990b; Hirrlinger et al., 2004; Witcher et al., 2007, 2010). Variation in synapse strength and the degree to which substances escape the perimeter might determine whether astroglial processes grow toward and ensheath parts of some synapses and avoid or retract from others (Cornell-Bell et al., 1990b; Hatton and Parpura, 2004; Witcher et al., 2007). Astroglia also secrete substances that are critical to the formation and function of synapses during development (Mauch et al., 2001; Ullian et al., 2001, 2004; Christopherson et al., 2005; Goritz et al., 2005) and contain contact-mediated factors that influence synapse maturation (Mazzanti and Haydon, 2003; Murai et al., 2003; Hama et al., 2004).

Astrocytes also function as modulators of neurotransmitters. It is commonly accepted that astrocytes play a large role in glutamate uptake, mostly through the GLT-1 transporter (Danbolt, 2001), also known as the excitatory amino acid transporter-2 (EAAT2) transporter with assistance by additional uptake via the EAAT1 transporter (Shigeri et al., 2004). Astrocytes, through the regulation of extracellular glutamate diffusion, also appear to modulate

intersynaptic crosstalk (Oliet et al., 2001, 2006; Theodosis et al., 2006) and modulate synaptic function (Piet et al., 2004). Astrocytic transporter placement may only be contributory to these effects, however, as glutamine synthetase plays a key role in the metabolism of uptaken glutamate (Magistretti, 2006). While glutamine synthetase has been shown to be upregulated in reactive astrocytes (Eddleston and Mucke, 1993), it appears to be reduced in the epileptic hippocampus (Eid et al., 2004; van der Hel et al., 2005), indicating potential changes in the uptake potential of astrocytic transporters. Glutamate transporters have similarly been shown to be reduced in the epileptic hippocampus (Proper et al., 2002). Similarly, GABA release from interneurons may be proportionately increased by intracellular astrocytic calcium through activation of the kainic subtype of neuronal ionotropic glutamate receptors (Liu et al., 2004). Disruption of this cycle could lead to a decrease in GABA release, and thus decreased inhibition of neurons. GABA and glutamate imbalance is believed to potentially be a key mechanism in pathologies such as epilepsy. Thus, astroglia likely modulate function of synapses individually and within synaptic networks.

ASTROCYTIC CHANGE IN NEUROPATHOLOGY CAN ALTER FUNCTION OF NEURONAL NETWORKS

Multiple pathologically induced changes in astrocytic function have been described. For example, neuronal calcium homeostasis may be modulated by growth factors such as NGF, known to be released by astrocytes in response to excitotoxic injection, providing a means of protecting cultured neurons against excitotoxicity (Eddleston and Mucke, 1993). Extracellular levels of calcium, as well as the propagation of multicellular calcium waves may be tied intricately to mechanically induced stimulation in astrocytes (Ostrow and Sachs, 2005). Under pathological conditions, mechanical stimulation could be provided in the injured CNS by reactive gliosis, swelling, mass effects, or tissue hypertrophy (Ostrow and Sachs, 2005). Astrocytic dysregulation of the vascular system has also been implicated in the epileptic brain. For example, altered calcium signaling by astrocytes can result in changes in local vascular tone (Zonta et al., 2003; Gomez-Gonzalo et al., 2011). Additionally, the upregulation or induction of multiple inflammatory factors within perivascular astrocytes, including IL-1β, complement components, and plasminogen activator in the epileptic brain may also have dramatic effects on the vascular system leading to disruption of the blood brain barrier (as reviewed in Aronica et al., 2012). Dysfunctional astrocytic calcium signaling may also underlay the development of a recurrent excitatory loop sustaining ictal discharge (Gomez-Gonzalo et al., 2010). Astrocytic dysfunction can also result in neuronal oxidative stress (Takuma et al., 2004), as failed support of dopaminergic neurons has been detected after astrocytes were experimentally deprived of glutathione (Drukarch et al., 1997).

Pathological dysfunction has also been observed in the gap-junctional coupling of astrocytes. Along these lines, increases have been noted in the expression of connexin-43 in experimental excitotoxic injuries (Haupt et al., 2007) as well as from epileptic patient specimens (Naus et al., 1991). Upregulation may result in increased intercellular signaling through calcium waves (Scemes and Giaume, 2006), an increased potential for astrocytic glutamate release (Parpura et al., 2004), and may play a role in the potassium buffering capacity of astrocytes (Wallraff et al., 2006). Through gap junctions, astrocytes can exert neurotrophic and neuroprotective influences (Naus et al., 2001; Takuma et al., 2004). Conversely, gap junction communication can be reversed in pathological conditions such as hypoxia (Martinez and Saez, 2000), potentially resulting in increased neuronal injury (Ozog et al., 2002).

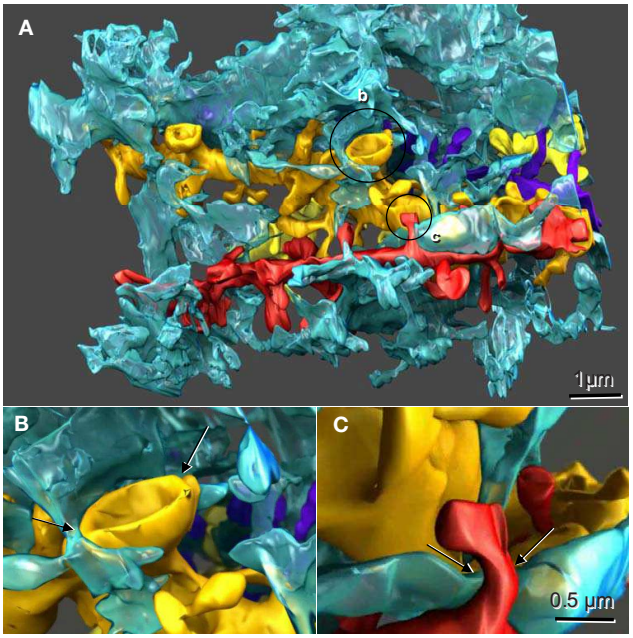

FIGURE 1 | Reconstructed dendrites, synapses, and associated astroglial processes from rodent hippocampus. (A) Three-dimensional reconstruction of a single astroglial process (blue) interdigitating among many dendrites, four of which are reconstructed here (gold, yellow, red, and purple). Axonal boutons are not displayed. **(B)** Approximately 50% of the Axon-Spine Interface (ASI) of a mushroom spine was apposed by astroglia (arrows). **(C)** Only the neck of this thin dendritic spine was apposed by astroglia (arrows). Scale bar in **(C)** is for **(B)** and **(C)**. Figure reproduced with permission from John Wiley and Sons.

It is therefore clear that astroglial cells function to support the microenvironments of neuronal cells and to modulate neural networks. Given their unique structural interactions with multiple neuronal contacts simultaneously (**Figure 1**; originally published in Witcher et al., 2007; and Movie S1 in Supplementary Material), it is evident that astrocytes can readily integrate into a functional network. Astroglial cells may even function as separate, parallel networks in roles not currently understood. It is highly likely that astroglial networks have a fundamental role in disease states addressed through neurosurgical approaches. Specifically, astrocytes play a vital role in the epileptic brain, the treatment algorithm of which often ends in neurosurgical resection, transection, or disconnection of cellular networks. Similarly, multiple neurosurgical entities currently depend on the modulation of cerebral networks through introduction and manipulation of neuromodulatory devices such as deep brain stimulators. These techniques have analogous implications for astrocytes and the astroglial network. These disease processes include dystonia, movement disorders

such Parkinson's disease and psychological disorders dependent on appropriate modulation of network function such as Obsessive-Compulsive Disorder, depression, and others. Through a better understanding and manipulation of the astrocytic networks of the human brain, we hypothesize that new targets and modulatory therapies can be developed in the treatment of epilepsy.

IMPLICATIONS IN EPILEPSY

One of the most consistently reported findings associated with seizure activity, whether a single event caused experimentally by focal application of convulsants (Jiang et al., 1998) or by repeated epileptic seizures *in vivo* (Scheibel and Scheibel, 1977), is hippocampal neuronal loss, subsequent deafferentation of dendritic partners, and loss of dendritic spines. Hippocampal alterations in mesial temporal lobe epilepsy (MTLE) include neuronal loss in the hippocampus, gliosis, and reorganization of subsets of neurons in the hippocampus (Sommer, 1880; Spencer, 2002). Lesions of CA1, in particular, are associated with the classically described Ammon's Horn sclerosis (Mathern et al., 1997; Duvernoy, 2005), and greater loss of spines is associated with a greater degree of pathology. Other findings consistently reported in epileptic tissue include denuding of dendritic segments, as well as the formation of varicosities along dendritic shafts (Multani et al., 1994). The degree of synaptic loss can vary along individual dendrites (Scheibel and Scheibel, 1977). As reviewed by Fiala et al. (2002), these pathologies are also consistent with other neurodegenerative disorders, many of which cause deafferentation of dendrites. This observation led to the hypothesis that deafferentation may be the cause of dendritic spine pathology associated with disorders including epilepsy (Swann et al., 2000; Fiala et al., 2002). Structural evidence including the presence of giant spines, tortuous spines, an appearance of axon-free post synaptic densities (PSD) in dendritic spines, and aberrant synaptic partners (Raisman and Field, 1973; Baloyannis and Kim, 1979; Chen and Hillman, 1982) could support this hypothesis.

While recent attention has focused on changes in glial function in neurological diseases such as epilepsy (Tian et al., 2005; Binder and Steinhauser, 2006; Lee et al., 2006b; Matute et al., 2006), associated changes in perisynaptic astrocytic structure have been less of a focus, though changes in the amount of synaptic astrocytic contact would also be expected in the epileptic hippocampus. Important findings established or implicated in this form of epilepsy include increases in extracellular glutamate (During and Spencer, 1993), decreases in glutamate metabolism (Malthankar-Phatak et al., 2006), decreases in glutamate-stimulated GABA release (During et al., 1995), increased GABA transporter expression (Lee et al., 2006b), and increased lactate levels (Cavus et al., 2005), all of which can be associated with astrocytic dysfunction. While multiple studies have addressed the morphological changes of reactive astrocytes (Krishnan et al., 1994; van Paesschen et al., 1997; Mitchell et al., 1999; Briellmann et al., 2002) ultrastructural alterations remain understudied. Of primary importance is evidence which contradictorily supports both increased and decreased association between synaptic partners and astrocytic processes.

Multiple lines of evidence would support the possibility of increased perisynaptic astroglial apposition in the epileptic brain. The first is that in the epileptic hippocampus, microdialysis studies indicate that extracellular glutamate levels rise prior to and during ictal onset, peaking at levels associated with potential neurotoxicity (During and Spencer, 1993). Previous work has shown that astrocytes are responsible for the removal of extracellular glutamate, and that astrocytic presence can be neuroprotective (Rosenberg and Aizenman, 1989). Increased presence of perisynaptic astrocytic processes could therefore potentially serve as a mechanism by which to lower glutamate concentrations to baseline levels. It has also been shown that astrocytic processes are attracted to the neurotransmitter glutamate (Cornell-Bell et al., 1990b). The source of extracellular glutamate is likely synaptic in nature. It is therefore plausible that astrocytic processes would be drawn to these remaining synapses.

Further supporting increased apposition are the findings that astroglial contact stabilizes larger synapses (Witcher et al., 2007) and increases the efficacy of synaptic transmission (Pfrieger and Barres, 1997). The effects of increasing the amount of apposition at individual synapses is currently unknown, but previous research suggests that the amount of surround is not correlated to synapse size (Ventura and Harris, 1999) and that any amount of synaptic contact may be significant (Witcher et al., 2007). As synaptic loss is a known consequence of epilepsy, astrocytic apposition could function to stabilize remaining synapses.

Finally, previously reported increases in individual neurotransmitter receptor could support increased apposition, since MTLE is associated with an increase in glutamate receptors in the epileptic hippocampus. Human hippocampal astrocytes, similar to neurons, contain a wide variety of glutamate receptors, including AMPA receptors (Seifert et al., 2004; Matute et al., 2006), which are increased in CA1 in human epileptic tissue (Brines et al., 1997). Given the established concomitant decrease in neuronal density in epileptic hippocampi, it is plausible that this glutamate receptor increase is in the astrocytic component of the neuropil.

In contrast to the preceding evidence, other findings support the likelihood that perisynaptic astrocytic apposition could instead be decreased in epilepsy. One line of evidence lies in the morphological changes which take place subsequent to neuronal deafferentation. Due to the decrease in hippocampal neuronal density and concomitant dendritic pathologies consistently reported in both experimental and human epilepsy (Scheibel and Scheibel, 1977; Belichenko and Dahlstrom, 1995; Drakew et al., 1996; Thompson et al., 1996), it has been hypothesized that epilepsy serves as a model of neuronal deafferentation (Swann et al., 2000; Fiala et al., 2002). In the hippocampus, Schaffer collateral axons provide excitatory input from CA3 pyramidal neurons to CA1 pyramidal neurons via apical dendrites in CA1 stratum radiatum. In MTLE, where neuronal loss is prominent in both the hippocampal subfields of CA3 and CA1, loss of CA3 pyramidal neurons results in deafferentation of CA1 pyramidal neurons. As reported from other brain regions, astrocytic withdrawal can result in increased signaling between postsynaptic and presynaptic partners (Oliet et al., 2001), as well as increased signaling between adjacent synapses (Piet et al., 2004). Similarly, astrocytic withdrawal has been shown to regulate synaptic connectivity in the arcuate nucleus (Fernandez-Galaz et al., 1997) and similar effects have been implicated in the hippocampal dentate hilus (Luquin et al., 1993; Klintsova et al., 1995). Therefore, decrease in astrocyte

surround at the synapse could be a compensatory mechanism useful for increasing axonal input to deafferented dendrites.

Modifications in the pattern of glutamate transporter expression in the sclerotic human hippocampus provide further support for astrocytic withdrawal from synapses. As many as five types of glutamate transporters have been described as present, and are designated as EAAT1–5, as reviewed by Danbolt (2001). The major glutamate transporters associated with hippocampal astrocytes include EAAT1 (GLAST), EAAT2 (GLT), and EAAT3 (EAAC) while the other EAAT subtypes are typically associated only with neurons (Danbolt, 2001). In addition to the astrocytic distribution of EAAT2, a presynaptic neuronal distribution was also shown in a subpopulation of excitatory hippocampal terminals, including the CA1 (Chen et al., 2002, 2004). In the CA1 subfield, approximately 60% of astrocytic membranes contain EAAT2 transporter proteins compared to approximately 30% of neurons (Chen et al., 2004). EAAT2 localizes in the cellular membrane of astrocytes (Danbolt, 2001), while in neurons it appears to label in the membrane as well as the cytoplasm (Chen et al., 2002, 2004). EAAT3 transporters, by contrast, appear to have a cytoplasmic distribution in both astrocytes and neurons (Danbolt, 2001). The distribution of EAAT1, however, is strictly astrocytic and localized almost exclusively to cytoplasmic membranes (Danbolt, 2001).

The EAAT1 transporter concentration does not vary between the soma and processes of astrocytes, but varies relative to neighboring structures, where concentration is increased along membranes apposing neuropil, and decreased in membrane apposing cellular somata, pial surfaces, or capillary endothelium (Danbolt, 2001). The EAAT1 transporter is therefore a reliable marker of astrocytic membrane in the neuropil, and can be particularly useful in the distal processes of astrocyte where GFAP filaments do not extend (Bushong et al., 2002, 2004). While the EAAT2 transporter is associated with the majority of glutamate uptake from the hippocampus, and a causative relationship has been shown between its knockout and the development of lethal, spontaneous seizures (Tanaka et al., 1997), its expression in neurons makes it non-specific to astrocytic membranes.

In the sclerotic human hippocampus, expression of the EAAT2 transporters are significantly decreased in CA1, and EAAT1 levels show a similar trend (Proper et al., 2002). Paradoxically, a well-known consequence of sclerosis is the hypertrophy of astrocytes, demonstrated repeatedly through expansion of the GFAP protein unique to astrocytic soma and perisomatic processes. This protein, however, is absent in small, distal astrocytic processes, such as those that appose synaptic membranes (Bushong et al., 2002). As the EAAT1 and EAAT2 transporters are localized in all portions of the astrocytic membrane regardless of distance from the soma, they serve as indicators of the astrocytic processes distal to the appearance of GFAP. While the decrease in EAAT2 is significant in TLE (Proper et al., 2002), the trend toward a concomitant decrease in astrocyte-specific EAAT1 suggests that the decrease is likely contributed to by astrocytic changes. Decrease in these transporters suggests a decrease in non-GFAP containing perisynaptic astrocytic processes, thereby supporting a potential withdrawal of these processes.

Using three-dimensional unbiased brick analysis, multiple interesting findings were recently shown from the human epileptic hippocampus (Witcher et al., 2010). Specifically, it was shown that synaptic loss was indeed associated with the process of epileptogenesis, and that synaptic density decreased as gliosis increased. It was also shown that the morphology of remaining synapses was altered, and in the severe epileptic state, normal-appearing neuronal spines were replaced by abnormal giant spines which likely have unique physiological properties. Remaining synapses did not show increased numbers of vesicles, refuting the idea that high extracellular glutamate (During and Spencer, 1993; Cavus et al., 2005) is likely based on decreased uptake of the neurotransmitter. Synapses remaining in the epileptic hippocampus were not restricted from intersynaptic communication (Witcher et al., 2010). Despite these changes, however, it was clear that the apposition of perisynaptic astroglia supported larger synapses (Witcher et al., 2007, 2010). Therefore, while the astroglia and synapses show fundamental changes in the pathologic brain, it is clear that positive benefits arise from the relationship.

Neurosurgical management of epilepsy is useful in patients who are refractory to optimized medical treatment (Engel et al., 2003; Lee et al., 2005b). Current modalities are dominated by resection or disconnection of epileptic cellular networks. Descriptions of modern methods and their psychosocial implications occupy a vast literature and include lesionectomies, anterior temporal lobectomy, amygdalohippocampectomy, extratemporal resection, and corpus callosotomy (Feindel et al., 2009; Wilson and Engel, 2010). There is also a developing role for stereotactic radiosurgery (Quigg et al., 2012). In spite of improvements in surgical technique, approximately 4% of patients will suffer death or permanent neurologic disability (ILAE, 1997). Moreover, more than one-third of patients will not be candidates for surgical resection (Kwan and Brodie, 2000). For patients who are not candidates for resective surgery, there are limited options. Neuromodulatory treatment, which consists of administering electrical pulses to neural tissue to modulate its activity leading to a beneficial effect (Witcher and Ellis, 2011), can be effective for these patients.

NEUROMODULATION AND ASTROGLIAL IMPLICATIONS

The interest in neuromodulation for neurological disorders is driven by a desire to discover less invasive surgical treatments, as well as new treatments for patients whose medical conditions remain refractory to existing modalities (Witcher and Ellis, 2011). Interestingly, the unique characteristics of astrocytes make them interesting targets in the developing field of neuromodulation. Specifically, the use of these technologies requires modulation of large neural networks, and likely involve modulation of or through astrocytes or astroglial networks. These mechanisms likely involve reactive astrocytosis, network manipulation, and modulation of the release of gliotransmitters.

Astrocytic processes are ubiquitous between cells and around excitatory synapses throughout the CNS (Witcher et al., 2007, 2010). This proximity of astrocytic processes to synapses allows synaptic placement of glutamate transporters at sites of glutamate release and also the ability to limit or delimit interactions between neighboring synapses (Witcher et al., 2007, 2010). Astrocytes have also been shown to express metabotropic receptors for many neurotransmitters, including glutamate, GABA, norepinephrine, and acetylcholine (Tritsch and Bergles, 2007). Astrocytic responses

to the activation of these receptors implies sensing of neuronal function and results in oscillations or repetitive spikes in Ca2+, which likely has influence over neuronal network function (Di Castro et al., 2011; Takata et al., 2011). Gliotransmitters, namely ATP, D-serine, and glutamate are release in response to neuronal and astrocytic stimulation which also could cause neuronal network effects (Santello and Volterra, 2009; Halassa and Haydon, 2010). Mechanisms eliciting neuronal responses have been studied in both culture and slice models and include stimulation of metabotropic receptors, photolysis of caged IP3 or infused caged Ca2+, and repetitive depolarization of the astrocyte membrane presynaptic (Araque et al., 1998; Parri et al., 2001; Fiacco and McCarthy, 2004; Fellin et al., 2004; Jourdain et al., 2007).

Vagal nerve stimulation (VNS) is one example of neuromodulation that was developed in the 1980s, and which is now routinely available (Ben-Menachem, 2002). VNS, as an adjunct to medical management, may yield up to a 50% reduction in seizure frequency (VNSSG, 1995) although most of these patients will not be seizure free. Deep brain stimulation (DBS) is another example of neuromodulation. Given the significant experience and success of DBS for movement disorders (Krack et al., 2003) combined with its reversibility, programmability, and low risk of morbidity, there has been a resurgence of interest in using DBS devices for treating medically refractory epilepsy. Responsive neurostimulation (RNS) is a technology that detects seizure activity at a previously defined focus and applies an electrical stimulus to the site of seizure onset to terminate the seizure. Lastly, transcranial magnetic stimulation (TMS) is a nearly 25-year-old technology initially introduced as a means to non-invasively investigate corticospinal circuits. Currently, TMS is used primarily in clinical neurophysiology. Importantly, TMS can be used to evaluate and manipulate excitatory and inhibitory intracortical circuits with poststimulatory effect, allowing for a developing use in epileptic neuromodulation. A growing body of literature supports the involvement of astrocytes in the realization of therapeutic goals for each of these modalities, and will be reviewed below.

VAGAL NERVE STIMULATION

The vagal nerve has a complex anatomical arrangement which projects to the autonomic and reticular structures and well as limbic and thalamic neurons. Stimulation of the vagus-nerve and its bilateral multisynaptic targets has become a common technology for the treatment of epilepsy. Over 50,000 patients have been treated with the technology, and current reports indicate an approximately 50% efficacy in seizure reduction, rivaling the efficacy of antiepileptic treatment, and often decreasing dependence on them (Labar, 2002). Efficacy has also been shown to increase over time (Vonck et al., 1999). The low side effect profile of VNS (Morris and Mueller, 1999) has also proven to be advantageous for users.

The mechanism of efficacy remains unknown, though certain structures within the brain appear to be affected by VNS. As evidenced by studies using positron-emission technology (PET), the thalamus is consistently affected by VNS stimulation, and blood flow to the cerebellum and cerebral structures is consistently altered (Ko et al., 1996; Henry et al., 1998, 1999; Ben-Menachem, 2002). Thalamic involvement has also been supported through SPECT (Van et al., 2000; Vonck et al., 2000) and functional MRI (Narayanan et al., 2002; Liu et al., 2003) analysis.

Studies of VNS have been reported from multiple vertebrate models including rodents (McLachlan, 1993), canines (Zabara, 1992), and lower primates (Lockard et al., 1990). In the rodent penicillin/pentylenetetrazol model, interictal spike frequency was reduced by 33% (McLachlan, 1993), the effect of which was later found to be greatest in continuous stimulation and reduced in a time-dependent fashion after stimulation (Takaya et al., 1996). Later tests showed that cortical excitability in rats can be modulated through VNS (De Herdt et al., 2010). Canine strychnine and pentylenetetrazol models show similar efficacy with lasting reduction in motor seizures and tremors (Zabara, 1992). In the alumina gel monkey model, seizures were eliminated in half of test animals during stimulation periods with some persistence into post-stimulation period (Lockard et al., 1990). Clinical trials have indicated seizure reduction at both low and high stimulation paradigms, with significantly greater reduction in the high stimulation group (Handforth et al., 1998) and overall efficacy showed a mean seizure reduction of approximately 35–45% (Morris and Mueller, 1999).

Astrocytic involvement in the regulation of the vagal nerve nuclei supports their importance in the efficacy of VNS. McDougal et al. (2011) recently demonstrated the activation of astrocytes within the nucleus of the solitary tract (NST) when afferent stimulation of the vagal nerve was applied. Using confocal, live-cell calcium imaging of brainstem slices, they showed that afferent activation of the vagal nerve resulted in increases in astrocytic intracellular calcium concentrations as well as in neurons. They then showed that the effect on astrocytes was blocked by the AMPA receptor antagonism and was unaffected by antagonism of NMDA and metabotropic glutamate receptors. This activation was dependent on extracellular Ca^{2+} influx through AMPA receptors. This Ca^{2+} influx was further amplified by calcium-induced calcium release via the ryanodine receptor. Selective staining verified the presence of the AMPAR subunit GluR1 on astrocytes. Taken together, they concluded that NST astrocytes may be active participants in the regulation of vagal activity (McDougal et al., 2011). This supports previous work which concluded that neurons in the NST are regulated via astrocytic glutamate signaling under pathologic and potentially physiologic conditions (Hermann et al., 2009).

DIRECT NEURAL STIMULATION

Neuromodulation through the direct implantation of chronic stimulating electrodes has become a standard of treatment in many neurological disorders. DBS lead implantation within the anterior nucleus of the thalamus (ANT), as well as other central nervous system (CNS) targets – including the caudate nucleus, centromedian nucleus of the thalamus, cerebellum, hippocampus, and subthalamic nucleus – results in seizure reduction in selected patients (Shandra and Godlevsky, 1990; Vercueil et al., 1998; Bragin et al., 2002; Lee et al., 2006a). In these studies, stimulation was delivered in an open-loop fashion, that is, in a pre-defined manner, independent of the momentary physiological activity of the brain. The exact mechanism of action of DBS in reducing seizure activity is, however, unknown. It is known that stereotactic

lesions of the ANT in humans can result in reduced seizure frequency (Mullan et al., 1967). DBS may interfere with synchronized oscillations by neurotransmitter release (Lee et al., 2005a). Other evidence suggests that the most likely mechanism may involve stimulation-induced modulation of pathologic neural networks (McIntyre et al., 2004). High frequency DBS appears to reproduce the clinical effect of ablative procedures (Benabid et al., 1987). Moreover, at high frequencies, DBS may abolish cortical epileptiform activity (Lado et al., 2003). A microthalamotomy effect has been postulated based on the observation that some patients obtain reduction in seizure frequency prior to activation of the pulse generator (Andrade et al., 2006; Lim et al., 2007).

Although the precise mechanism by which DBS reduces seizure activity is unclear, inhibition of neurons immediately adjacent to the area of applied current is likely involved. A "reversible functional lesion" may be generated in structures integral to initiating or sustaining epileptic activity (Boon et al., 2007). The applied current may inhibit neurons with a pathologically lowered threshold of activation. Alternatively, DBS may act on neuronal network projections to nearby or remote CNS structures originating from the area of stimulation. This might take place through either activation of inhibitory projections or through the inhibition of excitatory projections.

As reviewed recently by Vedam-Mai et al. (2012), high frequency stimulation shows effect on astrocytic activity which has important implications in the role of astroglia in this modality. Astrocytes can be directly depolarized by stimulation (Kang et al., 1998) and have the potential to modulate local and distant neural networks using clinically relevant stimulation paradigms via the release of gliotransmitters including ATP and glutamate (Bekar et al., 2008; Tawfik et al., 2010).

Another important mechanism implicating astrocytes in clinical efficacy is reactive astrocytosis. Reactive astrocytosis is a well described phenomena of astrocytes at stimulator implant sites, and is defined as astroglial hypertrophy and upregulation of GFAP and other astrocytic proteins (Pekny and Nilsson, 2005). This finding was described initially in cats (Stock et al., 1979) and has since been described in multiple species including rats (Kraev et al., 2009), non-human primates (Griffith and Humphrey, 2006), and humans (Moss et al., 2004; Sun et al., 2008a; DiLorenzo et al., 2010; Vedam-Mai et al., 2011). In these series, reactive elements including multinucleated giant cells and macrophages were common findings. In human studies, common elements included thin glial rims surrounding the electrode tract; lymphocytes and monocytes have also been described near the electrode (Moss et al., 2004; DiLorenzo et al., 2010). The volume of the glial surround, which could greatly impact electrode function, is, however, not known (Moss et al., 2004). Investigation in rats, however, indicates regional variability in astroglial reactivity to implanted electrodes (Hirshler et al., 2010).

The effects on network activity by reactive astrocytosis then becomes an important focus of their overall effect. Reactive astrocytes display marked functional changes which could include direct neurotrophic effects though modified energetics or neurotrophic factor release, enhanced glutamate uptake, reorganization of metabolic pathways, and modulation of synaptic transmission (Liberto et al., 2004; Sofroniew, 2005; Escartin and Bonvento, 2008). Recently, a model using selective virus-induced reactive astrocytosis in rat hippocampal area CA1 demonstrated that astrocytosis resulted in specific deficits in inhibitory synaptic transmission, and caused disruptions in functional regulation of circuits resulting in enhanced excitability of the local network (Ortinski et al., 2010). Specifically, a reduction was found in elicited monosynaptic inhibitory responses, which led to a reduction in basal inhibitory neurotransmission without affecting intrinsic neuronal properties. This resulted from an alteration in the astrocytic glutamate/glutamine cycle which resulted in reduced synaptic GABA availability (Ortinski et al., 2010). Thus, a growing body of evidence supports that reactive astrocytosis at the electrode site could readily alter network effects of targeted neural circuits.

In contrast to open-loop stimulation, contingent or closed-loop stimulation is designed to suppress epileptiform activity by stimulating a defined epileptogenic target directly in response to detection of abnormal EEG activity. This form of closed-loop, responsive neural stimulation (RNS), has preliminarily been shown safe and efficacious (Sun et al., 2008b), and is currently being evaluated in a randomized trial to assess safety and efficacy in epileptic patients. While its experimental and clinical trial background are beyond the scope of this review, its' similarities to DBS, notably contacting electrodes, pulse delivery, and network neuromodulatory effects imply a likelihood of astrocytic involvement analogous to open-loop stimulation.

TRANSCRANIAL MAGNETIC STIMULATION

Transcranial magnetic stimulation of cortical tissues was initially reported by Barker et al. (1985) and quickly found acceptance as a research vehicle for neurophysiologists. TMS was initially applied to the study of the motor system (Barker et al., 1985) and has since expanded to include investigations in psychiatric conditions (Pascual-Leone et al., 1996), and migraine headache (Lipton and Pearlman, 2010). Importantly, it has also become a viable option for the treatment of drug resistant epilepsy. TMS exerts its effects through repetitive non-invasive stimulation in which a pulsed magnetic field creates current flow in the brain which can temporarily excite or inhibit target areas (Hallett, 2000).

The basis of TMS as a therapeutic neuromodulatory is derived from the lasting effects from the application of a train of transcranial stimuli. Theoretically, the lasting effects of TMS can be used to modulate activity in focal areas of cortex (Fregni and Pascual-Leone, 2007). The induced effect depends on the nature of the stimulation; that is, the frequency, the timing, the focus, and the intensity of the repetitive stimulation (Kimiskidis, 2010). While some paradigms have been studied using animal models, the numbers of basic studies particular to epilepsy are somewhat limited.

Early study within the mouse hippocampal-entorhinal cortex slice model indicated that repetitive direct (i.e., non-transcranial) stimulation at 1 Hz can depress the generation of ictal activity in a 4-aminopyridine model (Barbarosie and Avoli, 1997), in a frequency-dependent manner (D'Arcangelo et al., 2005). This frequency dependence has been replicated in TMS. Low-frequency TMS stimulation shows the tendency to lower seizure

activity (Akamatsu et al., 2001; Godlevsky et al., 2006; Rotenberg et al., 2008). High frequency stimulation has been shown to potentially have both protective and inductive effects dependent on the chronicity of treatment and potentially other, unexplored, factors (Jennum and Klitgaard, 1996; Ebert and Ziemann, 1999).

Similar results have been identified in human studies. High frequency TMS has been shown to enhance cortical excitability at high intensities (Berardelli et al., 1998), while low-frequency TMS has been shown to reduce cortical excitability (Cincotta et al., 2003) as well as decreased strength of neuronal signaling (Muellbacher et al., 2000). As detailed by Kimiskidis (2010), the clinical effects are theoretically similar to long term potentiation (LTP) and long term depression (LTD) elicited by high- and low-frequency electrical stimulation, respectively. It is therefore possible that TMS at lower frequencies may exert its effect through the initiation of LTD, while at higher frequencies, the proconvulsant effect may be initiated through the induction of an LTP-type effect (Ziemann, 2005).

Direct evidence for astrocytic involvement in the neuromodulatory therapy is limited. Early work in a murine model found that high frequency TMS had a dramatic effect in the upregulation of astroglial gene expression (Fujiki and Steward, 1997) Following multiple high frequency trains (25 Hz), GFAP mRNA levels were significantly increased in the hippocampal dentate gyrus to levels similar to that following electroconvulsive seizures, indicating induction of an astrocytic reactive response (Fujiki and Steward, 1997). Indirectly, the analogous effects to LTD and LTP have important implications for astrocyte involvement, as the important contributions of astrocytes and gliotransmitters to synaptic plasticity have been described in multiple neuronal circuits (Yang et al., 2003; Witcher et al., 2007; Henneberger et al., 2010; Ben Menachem-Zidon et al., 2011; Bonansco et al., 2011; Navarrete et al., 2012).

CONCLUSIONS AND FUTURE DIRECTIONS

In spite of optimal medical management, many patients with epilepsy remain medically refractory and suffer from debilitating seizures. Some of these patients may benefit from neuromodulatory treatment. As the evidence above indicates, it is very likely that modulation of astroglial function is important to the efficacy of neuromodulation. Additional studies are needed to identify the appropriate patient populations for neuromodulation, optimal targets, optimal stimulation modalities, and paradigms. It is also critical that the cellular and network mechanisms underlying the effects of these treatments must be better elucidated. Further studies are needed to determine the contribution of neural and glial components of the nervous system, and future modalities must be developed which optimize both. Understanding these relationships may enable future technologies, perhaps even nanotechnologies, to flourish in the developing field of therapeutic neuromodulation.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://www.frontiersin.org/Computational_Neuroscience/10.3389/fncom.2012.00061/abstract

Movie S1 | Reconstructed dendrites, synapses, and associated astroglial processes from rodent hippocampus. Note the spatial relationship of a single astrocytic process (blue) interdigitating among multiple unique dendrites (gold, yellow, red, and purple). Axonal boutons are not displayed. These spatial relationships likely allow for the interactions of astrocytes with neurons, the regulation of neuronal communication, and form the basis of the astroglial-neuronal network. Figure acknowledgment to Cosmocyte, Inc. (Savage, MD, USA) for production in rendering and display.

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Self-repair in a bidirectionally coupled astrocyte-neuron (AN) system based on retrograde signaling

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In this paper we demonstrate that retrograde signaling via astrocytes may underpin self-repair in the brain. Faults manifest themselves in silent or near silent neurons caused by low transmission probability (PR) synapses; the enhancement of the transmission PR of a healthy neighboring synapse by retrograde signaling can enhance the transmission PR of the "faulty" synapse (repair). Our model of self-repair is based on recent research showing that retrograde signaling via astrocytes can increase the PR of neurotransmitter release at damaged or low transmission PR synapses. The model demonstrates that astrocytes are capable of bidirectional communication with neurons which leads to modulation of synaptic activity, and that indirect signaling through retrograde messengers such as endocannabinoids leads to modulation of synaptic transmission PR. Although our model operates at the level of cells, it provides a new research direction on brain-like self-repair which can be extended to networks of astrocytes and neurons. It also provides a biologically inspired basis for developing highly adaptive, distributed computing systems that can, at fine levels of granularity, fault detect, diagnose and self-repair autonomously, without the traditional constraint of a central fault detect/repair unit.

Keywords: astrocyte, calcium wave, endocannabinoid, IP3, neuron, self-repair, synapse

INTRODUCTION

Traditionally, communication, information transfer, and plasticity within the brain have been the sole province of the chemical synapses made by pre- and post-synaptic neurons. However, current research has challenged this view of synaptic physiology where it is now believed that astrocytes, a type of glial cell found in the central and peripheral nervous system can encapsulate $\sim 10^5$ synapses and can connect to multiple neighboring neurons (Bushong et al., 2002; Halassa et al., 2007); this intimate connection between astrocytes and neurons is named the *tripartite synapse* (Araque et al., 1999).

Although astrocytes cannot elicit propagating action potentials (APs) like neurons do, their "unit of excitation" is the transient increase in intracellular calcium (Ca^{2+}) levels that is elicited by various neurotransmitters (e.g., glutamate, ATP, GABA, etc.) following binding to their respective receptors on the astrocytic membrane. These astrocytic Ca^{2+} transients in turn lead to astrocytic release of transmitters (often referred to as "gliotransmitters") and to propagating Ca^{2+} waves (Dani et al., 1992; Porter and McCarthy, 1996). Although the propagation of intracellular Ca^{2+} is not fully understood, the process is believed to be facilitated by signaling proteins between microdomain clusters of inosotil 1, 4, 5-trisphosphate Receptors (IP_3Rs) (Weerth et al., 2007; Agulhon et al., 2008). Astrocytes also communicate in a feedback mode with neurons. In response to elevated levels of intracellular Ca^{2+}, astrocytes can release gliotransmitters such as glutamate which bind to extrasynaptic receptors on the post synaptic neuron (Corlew et al., 2008). This bidirectional communication between astrocytes and neurons results in various forms of synaptic modulation.

Alongside the astrocyte's role in synaptic regulation they are also implicated in synaptogenesis and synaptic maintenance (Slezak and Pfrieger, 2003) which may have a role in how the brain carries out repairs. This is further supported by the recent finding that astrocytes possess binding sites for endocannabinoids, a type of retrograde messenger released post-synaptically during neuronal depolarization (Alger, 2002). Similar to neurotransmitter uptake, this leads to the oscillation of Ca^{2+} levels within the astrocyte and the release of glutamate. Such a signaling pathway acts to modulate the transmission probability (PR) of the synapse and is a potential candidate for self-repair of damaged or low PR synapses (Navarrete and Araque, 2010).

Understanding the mechanisms that underpin the brain's distributed and fine-grained repair capability still however remains a key challenge. To this end, we propose a simple computational model for self-repair based on bidirectional interactions between astrocytes and neurons (Araque et al., 2001; Perea and Araque, 2005). This paper is an extension of our previous work (Wade et al., 2011a,b) but now demonstrates self-repair through modulation of synaptic release PR where we consider a fault to be a condition which results in silent or near silent neurons caused by low transmission PR synapses; the enhancement of the transmission PR of a "faulty" synapse by retrograde signaling can enhance the transmission PR (repair)

and we show that a key mechanism underlying PR is indirect signaling through retrograde messengers such as endocannabinoids.

MATERIALS AND METHODS

ENDOCANNABINOID MEDIATED SELF-REPAIR

Upon the arrival of an AP at the pre-synaptic axon, neurotransmitter (glutamate) is released across the cleft and binds to receptors on the post-synaptic dendrite causing a depolarization of the post-synaptic neuron. When the post-synaptic neuron is sufficiently depolarized (e.g., emits an output spike), voltage gated channels on the dendrite allow the influx of Ca^{2+} into the dendrite causing endocannabinoids to be synthesized and subsequently released from the dendrite. However, the exact release machinery underlying this process is not fully understood (Alger, 2002). Endocannabinoids are a type of retrograde messenger which travel back from the post-synaptic terminal to the pre-synaptic terminal. The release of 2-arachidonyl glycerol (2-AG), a type of endocannabinoid, is known to feed back to the pre-synaptic terminal in two ways:

1. **Directly:** 2-AG binds directly to type 1 Cannabinoid Receptors (CB1Rs) on the pre-synaptic terminal. This results in a decrease in transmission PR and is termed Depolarization-induced Suppression of Excitation (DSE) (Alger, 2002).
2. **Indirectly:** 2-AG binds to CB1Rs on an astrocyte which enwraps the synapse increasing IP_3 levels within the astrocyte and triggering the intracellular release of Ca^{2+}. This results in the astrocytic release of glutamate which binds to pre-synaptic group I metabotropic Glutamate Receptors (mGluRs). Such signaling results in an increase of synaptic transmission PR termed e-SP (Navarrete and Araque, 2010).

Experimental evidence shows that local synapses (i.e., synapses where post-synaptic firing results in both direct and indirect signaling) exhibit DSE and PR is reduced by ∼50%. This is thought to be a result of the direct signaling pathway overpowering the indirect pathway. The direct signaling pathway is very local since 2-AG can only travel ∼20 nm within the extracellular fluid and therefore binds only with a few neighboring synapses. The indirect signaling pathway is however far reaching and can affect distant synapses (Navarrete and Araque, 2010). Since astrocytes can enwrap very many ($\sim 10^5$) synapses and contact ∼6 neurons within the cortex and hippocampus (Halassa et al., 2007), the indirect signaling pathway has the potential to reach many synapses via the astrocyte. Distal silent synapses expressing indirect signaling via the astrocyte only, exhibit e-SP where PR increases by ∼200% (Navarrete and Araque, 2010). The repair mechanism proposed and modeled utilizes both DSE and the e-SP signal via an astrocyte to increase PR in neighboring synapses.

Given the known properties of endocannabinoids for the modulation of synaptic transmission PR, we hypothesize here that the indirect signaling pathway is the catalyst for self-repair of damaged or low PR synapses. For instance, consider the case where a synapse is damaged with a low PR insufficient to cause post-synaptic activity. Because this neuron is not emitting 2-AG its associated synapses will experience an increase in PR due to the release of 2-AG from neighboring neurons. This messenger causes the release of glutamate from the astrocyte cell activating type I mGluRs in the pre-synaptic terminal.

The proposed self-repairing model builds on two biophysically motivated models which describe the interactions between astrocytes and neurons in a tripartite synapse: namely the gatekeeper model (Volman et al., 2007) and the Nadkarni and Jung model (Nadkarni and Jung, 2004, 2007). Both of these models use the Li-Rinzel model (Li and Rinzel, 1994) to describe the evolution of synapse driven Ca^{2+} within the astrocyte; Ca^{2+} regulates synapse transmission via the release of glutamate which binds to pre-synaptic receptors.

ENDOCANNABINOID DYNAMICS

The gatekeeper (Volman et al., 2007) and Nadkarni and Jung (2004, 2007) models describe the interaction of astrocytes and neurons via the tripartite synapse. In a tripartite synapse an astrocyte process connects with the axon and dendrite of the pre- and post-synaptic neurons and is sensitive to the neurotransmitters within the extracellular fluid in the synaptic cleft (Araque et al., 1999). However, the tack taken in the current work is to model the astrocytes sensitivity to 2-AG instead of neurotransmitter. **Figure 1** illustrates a tripartite synapse with 2-AG signaling.

When neurotransmitter, e.g., glutamate, is released into the synaptic cleft and the post-synaptic neuron is sufficiently depolarized, 2-AG is released from the dendrite and binds to CB1Rs on the astrocyte process. This in turn initiates the creation and

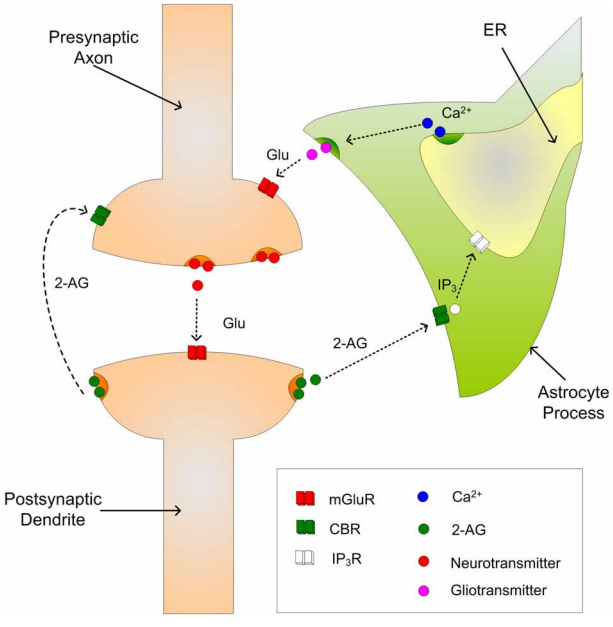

FIGURE 1 | The tripartite synapse showing indirect and direct signaling of 2-AG.

release of IP_3 into the cytoplasm of the astrocyte which subsequently binds to IP_3Rs on the Endoplasmic Reticulum (ER); the ER is a long network of tubes and vesicles used to store calcium within the cell (Kurosinski and Gotz, 2002). The binding of IP_3 with IP_3Rs opens channels that allow the release of Ca^{2+} from the ER in to the cytoplasm. While individual Ca^{2+} releases are incapable of propagating intracellularly, several releases can raise Ca^{2+} levels in the cytoplasm beyond a threshold and an oscillating Calcium Induced Calcium Release (CICR) propagation can be observed (Marchant et al., 1999); the threshold is believed to be of the order 0.2–0.4 μM (Bezprozvanny et al., 1991). The increase in cytosolic Ca^{2+} then causes the release of gliotransmitter back into the synaptic cleft which binds to pre-synaptic group I mGluRs, i.e., indirect signaling. The 2-AG also binds directly (direct signaling) to the pre-synaptic CB1Rs which causes a decrease in PR.

To model 2-AG release we assume each time a post synaptic neuron fires, 2-AG is released and can be described as follows:

$$\frac{d(\mathrm{AG})}{dt} = \frac{-\mathrm{AG}}{\tau_{\mathrm{AG}}} + r_{\mathrm{AG}}\delta(t - t_{\mathrm{sp}}) \tag{1}$$

where AG is the quantity of 2-AG, τ_{AG} is the decay rate of 2-AG, r_{AG} is the 2-AG production rate ($= 0.8\,\mu\mathrm{Ms}^{-1}$) and t_{sp} is the time of the post-synaptic spike. To our best knowledge no data are available in the literature on the magnitude of τ_{AG} and therefore we have assumed that the lifetime of 2-AG is consistent with other time constants: for example the effects of e-SP are known to have a rise time of ∼100 s and a decay time of ∼200 s (Navarrete and Araque, 2010), therefore a value of 10 s is assumed for τ_{AG}.

CALCIUM DYNAMICS

In the present model, 2-AG binds to CB1Rs on the astrocyte process and the generation of IP_3 is achieved in a similar manner to the gatekeeper model (Volman et al., 2007). This process is assumed to be dependent on the amount of 2-AG released. The generation of IP_3 is given by:

$$\frac{d(\mathrm{IP}_3)}{dt} = \frac{\mathrm{IP}_3^* - \mathrm{IP}_3}{\tau_{\mathrm{ip3}}} + r_{\mathrm{ip3}}\mathrm{AG} \tag{2}$$

where IP_3 is the amount within the cytoplasm, r_{ip3} is the production rate of IP_3 and is set at $0.5\,\mu\mathrm{Ms}^{-1}$, IP_3^* is the baseline of IP_3 within the cytoplasm when the cell is in a steady state and receiving no input, and τ_{ip3} is the IP_3decay rate.

The Li-Rinzel model uses three channels to describe the Ca^{2+} dynamics within a cell: J_{pump} models how Ca^{2+} is stored within the ER by pumping Ca^{2+} out of the cytoplasm into the ER via Sarco-Endoplasmic-Reticulum Ca^{2+}-ATPase (SERCA) pumps, J_{leak} describes Ca^{2+} leakage into the cytoplasm from the ER and J_{chan} models the opening of Ca^{2+} channels by the mutual gating of Ca^{2+} and IP_3 concentrations. Since the model only considers the case of a single cell which exists in a Ca^{2+}-free extracellular environment, no account is taken of any Ca^{2+} flux across the cell membrane (De Pittà et al., 2008). The Li-Rinzel model is described using the following equations (a full derivation of these equations is provided by De Pittà et al., 2009):

$$\frac{d(\mathrm{Ca}^{2+})}{dt} = J_{\mathrm{chan}}\left(\mathrm{Ca}^{2+}, h, \mathrm{IP}_3\right) + J_{\mathrm{leak}}\left(\mathrm{Ca}^{2+}\right) - J_{\mathrm{pump}}\left(\mathrm{Ca}^{2+}\right) \tag{3}$$

$$\frac{dh}{dt} = \frac{h_\infty - h}{\tau_h} \tag{4}$$

where J_{chan} is the IP_3 and Ca^{2+}-dependent Ca^{2+} release, J_{pump} is the amount of Ca^{2+} pumped from the cytoplasm into the ER via the SERCA pumps, J_{leak} is the Ca^{2+} which leaks out of the ER and h is considered to be the fraction of activated IP_3Rs. The parameters h_∞ and τ_h are given by:

$$h_\infty = \frac{Q_2}{Q_2 + \mathrm{Ca}^{2+}} \tag{5}$$

and

$$\tau_h = \frac{1}{a_2\left(Q_2 + \mathrm{Ca}^{2+}\right)} \tag{6}$$

where

$$Q_2 = d_2\frac{\mathrm{IP}_3 + d_1}{\mathrm{IP}_3 + d_3} \tag{7}$$

The description of the J_{chan} channel is given by:

$$J_{\mathrm{chan}} = r_C m_\infty^3 n_\infty^3 h^3\left(C_0 - (1 + c_1)\,\mathrm{Ca}^{2+}\right) \tag{8}$$

where r_C is the maximal CICR rate, C_0 is the total free Ca^{2+} cytosolic concentration, C_1 is the ER/cytoplasm volume ratio and m_8 and n_8 are the IP_3 Induced Calcium Release (IICR) and CICR channels, respectively, and are given by:

$$m_\infty = \frac{\mathrm{IP}_3}{\mathrm{IP}_3 + d_1} \tag{9}$$

and

$$n_\infty = \frac{\mathrm{Ca}^{2+}}{\mathrm{Ca}^{2+} + d_5} \tag{10}$$

The remaining channels are given by:

$$J_{\mathrm{leak}} = r_{\mathrm{L}}\left(C_0 - (1 + c_1)\,\mathrm{Ca}^{2+}\right) \tag{11}$$

and

$$J_{\mathrm{pump}} = \nu_{\mathrm{ER}}\frac{\left(\mathrm{Ca}^{2+}\right)^2}{k_{\mathrm{ER}}^2 + \left(\mathrm{Ca}^{2+}\right)^2} \tag{12}$$

where r_{L} is the Ca^{2+} leakage rate, ν_{ER} is the maximum SERCA pump uptake rate and k_{ER} is the SERCA pump activation constant.

ENDOCANNABINOID-MEDIATED SYNAPTIC DEPRESSION/ POTENTIATION (DSE/e-SP)

There is no clear consensus in the literature on the relationship between DSE and the level of 2-AG released by the post synaptic

neuron. In the present case we assume a linear correspondence given by:

$$\mathrm{DSE} = \mathrm{AG} \times K_{\mathrm{AG}} \tag{13}$$

where AG is the amount of 2-AG released by the post-synaptic neuron and is found from Equation (1) and K_{AG} ($= -4000$) is a scaling factor used to convert the level of 2-AG into the desired negative range. The intracellular astrocytic calcium dynamics are used to regulate the release of glutamate from the astrocyte which drives e-SP. To model this release, we assume when Ca^{2+} crosses the CICR threshold from below that a quantity of glutamate targeting group I mGluRs is released every 300 ms and is given by:

$$\frac{d(\mathrm{Glu})}{dt} = \frac{-\mathrm{Glu}}{\tau_{\mathrm{Glu}}} + r_{\mathrm{Glu}}\delta(t - t_{\mathrm{Ca}}) \tag{14}$$

where Glu is the quantity of glutamate, τ_{Glu} is the decay rate of glutamate ($= 100$ ms), r_{Glu} is the glutamate production rate ($= 10\,\mu\mathrm{Ms}^{-1}$) and t_{Ca} is the time of the Ca^{2+} threshold crossing. It is believed that Ca^{2+} oscillations can be initiated within discrete microdomains (Panatier et al., 2011) and can be localized or propagated intracellularly by activating neighboring microdomains of storage (Pasti et al., 1997; Carmignoto, 2000; Weerth et al., 2007; Agulhon et al., 2008; Di Castro et al., 2011). Therefore, the level of Ca^{2+} within the cell differs depending on spatial location. However, for simplicity in the present model we assume that the instantaneous level of Ca^{2+} remains the same everywhere; therefore the release of glutamate is also assumed to be instantaneous.

To model e-SP we use the following:

$$\tau_{\mathrm{eSP}}\frac{d(\mathrm{eSP})}{dt} = -\mathrm{eSP} + m_{\mathrm{eSP}}\mathrm{Glu}(t) \tag{15}$$

where τ_{eSP} is the decay rate of e-SP ($= 40$ s) and m_{eSP} is a weighting constant ($= 55 \times 10^3$) used to control the height of e-SP. From Equation (15) it is clear that the level of e-SP is dependent on the quantity of glutamate released by the astrocyte.

Parameters and initial variable values used throughout this paper can be found in **Tables A1–A3**. The initial variable values represent the system in a quiescent state. Note that initial values for h and Ca^{2+} were found experimentally by initializing Ca^{2+} and h to 0.5 and 0.06 μM, respectively. The model was then simulated with IP_3 levels clamped at 0.16 μM (IP_3^*) until Ca^{2+} and h stabilized.

SELF REPAIRING PARADIGM

Our self repairing paradigm is proposed with reference to **Figures 2(A)** and **(B)**, where **(A)** shows a simple SNN network fragment with no faults and **(B)** show the same network where the PR has been reduced (to simulate a fault) in the synapse associated with the post-synaptic neuron N2.

To illustrate the self repairing concept we first consider the case where both synapses are healthy, as depicted in **Figure 2A**: both direct and indirect feedback signals compete at each synapse to alter PR and enable a stable state to be reached (signal conditions for the non-faulty state will be verified later on). In **Figure 2B** a fault is introduced into the synapse associated with N2 and hence both direct and indirect retrograde feedback from neuron N2 ceases. This creates an imbalance in PR at the synapse associated with N2 with the result that PR increases to restore functionality due to indirect retrograde feedback from N1. This is the self-repairing mechanism proposed here and is verified by the computational results shown later in **Figure 5**. Our simulations demonstrate that when faults occur in synapses associated with a neuron, indirect feedback from other neurons implements repair by increasing the PR across all synapses (faulty and non-faulty) associated with the neuron to restore the original functionality.

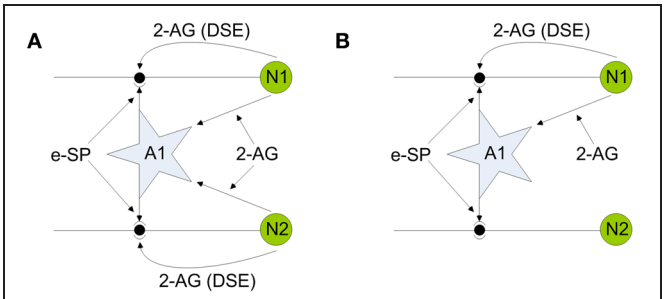

FIGURE 2 | Network fragments illustrating endocannabinoid mediated self-repair. (A) Network before fault. **(B)** Network after fault. Note 2-AG is a local signal associated with each synapse connected to either neuron N1 or N2, whereas e-SP is a global signal associated with all synapses connected to the astrocyte A1.

NEURON MODEL

Although many neuron models exist such as the Hodgkin–Huxley model (Hodgkin and Huxley, 1952), the simplified counterparts such as (FitzHugh, 1961; Nagumo et al., 1962; Morris and Lecar, 1981) are often preferred (Gerstner and Naud, 2009). Nevertheless, these models are still computationally expensive and require a great deal of parameter tuning. However, one of the most widely used neural models is the Leaky Integrate and Fire (LIF) model (Gerstner and Kistler, 2002) which has relatively few parameters (Gerstner and Naud, 2009) and requires relatively little computational effort due to its simplistic nature. Consequently the LIF is more suited to large network simulations (Bugmann, 1997). The LIF model used in this work is the passive model (Gerstner and Kistler, 2002) described by:

$$\tau_m\frac{dv}{dt} = -v(t) + R_m\sum_{i=1}^{n} I_{\mathrm{syn}}^i(t) \tag{16}$$

where τ_m is the membrane time constant, v is the membrane potential and R_m is the membrane resistance and I_{syn}^i is the current injected to the neural membrane at synapse i. If v is greater than the firing threshold (v_{th}) then v is clamped at 0V for 2 ms thereby implementing the refractory period of the neuron.

SYNAPSE MODEL

The synapse model used here is probabilistic-based where each time a pre-synaptic spike is presented to the synapse a uniformly distributed pseudorandom number generates a number between

0 and 1 (*rand*). If the value of the random number is less than or equal to the release PR a fixed current I_{inj} (= 6650 pA) is injected into the LIF given by:

$$I^i_{syn}(t) = \begin{cases} I_{inj} & \text{rand} \leq \text{PR} \\ 0 & \text{rand} > \text{PR} \end{cases} \quad (17)$$

All synapse and neuron parameters can be found in **Table A3**. If we now consider the case where the network is functioning without fault (**Figure 2A**) then the associated PR of each associated synapse is governed by the following:

$$\text{PR}(t) = \left(\frac{\text{PR}\,(t_0)}{100} \times \text{DSE}(t)\right) + \left(\frac{\text{PR}\,(t_0)}{100} \times \text{eSP}(t)\right) \quad (18)$$

where $\text{PR}(t_0)$ is the initial PR of each associated synapse. The variables of DSE and e-SP have been tuned so that Equation (18) results in an overall depression of each of the synapses by ∼50% in accordance with Navarrete and Araque (2010). However, if we consider the case in which a number of synapses become faulty and therefore DSE decreases, then the depression of each synapse is decreased as e-SP starts to overpower DSE. In extreme faults the PR is given by Equation (19), which is ∼200% and the associated neuron (N2 in **Figure 2B**) exhibits a significantly reduced firing rate with no appreciable direct or indirect impact on the synapse.

$$\text{PR} \rightarrow \left(\frac{\text{PR}\,(t_0)}{100} \times \text{eSP}\,(t)\right) \quad (19)$$

However, indirect feedback from N1 via the astrocyte increases e-SP, and *PR* at the synapses is proportionally increased. We view this as a repair mechanism. Therefore, the value of PR at time t, $\text{PR}(t)$, is a percentage of the initial value of $\text{PR}(t_0)$ and is governed by the indirect signaling pathway between the astrocyte and neuron (e-SP). Note that when a fault is simulated the value $\text{PR}(t_0)$ is set to the fault PR value of the synapse.

RESULTS

Here we present results of simulations that highlight the dynamics of our model and demonstrate how self-repair can occur at synapses. In these simulations the network shown in **Figure 2** is used with each neuron receiving input from 10 synapses. All synapses have an initial PR of 0.5 and are simulated with a unique Poisson distributed spike train with an average firing rate of 10 Hz. The Matlab 2009a simulation environment was employed throughout and the Euler method of integration with a fixed time step of $\Delta t = 1$ ms was used in all simulations. Results remained unchanged using a time step of $\Delta t = 0.1$ ms (data not shown).

SIMULATION WITH NO FAULT

Consider the case where neurons N1 and N2 are firing as a result of the presented pre-synaptic stimuli and coupling with the astrocyte occurs via the 2-AG signal. This causes the release of glutamate which acts on mGluRs receptors on the pre-synaptic terminals of both neurons (see **Figure 2**). Both neurons are simulated for a period of 200 s. From **Figure 3(A)** it can be seen that the e-SP function is global to both N2 and N2 while DSE is local to the synapses associated with the individual neurons. Thus, the synapse of N1 receives a different DSE signal to that of N2 [**Figure 3(B)**]. **Figure 3(C)** presents the PR of a synapse associated with N1 (N2). Note how PR is reduced by ∼50% due to the summation of e-SP and DSE at the synapse. Furthermore each of the synapses of N1 and N2 receives the same PR value as depicted in **(C)**. Finally, **Figure 3(D)** depicts the average firing rates of both neurons, where it can be seen that the firing rates of both neurons are reduced from the initial firing rate. This results from the overall depression of all synapses by ∼50%.

SIMULATION PARTIAL FAULT (MODERATE REDUCTION OF PR)

Now consider the case where N1 and N2 are stimulated by multiple synapses (10 in this example) and that the PR associated with 80% of the synapse of N2 is deliberately reduced (simulating a fault) to 0.1 after 200 s. From **Figure 4** we can see that N1, as expected, is unaffected and the PR values of all synapses connected to N1 are depressed at ∼50% of their initial value.

This is not the case with synapses associated with N2 (**Figure 5**). After 200 s the reduction in PR of the faulty synapses causes a decrease in the output firing rate of N2 thereby reducing the associated DSE signal. This creates an imbalance in the dynamics and e-SP is allowed to enhance all synapses connected to N2. Note how the PR of the remaining healthy synapses (**Figure 5D**) is enhanced to a greater extent when compared to the faulty synapses (**Figure 5C**) suggesting that the introduction of faults perturbs the balance between DSE and e-SP such that an overall increase in PR results primarily in the healthy synapses. This is akin to redistributing the PR across the remaining 20% of healthy synapses. Indicative of the proposed repair process, **Figure 5D** shows that the average firing rate of N2 falls after 200 s but then increases again after a period of time due to the redistribution of PR across all synapses (repair process).

SIMULATION WITH COMPLETE FAULT (PR REDUCED TO 0)

Next we consider the case when 8 out of the 10 synapses connected to N2 exhibit a catastrophic failure such as their connecting axons being severed due to injury. This is simulated by reducing the associated PRs to 0 at 200 s. The total length of this simulation is 400 s. Again N1 is unaffected and the synapses have a reduction of ∼50% as in the previous cases (data not shown). **Figure 6** presents the PR from 50% of the synapses connected to N2: eight synapses had induced faults while two were left to function normally. It can be seen from plots **A–C** of **Figure 6** that no repair occurs, i.e., PR = 0. However, the PR values of the remaining two non-faulty synapses were again significantly increased beyond their initial value of 0.5 as repair takes place. Once again, the faults are tolerated by an increase in PR at the non-faulty synapses.

Figure 7 describes the average firing rate of N2 where it can be seen that the firing rate falls to ∼0 Hz at 200 s but recovers after a few seconds (albeit to a lower firing rate) when repair has taken place. Again, the repair shows partial recovery of the firing rate.

SIMULATION WITH NO e-SP

The results of the previous experiments suggest that when synapses within the Astrocyte-Neural Network become faulty,

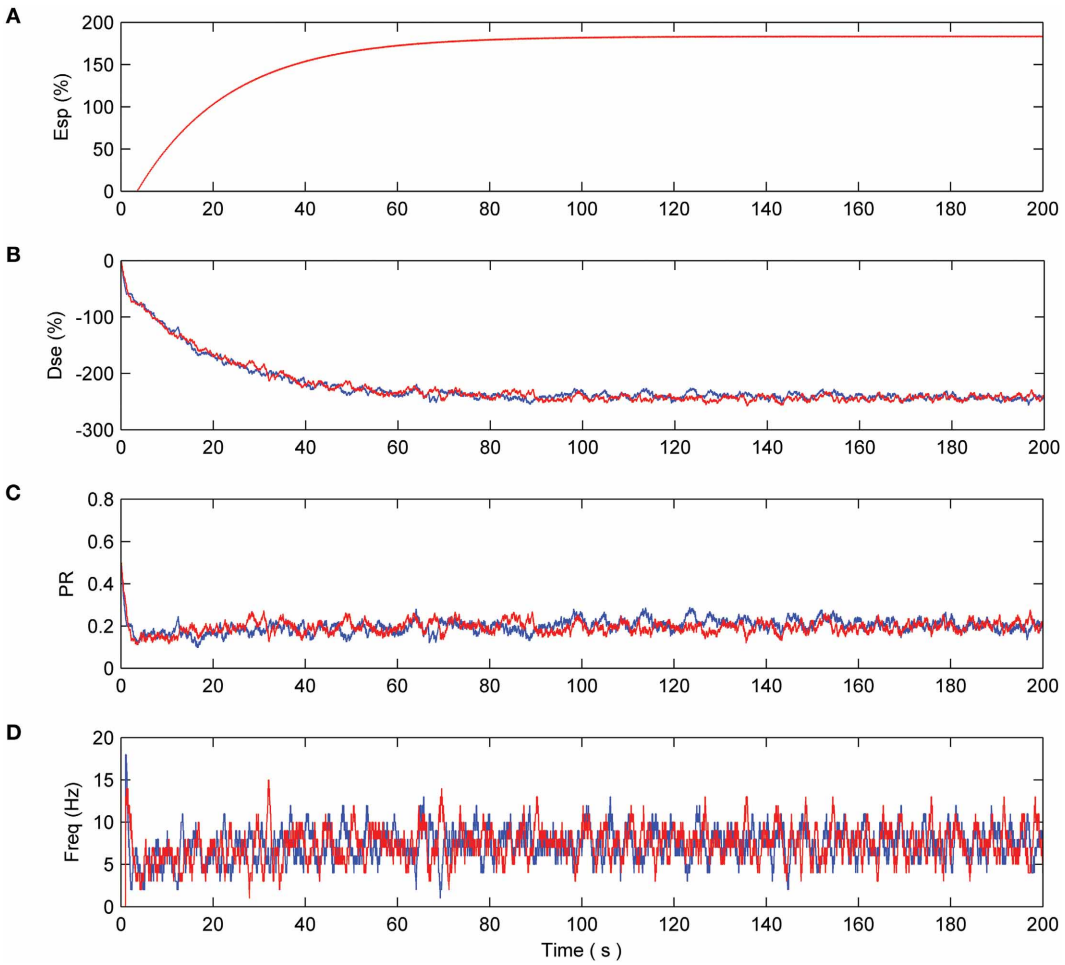

FIGURE 3 | Network with no fault. (A) e-SP function of both N1(blue) and N2 (red). Since e-SP is a global function and relates to all synapses connected by the astrocyte it is the same for N1 and N2 **(B)** DSE function of N1 and N2. Since DSE is only local to all synapses connected to a neuron, DSE is different in N1 and N2 and is driven by the output of each neuron. **(C)** The probability of a synapse connected to N1 and N2. This probability is the summation of e-SP and DSE presented to the neuron. Note how the probability is reduced by ∼50% which results in an overall reduction of the firing rate of N1 and N2 as seen in **(D)**.

repairs are implemented by enhancement of PR at other non-faulty synapses. To prove that no other mechanism is responsible for repair we repeat the above experiment in the absence of the astrocyte cell, i.e., without an indirect feedback signal (e-SP). The results showed a decrease in PR of all synapses of N1 and N2 in the order of ∼200% due to direct feedback. At the onset of induced faults at 200 s, the functioning synapse of N2 exhibited an increase in PR due to the reduction in DSE associated with N2's drop in firing activity (data not shown). Such PR increase is much less than that found in the previous simulation and is ineffective as a repair mechanism.

CALCIUM DYNAMICS

Figure 8 describes the astrocyte calcium dynamics for *no fault*, *partial fault,* and *catastrophic conditions* as described by the previous three simulations. Note there is a reasonable constant Ca^{2+} oscillation when there is no fault; however, when a fault is induced at 200 s the overall calcium levels drop and the oscillations continue. As long as the overall Ca^{2+} level remains above the threshold the astrocyte will release glutamate in an attempt to reinforce the PR of all synapses.

DISCUSSION

This work was motivated by the need to understand how the brain regulates itself to cope with injury. Exploiting the biological adaptive/repair mechanisms of the brain (Stevens, 2008) would provide a novel approach to fault tolerant computing, which goes beyond existing capabilities where reliable computations could then be realized using neural networks (Patterson et al., 2012), instead of traditional von Neumann computing architectures. Neural networks offer a fine-grained distributed computing architecture that captures to some degree high levels of parallel processing in the brain. The fine-grained parallelism provides the framework that enables fault tolerance to be realized at very low levels of granularity; i.e., computations are mapped across many neuron clusters permitting a "scattering" of faults without a significant level of computing degradation. However, high levels of parallelism are not the only contributor to fault tolerance as the

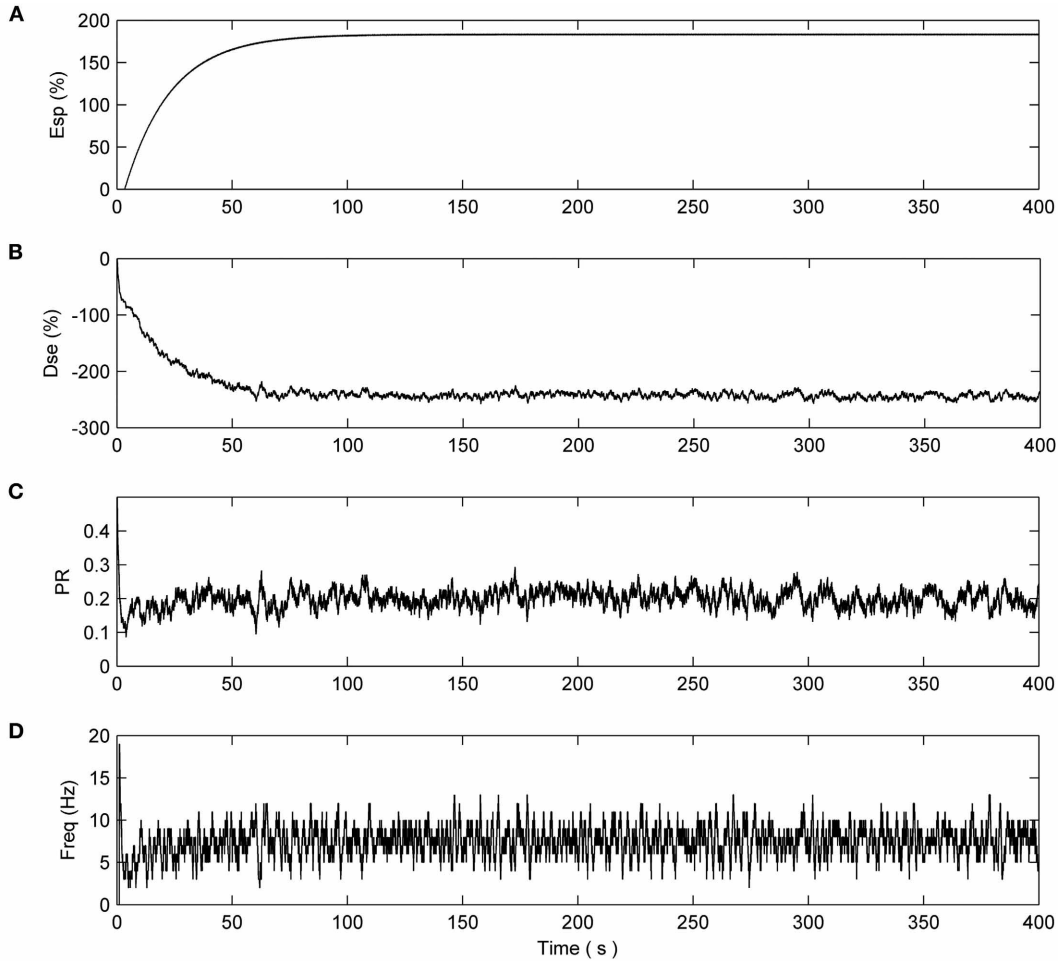

FIGURE 4 | Network with partial fault (N1). No synapse connected to N1 have a fault. **(A)** e-SP signal. **(B)** DSE signal. **(C)** PR of the first synapse connected to N1. The remaining synapses connected to N1 have the same PR dynamics. **(D)** Average firing rate of N1.

brain uses key repair mechanisms to continually adapt to conditions via re-wiring pathways to cope with decaying or damaged neurons (Slezak and Pfrieger, 2003). Therefore, we believe our model is the first step in addressing the key challenge which is to understand the mechanisms that underpin the brain's distributed and fine-grained repair capability. From a purely engineering point of view, modeling the interaction between cells at a network level may lead to a truly brain-inspired paradigm for fault tolerant computing beyond current self-repairing hardware architectures (Harkin et al., 2009). Traditionally, mission critical electronic systems demanded *design-for-reliability* due to the important function of the system (Ratter, 2004; Vladimirova and Paul, 2009; SEA, 2011). However this design challenge is now penetrating into non-critical systems where engineers must aim to realize reliable systems using unreliable computing fabrics (Barker et al., 2007; Beiu and Ibrahim, 2007; DeSyRe, 2012). Currently, bio-inspired techniques which utilize FPGAs (e.g., Glackin et al., 2004; Negoita and Hintea, 2009) provide adaptive repair but the levels of granularity are still insufficient. Furthermore, such systems also depend upon a central controller to make repair decisions thereby rendering the entire repair process ineffective if it develops a fault.

The present paper draws on numerous published experimental findings and a previous theoretical effort called the astrocyte-neuron (AN) model (Wade et al., 2011a,b) both of which suggest that astrocyte networks provide a more significant role in the function of nervous system than simply structural support. Rather, astrocytes are viewed as regulators of neural circuitry through coordination of transmission at the synapse. Current evidence indicates that retrograde messengers induced in the post-synaptic neuron are fed back either directly or indirectly via astrocyte cells to receptors on the pre-synaptic neuron (Navarrete and Araque, 2010). The present extension of our AN model captures the endocannabinoid interaction between astrocytes and neurons and demonstrates that positive feedback enhances the transmission PR in remote synapses during so-called fault conditions. Our hypothesis is that the emergence of low transmission PR synapses, which result in silent or near silent neurons, is how a "fault" is detected. Essentially a fault is detected when a neuron ceases sustained

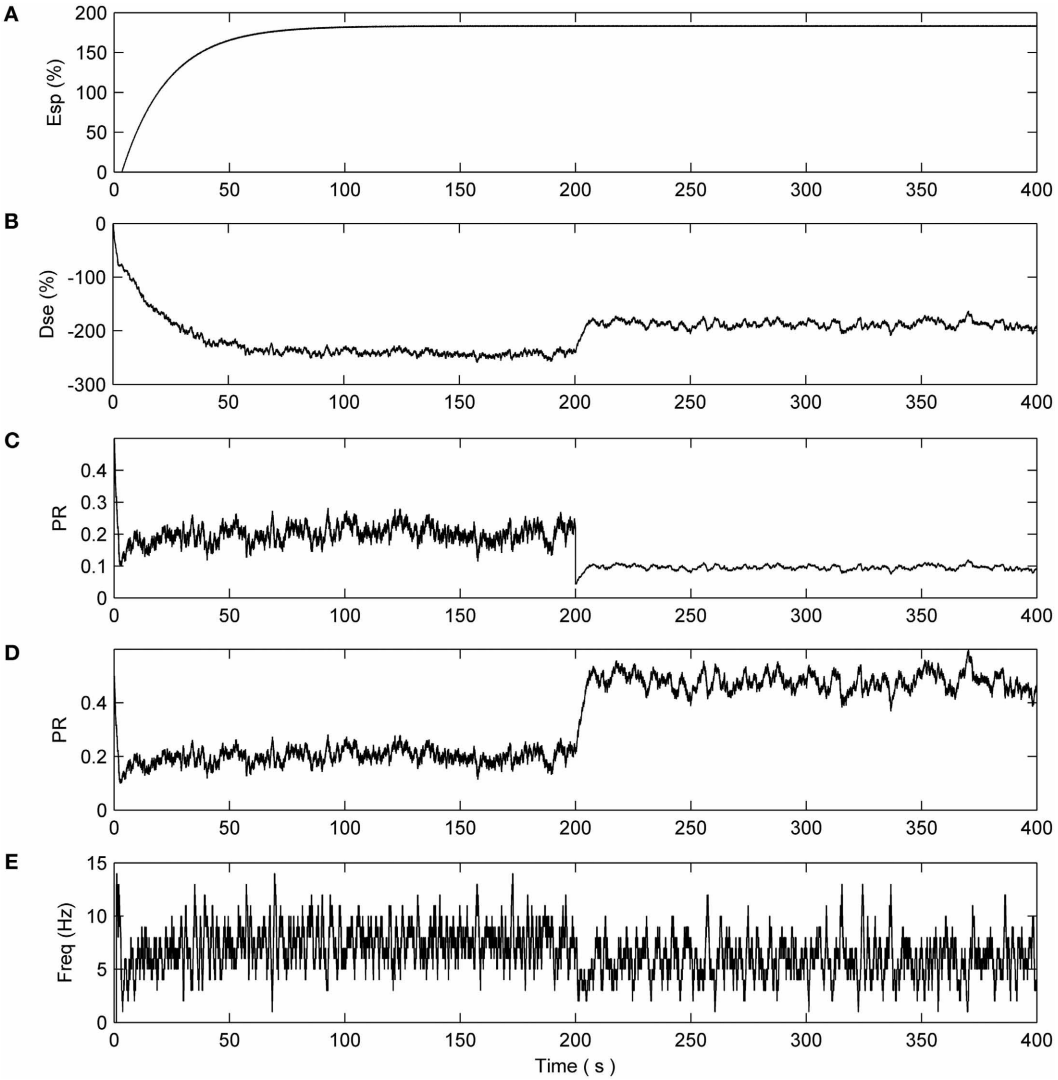

FIGURE 5 | Network with 80% of N2 synapses with PR reduced to 0.1 after 200 s. (A) e-SP signal. **(B)** DSE signal. **(C)** PR of the first synapse connected to N2. **(D)** PR of the non faulty synapses. **(E)** Average firing rate of N2 showing the rate falling off at 200 s and increasing thereafter due to the hypothesized repair process.

firing activity. The result is that endocannabinoid release, and therefore both direct and indirect feedback to associated synapses, is stopped or significantly reduced. However, the PR of release at synapses will slowly be enhanced again as a result of other active neuron signaling (e-SP) via astrocytes. Enhancement of the transmission PR of these synapses by indirect retrograde feedback from other active neurons is the proposed repair mechanism.

The self repairing concept minimizes degradation in the information processing capability of the network since the distribution in the weight vector, from the learning phase, is minimally altered by the repair process. If we consider the case where a number of active synapses are severed completely, the repair mechanism redistributes the synaptic weight associated with the lost synapses by increasing PR across the remaining healthy synapses. This is also the case where there is only a partial loss of the synapse. Therefore the repair process simply scales all the weights associated with the faulty neuron. Considering that a single axon can connect to a neuron via many synapses, where information is transmitted across these several synaptic paths with varied strengths/weights, the loss of several synapses will not result in the net information from the pre-synaptic neuron being completely diminished. The repair process will accommodate the loss of synapses by redistributing the original weighting of the lost synapses across the remaining healthy synapses. The result is that information from the pre-synaptic neuron still creates a similar net stimulation of the post-synaptic neuron. While more research is needed on the role of PR in information encoding, we would however, suggest that redistributing PR will result in a "redistribution of information coding" at the level of synapses

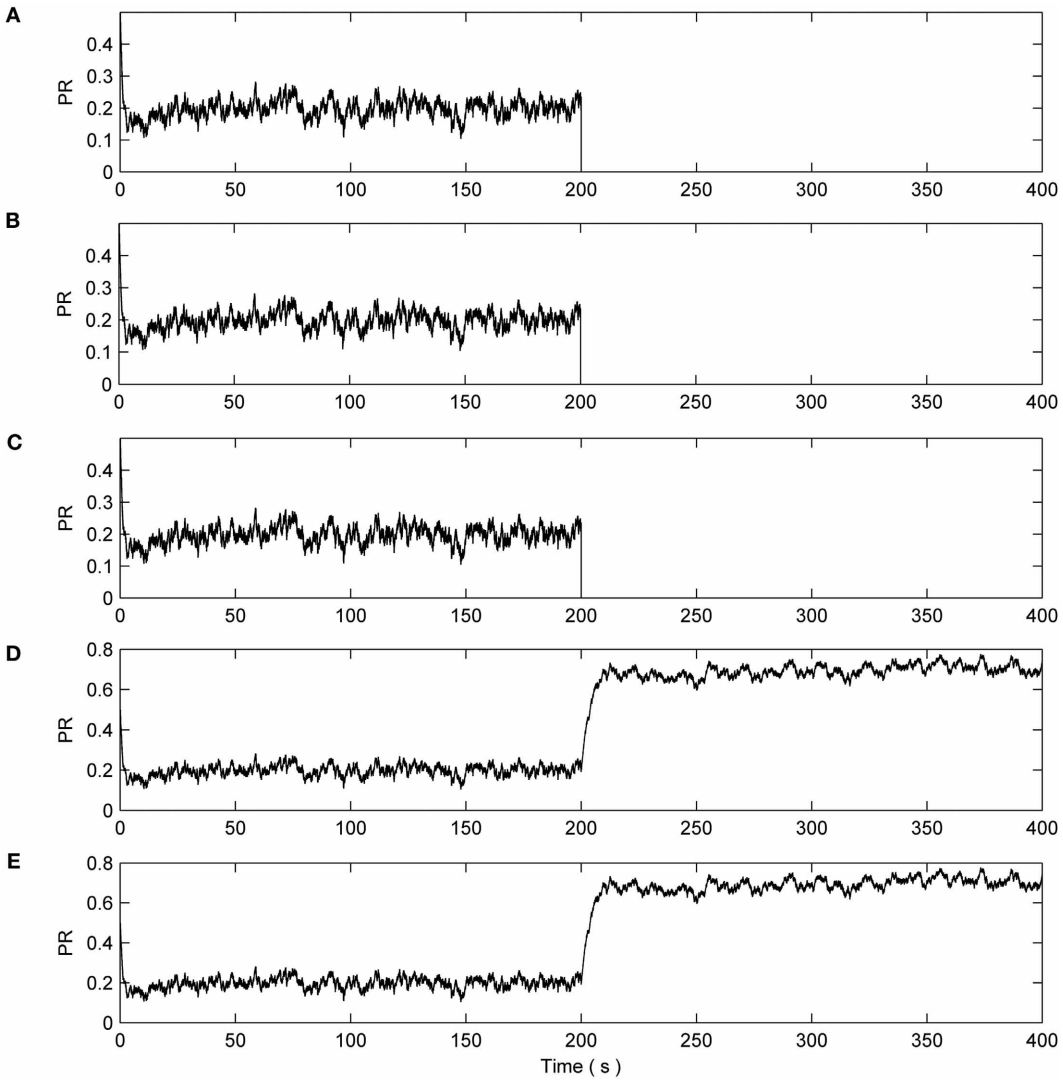

FIGURE 6 | PR values of synapses of N2. (A–C) Show three faulty synapses where the fault is induced at time 200 s. **(D,E)** Demonstrates the PR of the remaining non faulty synapses increasing to compensate for the net loss of **(A–C)**, thereby restoring the functionality of N2.

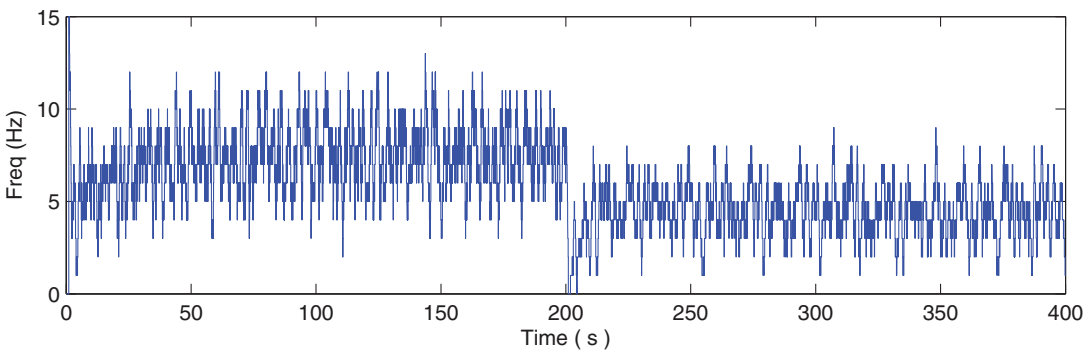

FIGURE 7 | Average firing rate of N2. Note how the output frequency of N2 falls to zero at 200 s as a result of the catastrophic failure of 80% of the synapses connected to N2. As the self-repair mechanism kicks in and increases the PR value of non-faulty synapses, the output frequency of N2 also increases.

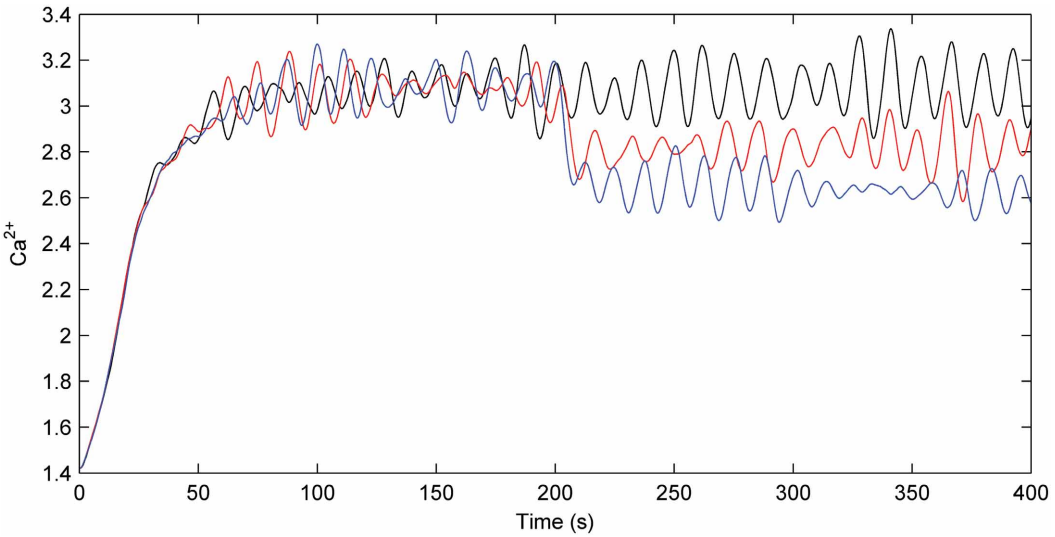

FIGURE 8 | Astrocyte calcium dynamics. Calcium dynamics for no fault (black), partial fault (red), and catastrophic fault (blue). When a fault occurs the Ca^{2+} levels drop within the astrocyte. However, as a result of the e-SP produced by the astrocyte, Ca^{2+} levels are not reduced substantially and oscillations continue.

to reestablish neural depolarization representative of that prior to the faulty condition. More importantly, the repair process exploits existing healthy synapses to take up the signaling effort which was originally sustained by the lost synapses thereby, removing the requirement for redundant synapses. This is a key attribute which enables efficient hardware implementations of the repair mechanism.

Clearly, further research is required to demonstrate self repair at the level of useful large-scale networks as our current network lacks some biological detail. For example, the model used to describe the functional relationship between PR and e-SP/DSE signal requires more experimental evidence to provide a more biophysical model, as does the functional dependency of the 2-AG signal on the activity of the post-synaptic neuron. Furthermore, the sustained firing activity required to create the DSE signal within our model lacks biological realism, firing of this nature is generally only found in motor neurons. However, we purposely implemented our DSE signal in this very simple way due to the lack of a clear understanding of the relationship between DSE and levels of 2-AG. Further investigations are required to underpin biological knowledge about the mechanisms of DSE before our model could faithfully capture the firing patterns found in other brain regions. Moreover, a more complete model would also take account of the spatial distribution of synapses from the delay perspective: delay associated with the astrocyte process. Despite these limitations our self repair network demonstrates the principle for local repair at the level of synapses and therefore provides a building block to develop upon and explore large-scale networks where global repair is possible via an astrocyte network. However, to extend this repairing paradigm a better understanding of astrocyte to astrocyte communication is required. While we are aware that these networks communicate using both gap junctions and ATP (Giaume et al., 2010), no formulation of these communications mechanisms has appeared in the literature. Consequently much research is required to support the modeling of large-scale repair.

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APPENDIX

Table A1 | Astrocyte parameters.

Astrocyte parameter	Parameter description	Value
IP_3^*	Baseline value of IP_3	0.16 μ M
r_{IP_3}	Rate of IP_3 production	0.5 μ M s^{-1}
τ_{IP_3}	IP_3 degradation time constant	7 s
τ_{Ca}	Decay rate of f controlled by level of Cytosolic Ca^{2+}	1 s
τ_{AG}	Decay rate of 2-AG	10 s
τ_{Glu}	Decay rate of Glutamate	100 ms
τ_{eSP}	Decay rate of eSP	40 s
r_C	Maximum rate of CICR	6 s^{-1}
r_L	Ca^{2+} leakage rate from ER	0.11 s^{-1}
r_{Glu}	Maximum rate of Glutamate production	10 μ M s^{-1}
v_{ER}	Maximum rate of SERCA uptake	0.8 μ M s^{-1}
c_0	Total free Ca^{2+} cytosol concentration	2 μM
k_{ER}	SERCA pump activation constant	0.1 μM
c_1	Ratio of ER volume to cytosol volume	0.185
d_1	IP_3 dissociation constant	0.13 μM
d_2	Ca^{2+} inactivation dissociation constant	1.049 μM
d_3	IP_3 dissociation constant	0.9434 μM
d_5	Ca^{2+} activation dissociation constant	0.08234 μM
a_2	IP3R Ca^{2+} inactivation binding rate	0.2 μM s^{-1}
m_{eSP}	e-SP weighting factor	55×10^3
Ca^{2+} Threshold	Astrocyte Glutamate release threshold	0.3 μM

Table A2 | Astrocyte initial variables.

Astrocyte variable	Initial value
Ca^{2+}	0.071006 μM
h	0.7791
J_{chan}	0 v
J_{pump}	0
J_{leak}	0
m_∞	0
n_∞	0
AG	0
Glu	0
eSP	0
IP_3	0.16 μM

Table A3 | Neuron and synapse parameters.

Neuron parameter	Parameter description	Value
v_{th}	Firing threshold voltage	9 mv
R_m	Membrane resistance	1.2 GΩ
τ_{mem}	Membrane time constant	60 ms
I_{inj}	Injected current	6650 pA

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Computational model of neuron-astrocyte interactions during focal seizure generation

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Empirical research in the last decade revealed that astrocytes can respond to neurotransmitters with Ca^{2+} elevations and generate feedback signals to neurons which modulate synaptic transmission and neuronal excitability. This discovery changed our basic understanding of brain function and provided new perspectives for how astrocytes can participate not only to information processing, but also to the genesis of brain disorders, such as epilepsy. Epilepsy is a neurological disorder characterized by recurrent seizures that can arise focally at restricted areas and propagate throughout the brain. Studies in brain slice models suggest that astrocytes contribute to epileptiform activity by increasing neuronal excitability through a Ca^{2+}-dependent release of glutamate. The underlying mechanism remains, however, unclear. In this study, we implemented a parsimonious network model of neurons and astrocytes. The model consists of excitatory and inhibitory neurons described by Izhikevich's neuron dynamics. The experimentally observed Ca^{2+} change in astrocytes in response to neuronal activity was modeled with linear equations. We considered that glutamate is released from astrocytes above certain intracellular Ca^{2+} concentrations thus providing a non-linear positive feedback signal to neurons. Propagating seizure-like ictal discharges (IDs) were reliably evoked in our computational model by repeatedly exciting a small area of the network, which replicates experimental results in a slice model of focal ID in entorhinal cortex. We found that the threshold of focal ID generation was lowered when an excitatory feedback-loop between astrocytes and neurons was included. Simulations show that astrocytes can contribute to ID generation by directly affecting the excitatory/inhibitory balance of the neuronal network. Our model can be used to obtain mechanistic insights into the distinct contributions of the different signaling pathways to the generation and propagation of focal IDs.

Keywords: computational model, epilepsy, excitation/inhibition balance, neuron-astrocyte interaction, tripartite synapse

INTRODUCTION

The intracellular Ca^{2+} elevations occurring in cultured astrocytes in response to a glutamate challenge (Cornell-Bell et al., 1990) was the initial observation that hinted at the existence of a form of excitability in astrocytes based on cytosolic Ca^{2+} concentration changes. A few years later, Ca^{2+} elevations in astrocytes from both cell cultures (Parpura et al., 1994) and brain slices (Pasti et al., 1997) were observed to result in Ca^{2+} increases in nearby neurons mediated by astrocytic glutamate. Considering that astrocytes occupy non-overlapping spatial territories (Bushong et al., 2002; Halassa et al., 2007b) and that the processes of a single astrocyte can contact hundreds of synapses (Ventura and Harris, 1999), it was suggested that astrocyte-to-neuron communication may play a fundamental functional role in the brain. It was also found that astrocytes establish extensive contacts with cerebral blood vessels (Simard et al., 2003), which added further complexity to the functional role of neuron-to-astrocyte signaling. This neuron-astrocyte-blood-vessel signaling pathway was revealed to be central in neurovascular coupling, the process by which episodes of intense neuronal activity at restricted brain regions trigger local increases in cerebral blood flow to satisfy the energy demand of active neurons (Zonta et al., 2003; Mulligan and MacVicar, 2004; Gordon et al., 2008).

These pioneering results lead to the idea that astrocytes and neurons establish a bidirectional communication in the brain which may play fundamental roles in the modulation of synaptic transmission and plasticity (Carmignoto, 2000; Haydon, 2001).

Over the last decade numerous studies provided evidence for the ability of astrocytes to listen and talk to the synapse by exerting both excitatory and inhibitory actions on neurons (Araque et al., 1999; Brockhaus and Deitmer, 2002; Zhang et al., 2003; Pascual et al., 2005; Panatier et al., 2006; Serrano et al., 2006; Jourdain et al., 2007; Perea and Araque, 2007). These studies revolutionized our view of how the brain works. The processing of sensory information in the brain, which has been for many years considered to be based exclusively on neuronal communication, is now viewed as a product of the dynamic signals that neurons and astrocytes constantly exchange in the brain network.

Such a bidirectional communication between neurons and astrocytes was conceptualized in the tripartite synapses in which the astrocyte composes with the pre-synaptic terminal and the post-synaptic target neuron, a third functional element of the synapse (Araque et al., 1999; Carmignoto, 2000; Halassa et al., 2007a; Perea et al., 2009).

The discovery that astrocytes are crucially involved in normal brain function raised the intriguing possibility that these cells may be involved also in brain dysfunction. The observation that glutamate released by astrocytes evokes episodes of synchronous activity in small groups of nearby neurons (Fellin et al., 2004, 2006), was the first clue suggesting that gliotransmission represent a relevant non-neuronal mechanism for neuronal synchrony, which may ultimately favor the generation of focal epileptiform activity (Kang et al., 2005; Tian et al., 2005). A new experimental protocol was recently developed by our group in rat enthorinal cortex (EC) slices in order to reproduce the spatial and temporal features of focal epileptiform discharges (Gomez-Gonzalo et al., 2010; Losi et al., 2010). In this model, a pharmacological stimulation of neurons from a restricted cortical region induces a propagating seizure-like ictal discharge (ID). The ability to emulate an epileptogenic focus allows us to study the early cellular events that take place during the generation of epileptiform activity as it arises at a focal site and propagates to the surrounding brain tissue. By using this experimental protocol we recently provided evidence that neurons engage astrocytes into an excitatory loop that pushes the neuronal network toward the ID generation threshold (Gomez-Gonzalo et al., 2010).

There are currently many computational models of seizures generation, development and cessation (Pitkänen et al., 2006). The level of description ranges from mean field models (Wendling et al., 2002; Suffczynski et al., 2004) to biophysically detailed models (Destexhe, 1998; Bazhenov et al., 2004; Traub et al., 2005). We used here a simplified approach in the description of the dynamics of single neurons and astrocytes. With this simplified dynamics we implemented a computational network model that allowed us to investigate the network mechanisms of focal ID generation and the role of astrocytes at the onset of the ID.

We found that the positive feedback provided by the astrocytes influences the dynamics of the system and favors the generation of epileptiform activities. The computational model quantitatively reproduces the spatial and temporal features of ID generation and propagation and provides mechanistic insights into the astrocyte contribution.

METHODS

NEURON MODEL

The computational model aims to reproduce the behavior of a brain network that in response to NMDA pulse stimulation generates a focal ID (Losi et al., 2010). The network consists of 320 excitatory and 80 inhibitory neurons randomly disposed and synaptically connected in a 2D configuration. As in our previous work (Reato et al., 2010), we used Izhikevich's model (Izhikevich, 2003) to describe the dynamics of single neurons. Briefly, the voltage dynamics of single neurons is characterized by four parameters: a, b, c, d as follows:

$$\frac{dv}{dt} = 0.04v^2 + 5v + 140 - u + I = f(v, u) + I$$
$$\frac{du}{dt} = a\,(bv - u) \tag{1}$$

With a reset of the dynamic variables u, v when a spike is generated:

$$\text{if } v \geq 50 \text{ mV, then } \begin{cases} v \leftarrow c \\ u \leftarrow u + d \end{cases} \tag{2}$$

The choice of values for the four parameters defines different spiking behaviors. The parameters were chosen to reproduce the behavior of a regular spiking neuron for excitatory neurons ($a = 0.02$, $b = 0.2$, $c = -65$, $d = 10$) and of a fast spiking neuron for inhibitory neurons ($a = 0.2$, $b = 0.26$, $c = -65$, $d = 0.5$). The variable I represents the sum of the synaptic current and the external stimulation.

The synaptic currents mimic AMPA, NMDA, $GABA_A$, and $GABA_B$ receptor activation following (Izhikevich and Edelman, 2008). Briefly, the synaptic conductances are described by first-order linear kinetics, $\frac{dg_x}{dt} = -\frac{g_x}{\tau_x} + \sum_j s_j \delta\left(t - t_f\right)$ (where $x =$ AMPA, NMDA, $GABA_A$, $GABA_B$) with, $\tau_{AMPA} = 1$ ms, $\tau_{NMDA} = 2000$ ms, $\tau_{GABA_A} = 6$ ms, $\tau_{GABA_B} = 150$ ms. Every time a pre-synaptic neuron m fires an action potential the conductance of the post-synaptic neurons increases instantaneously by $s = s_{exc}$ and $s = s_{inh}$ for pre-synaptic excitatory or inhibitory neurons respectively ($s_{exc} = 0.001$, $s_{inh} = 0.01$). The ratio of NMDA to AMPA receptors was set to be uniform at a value of 2, while $GABA_B$ to $GABA_A$ equal to 0.3 ($s_{exc} = 0.002$ for NMDA and $s_{inh} = 0.003$ for $GABA_B$). The synaptic current of a post-synaptic neuron is then given by:

$$\begin{aligned} I_{syn} &= I_{exc} + I_{inh} \\ I_{exc} &= g_{AMPA}\,(v_{exc} - v) \\ &\quad + g_{NMDA}\frac{[(v+80)/60]^2}{1 + [(v+80)/60]^2}\,(v_{exc} - v) \\ I_{inh} &= g_{GABA_A}\,(v_{inh} - v) + g_{GABA_B}\,(v_{inh} - v) \end{aligned} \tag{3}$$

Where v (function of time, Equation 1) is the voltage of the post-synaptic neuron and v_{exc} and v_{inh} are the reversal potentials for excitatory and inhibitory synapses. Here we chose $v_{inh} = -90$ mV, $v_{exc} = 0$ mV. Each neuron receives excitatory inputs from a square of maximum 48 neighbors, while inhibition from maximum eight neurons. Using these parameters a single excitatory pre-synaptic spike induces a depolarization of maximum ~0.1 mV, while an inhibitory pre-synaptic spike leads to maximum ~0.5 mV hyperpolarization. All the main parameters of the simulations (the a, b, c, d parameters describing the dynamics of single neurons for both excitatory and inhibitory neurons and the s parameters for synaptic connections) were selected from a normal distribution with standard deviation equal to 1% of the average value. To mimic the onset of an ID, a few parameters of the network were chosen in order to place the network in a hyperexcitable state. The

excitability of excitatory neurons was slightly increased by injecting depolarizing currents (amplitude equal to 2), that could mimic the effects of 4-AP (a K^+ channel blocker) used in the slice preparation. The high values chosen for both the conductance and the time constant of NMDA currents aim to reproduce the low Mg^{2+} experimental conditions. Without stimulation, both excitatory and inhibitory neurons are completely silent.

The NMDA stimulation that in experimental slice preparations evoked an ID was simulated in the model by depolarizing a set of neurons within a 7×7 square area above threshold for 500 ms (49 neurons). We refer to this as a simulated pulse (SimP). Alternatively, the NMDA pulses could have been simulated by activating NMDA currents. However, since we are interested in analyzing the effects on NMDA currents during the ID onset, this would have resulted in "stimulation artifacts" (the NMDA current induced by the pulse). Since we were also interested in studying the mechanisms leading to ID generation, the intensity of the stimulation was set to a value that not necessarily induced an ID in all the simulations (see **Figure 3D**).

In each simulation, nine SimPs were applied. In unsuccessful simulations, the average firing rate in the network increases during each SimP, but it rapidly comes back to zero between successive SimPs. An ID was considered to be successfully generated when the firing rate in neurons remains sustained above 1 Hz. The ID onset was then defined as the number of SimPs which starts this process.

The cessation of the ID was obtained by a modulation of the parameter b in a firing specific way. More specifically, we assumed that an elevated spiking activity decreases the excitability of single neurons. Possible physiological correlates of this event are the inactivation of Na^+ channels (Bazhenov et al., 2004), the activation of Ca^{2+}- or Na^+-dependent K^+ channels (Alger and Nicoll, 1980; Schwindt et al., 1989; Bazhenov et al., 2004; Timofeev et al., 2004) or the exhaustion of metabolic support (Yamada et al., 2001; Kirchner et al., 2006).

The equation used is:

$$\frac{db}{dt} = -mR(t) + (b_s - b) \tag{4}$$

Where $R(t)$ is the spike train of a single neuron low-pass filtered (time constant equal to 150 s), m is the coupling constant between the spiking activity and b (chosen here to be 15), and b_s the value of b in resting conditions (no spiking activity). The second term in the equation can be thought as a driving force to recover the normal neuronal functionality of the neuron, for example the metabolic support.

Because of the hyperexcitability of the network, i.e., neurons are firing intensively at ID onset, we had to integrate Izhikevich's equations with the method proposed in Izhikevich (2010) assuming the time step to be 1 ms:

$$v(t+1) = \frac{v(t) + f(v(t), u(t)) + g(t)E(t) + I}{1 + g(t)} \tag{5}$$

Where $E(t) = \sum \left(g_i(t)E_i\right)/g(t)$ with $g(t) = \sum g_i(t)$ (the total sum of conductances) and $E_i = v_{\text{exc}}, v_{\text{inh}}$ for excitatory and inhibitory connections, respectively. This method is efficient and stable even for large synaptic currents.

"Excitation" refers to the sum of excitatory currents (AMPA and NMDA) averaged across neurons, and similarly "inhibition" refers to the average summed inhibitory currents ($GABA_A$ and $GABA_B$). Excitatory and inhibitory firing rate indicate the firing rate of excitatory and inhibitory neurons, respectively. Where otherwise indicated, excitation, inhibition and firing rates of single simulations were always filtered with a moving average filter using a 50 ms time window for better visualization. Postictal refractory period was estimated as the time between the end of the seizure (average firing rate back to zero) and the time at which the b variable recovers to the 95% of the initial value.

Under the conditions described above, our computational model is able to generate a neuronal network activity which resembles several characteristics of experimental focal IDs (see later in the "Results"):

(i) the simulated ID originates from a small number of neurons in the network and propagates outside the focal area with a delay (Traub and Wong, 1982; Avoli et al., 2002);
(ii) the simulated ID arises from an unbalance between inhibitory and excitatory activity at the focal area (Bradford, 1995; Ben-Ari, 2002);
(iii) the simulated IDs have a cessation and a similar average duration (Jefferys, 1990; Traub et al., 1993; Pinto et al., 2005);
(iv) the network enters into a period of postictal refractoriness (Jefferys, 1990);
(v) the peak in the firing rate of the excitatory and inhibitory neurons during simulated IDs is compatible with that measured in the *in vitro* experimental models.

Our model failed to reproduce the bursting behavior which characterizes the firing discharges in individual neurons and the two main phases in ID development, i.e., the initial tonic and the delayed clonic activity. However, the main focus in this computational model was to include astrocytes in the neuronal network and gain insights into how these non-neuronal cells can affect the equilibrium between excitation and inhibition in the network.

ASTROCYTE MODEL

We introduce here a simple representation of astrocytes interacting with a neuronal network. The parameters related to the ability of astrocytes to respond to neuronal activity with cytosolic Ca^{2+} elevations were captured from results obtained in experiments performed both in brain slices (Pasti et al., 1997; Porter and McCarthy, 1997) and in the living brain (Hirase et al., 2004; Wang et al., 2006; Kuga et al., 2011). To simulate the Ca^{2+} dynamics of a single astrocyte we used a framework similar to the Izhikevich neuron model. The equations represent a dynamical system of two variables [(Ca^{2+}) and φ], without non-linear action potential or reset. The set of equation describing the Ca^{2+} concentration

has the following form:

$$\frac{d\left[Ca^{2+}\right]}{dt} = -\varphi + \sum_j \sigma_j \delta\left(t - t_f\right)$$
$$\frac{d\varphi}{dt} = \alpha\left(\beta\left[Ca^{2+}\right] - \varphi\right) \quad (6)$$

where φ is a recovery variable and σ_j is assumed here to be the neuronal input when an action potential is generated by the neuron j, since astrocytes respond to neuronal releases of glutamate (Pasti et al., 1997; Porter and McCarthy, 1997). Although the equations are dimensionless, the values σ_j where chosen to reproduce the pattern and amplitude of the Ca^{2+} elevations that are experimentally observed in astrocytes in response to neuronal activity (Porter and McCarthy, 1996; Pasti et al., 1997). The values of σ_j was chosen as been normally distributed with mean 0.00083 and standard deviation equal to 1% of the mean. Ca^{2+} concentration was restricted to be non-negative. Similarly to the dynamics described by the Izhikevich's single neuron, different values of α and β determine different behaviors (time constant of changes and coupling with the recovery variable). Here we chose $\alpha = 0.001$ and $\beta = 0.01$. This choice was made to reproduce the slow time course of Ca^{2+} changes in astrocytes (Kawabata et al., 1996). When astrocytes were included in the whole network, these values were chosen to be normally distributed with a standard deviation equal to 1% of the mean.

To describe the release of astrocytic glutamate triggered by Ca^{2+} elevations, we considered a first order dynamics (low pass filters), with a release of glutamate that can occur only when Ca^{2+} reach a threshold (Pasti et al., 1997; Parpura and Haydon, 2000; Pasti et al., 2001):

$$\mu\frac{d\left[glu\right]}{dt} = \begin{cases} -\left[glu\right] + \left(\left[Ca^{2+}\right] - \left[Ca^{2+}\right]_{th}\right) - \kappa\lambda \\ \qquad\qquad \text{if } \left[Ca^{2+}\right] > \left[Ca^{2+}\right]_{th} \\ -\left[glu\right] - \kappa\lambda \qquad \text{otherwise} \end{cases} \quad (7)$$
$$\eta\frac{d\lambda}{dt} = -\lambda + \left[glu\right]$$

Where $[Ca^{2+}]_{th} = 0.0018$ mM is the threshold for glutamate release, $\kappa = 200$ describes the coupling between the glutamate concentration [glu] and the recovery variable λ. Glutamate concentration was imposed to be non-negative. The time constants for the two variables were $\mu = 0.5$ s and $\eta = 10$ s. The value of $[Ca^{2+}]_{th}$ was set based on available data showing that an increase in astrocytic Ca^{2+} of a few hundreds of nM was able to trigger glutamate release (Parpura and Haydon, 2000). Assuming a value of 200 nM for a single synapse (Nadkarni and Jung, 2003) and considering that astrocytes in our model receive inputs from a maximum of nine neurons, the threshold value can be determined by multiplying the value for the single synapse by the number of inputs, as considered in other studies (Wade et al., 2011).

The set of parameters used for a single astrocyte reproduces basic features of Ca^{2+} dynamics and glutamate release in astrocytes. Increasing the input to an astrocyte, simulated as Poisson-distributed spike trains of increasing frequencies, leads to increasing Ca^{2+} concentrations (**Figures 1A1–A4**). The Ca^{2+} increase due to a single spike is less than 100 nM and lasts for about half a second (inset in **Figure 1A1**). These results are compatible with experimental evidences (Pasti et al., 1997; James et al., 2011) and previous computational models (Jefferys, 2003; Nadkarni and Jung, 2004, 2007; Wade et al., 2011). The linear dependence of Ca^{2+} increases as a function of simulated firing rate is reported in **Figure 1B**. Increasing the number of inputs by summing up Poisson-distributed spike trains (color scale from red to blue) also elevated Ca^{2+} concentrations. Since the release of glutamate due to the Ca^{2+} increases occurs only when Ca^{2+} is above a threshold, only strong activation can drive the release. As an example, nine spike trains at 10 Hz induced transient releases of glutamate (**Figures 1C1**,**C2**, Ca^{2+} threshold in red). **Figure 1D** summarizes the dependence of glutamate released by the astrocyte as a function of the firing rate and the number of inputs. For very low firing rates, there is no astrocytic glutamate release independently on the number of inputs. In the case of high firing rates, the release is linearly dependent on both the number of inputs and the firing rate. To further validate the parameters that we choose, we stimulated single astrocytes with a spike train from nine neurons from a simulated ID (see later in the "Results"). The neuronal activity leads to Ca^{2+} increases in the astrocyte (**Figure 1E1**) that caused a glutamate release (**Figure 1E2**) only after the second pulse (see also below). Interestingly, when the ID was fully developed, Ca^{2+} elevations reached a steady state value and glutamate was no longer released. It is known from experiments in cultures and in brain slices (Pasti et al., 1997; James et al., 2011) that upon intense stimulation the Ca^{2+} level in astrocytes increases rapidly and remains at an elevated steady-state value for tens of seconds (**Figure 1E1**). A single episode of glutamate release is experimentally observed only after the initial Ca^{2+} rise.

Astrocytes were included in the network with a 1:1 ratio with neurons. The ratio of glia to neurons increases in phylogenesis and is 1.65 in the human frontal cortex (Oberheim et al., 2006; Sherwood et al., 2006). Given that astrocytes account for about 50% of the total number of glial cells, a 1:1 ratio seems to represent an acceptable approximation. The input from neuronal activity, σ for each m astrocyte, was considered as the excitatory input from neurons firing $s_{exc}(m)$ (the excitatory component) in a 3×3 square (inputs from nine excitatory neurons). This choice was made considering that the feedback of astrocytes on neuronal activity is thought to be local with four to eight neuronal somata enveloped by a single astrocyte (Halassa et al., 2007b), but with the processes from a single astrocyte associated with up to 600 dendrites and many thousands of synapses (Bushong et al., 2002; Oberheim et al., 2006). The glutamate released by astrocytes was used as input to the same neurons to which the astrocyte is exposed. This glutamate generated NMDA currents in these neurons by activating the NMDA channel (in the same way than s_{exc}). In some simulations (see "Results") we considered the effects of inhibitory inputs from astrocytes. This was done by considering that the astrocytic response activate $GABA_A$ receptors instead of NMDA (so simulating the effect of GABA release).

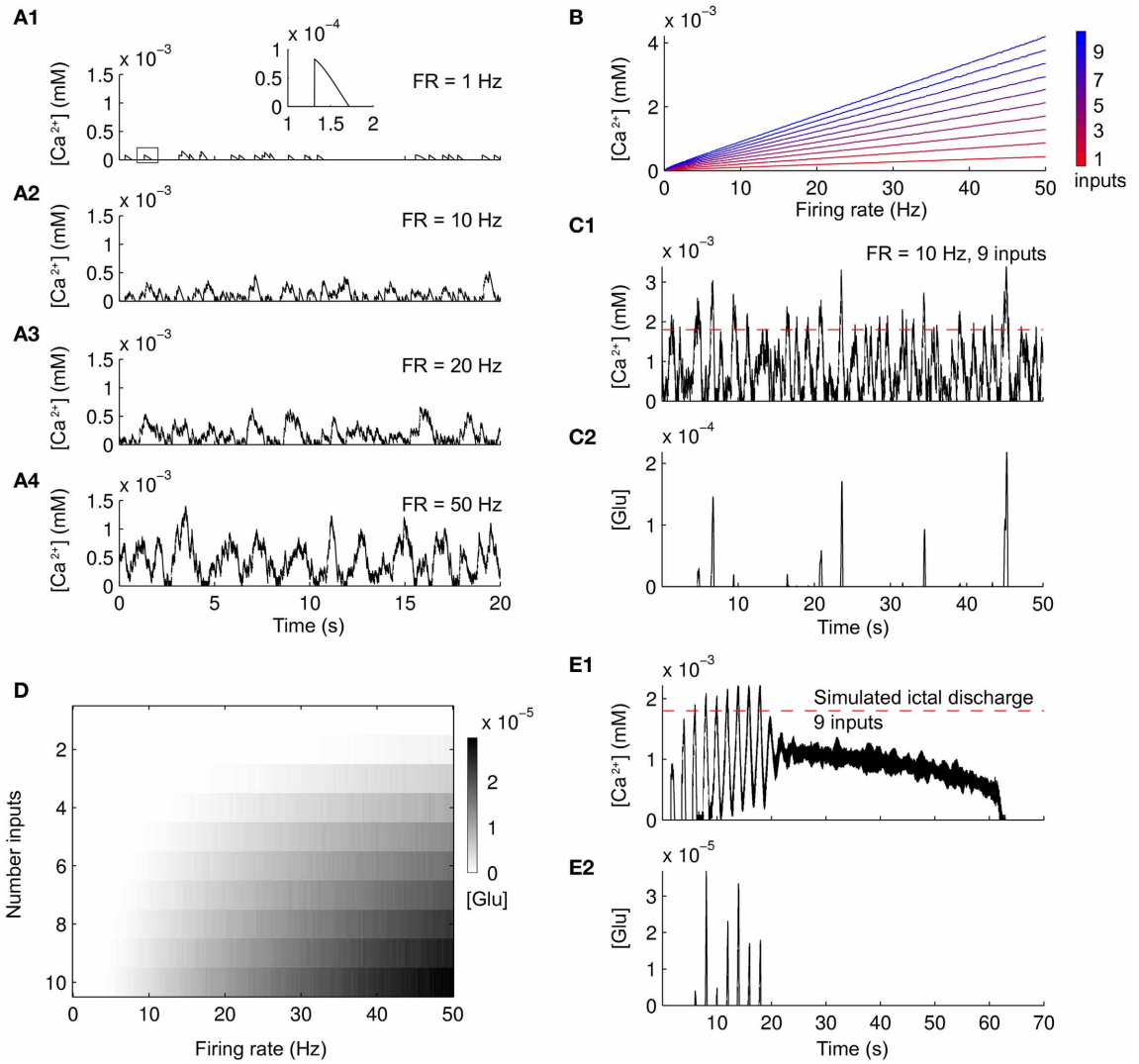

FIGURE 1 | Ca^{2+} changes and glutamate released from a simulated astrocyte in response to different patterns of neuronal firing. (A1–A4) Ca^{2+} change in the simulated astrocyte induced by Poisson-distributed spike trains of increasing frequencies of an individual neuron. A single Ca^{2+} increase is outlined by the box in **(A1)** and expanded within the inset. **(B)** Summary of the Ca^{2+} concentration in the simulated astrocyte as a function of an increasing number of Poisson-distributed neuronal inputs (colorbar from red to blue) and increasing firing frequency. **(C1,C2)** Ca^{2+} change and glutamate released from the simulated astrocyte in response to a Poisson-distributed spike trains of nine inputs. Glutamate is released only when Ca^{2+} is above a threshold (red dashed line). **(D)** Summary of the glutamate released from the simulated astrocyte (gray scale colorbar) in response to the same neuronal stimulation as in **(B)**. **(E1,E2)** Ca^{2+} change and glutamate released from the simulated astrocyte during a simulated ID.

All the simulations were performed using MATLAB (Mathworks), and the code is available at www.neuralengr.com/code.

RESULTS

FOCAL ID GENERATION IN ENTORHINAL CORTEX SLICES

As we previously reported (Losi et al., 2010), an episode of neuronal hyperactivity can generate a focal ID in EC slices perfused with the K^+ channel blocker 4-aminopyridine (4-AP) and low Mg^{2+}. **Figure 2** illustrates a typical ID that was generated in cortical layer V-VI by a double brief pressure pulse applied to an NMDA-containing glass pipette (**Figures 2A,B**). Dual patch-clamp recordings revealed that the firing in neurons located within the focus (**Figure 2C**, neuron 1) evolved into a focal ID with some delay after the NMDA double pulse. Following the ID generation at the focus (**Figure 2A**, gray circle), neurons outside the focus (<400 μm from the NMDA pipette tip) were also recruited and exhibited a similar pattern of action potential firing (**Figure 2C**, neuron 2). Given that the somatic Ca^{2+} change in neurons reflects faithfully the action potential firing in these cells, in slices loaded with the Ca^{2+} sensitive dye Oregon Green BAPTA1-AM (OG-B1-AM) we could monitor the activity of tens of neurons and follow how a focal ID is generated in the neuronal network. These experiments revealed that the NMDA stimulation

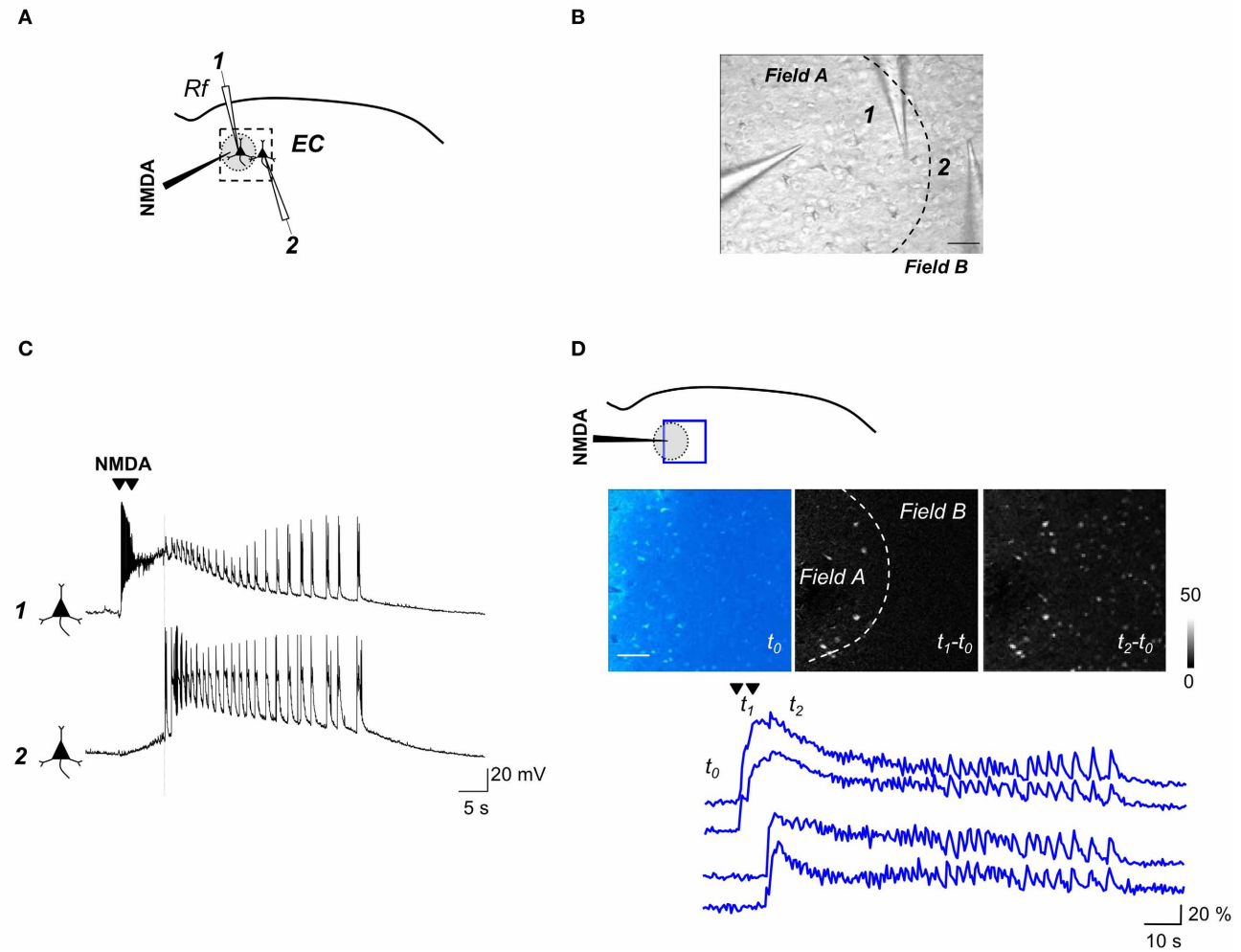

FIGURE 2 | Experimental protocol for inducing focal IDs in brain slices. **(A)** Schematic of a dual voltage-clamp recording from EC deep layers pyramidal neurons (PyNs) with indication of the NMDA pipette and the patched neurons (1,2), one within the region directly activated by NMDA (gray circle) and the other immediately outside (~200 and ~350 μm from the NMDA pipette respectively). **(B)** DIC image of the area marked by the dashed box in **(A)**. Scale bar 100 μm. **(C)** Dual current-clamp recording from two PyNs during a focal ID evoked by a double NMDA pulse (arrowheads). The vertical dashed line marks the generation of ID in both cells. Note that in neuron 1 the direct NMDA response precedes and partially masks the ID onset. **(D)** Schematic of an experiment similar to that reported in **(A)** showing the region of Ca^{2+} signal imaging (blue box). The image of the basal OGB1 fluorescence in the EC (t_0) and the difference images of the fluorescence signal change captured at different times (see lower traces) during an evoked ID (t_1–t_0; t_2–t_0) are reported. The NMDA-pipette is visible on the left. The dashed line in the image t_1–t_0 marks the region directly activated by NMDA. Scale bar 100 μm. Lower traces show the Ca^{2+} signal change from two neurons that were directly activated by NMDA (upper traces) and two other neurons, located outside the region directly activated by NMDA, that exhibited the ID only (lower traces). The timing that corresponds to the different images (t_0, t_1, and t_2) is reported.

evoked a rapid Ca^{2+} elevation in neurons located within the focal area, while neurons from the surrounding network were recruited into the ID only after a delay of 10.9 ± 0.8 s (30 IDs from 15 slices).

FOCAL ID GENERATION IN THE NEURONAL NETWORK MODEL

In the model we first examined how the neuronal network responds to a sequence of simulated NMDA pulses in the absence of astrocytes. To mimic the NMDA pulses at the focus, a depolarizing current pulse was injected for 500 ms in an area of 7×7 neurons (see "Methods"). The first SimP evoked robust spiking activity that remained restricted to neurons of the focus (**Figure 3A1**). Upon successive SimPs the firing activity spread from the focus to the surrounding neurons approximately 10 s after the SimP onset (**Figures 3A1–A4,B1**). The neuronal firing discharge remained high thereafter for tens of seconds (61 ± 2 s) before a sudden cessation (**Figure 3B1**). A postictal refractory period was observed with an average duration of 266 ± 1 s (see "Methods"). This pattern of activity resembles the focally evoked ID in slice preparations (see **Figure 2D**). A raster plot of the activity in a subpopulation of excitatory and inhibitory neurons within and outside the focus revealed that inhibitory neurons fire more intensively as compared to excitatory neurons during the SimPs, while the spiking activity in excitatory neurons increases with successive SimPs (**Figure 3B2**). The peak of the activity in the whole network was reached

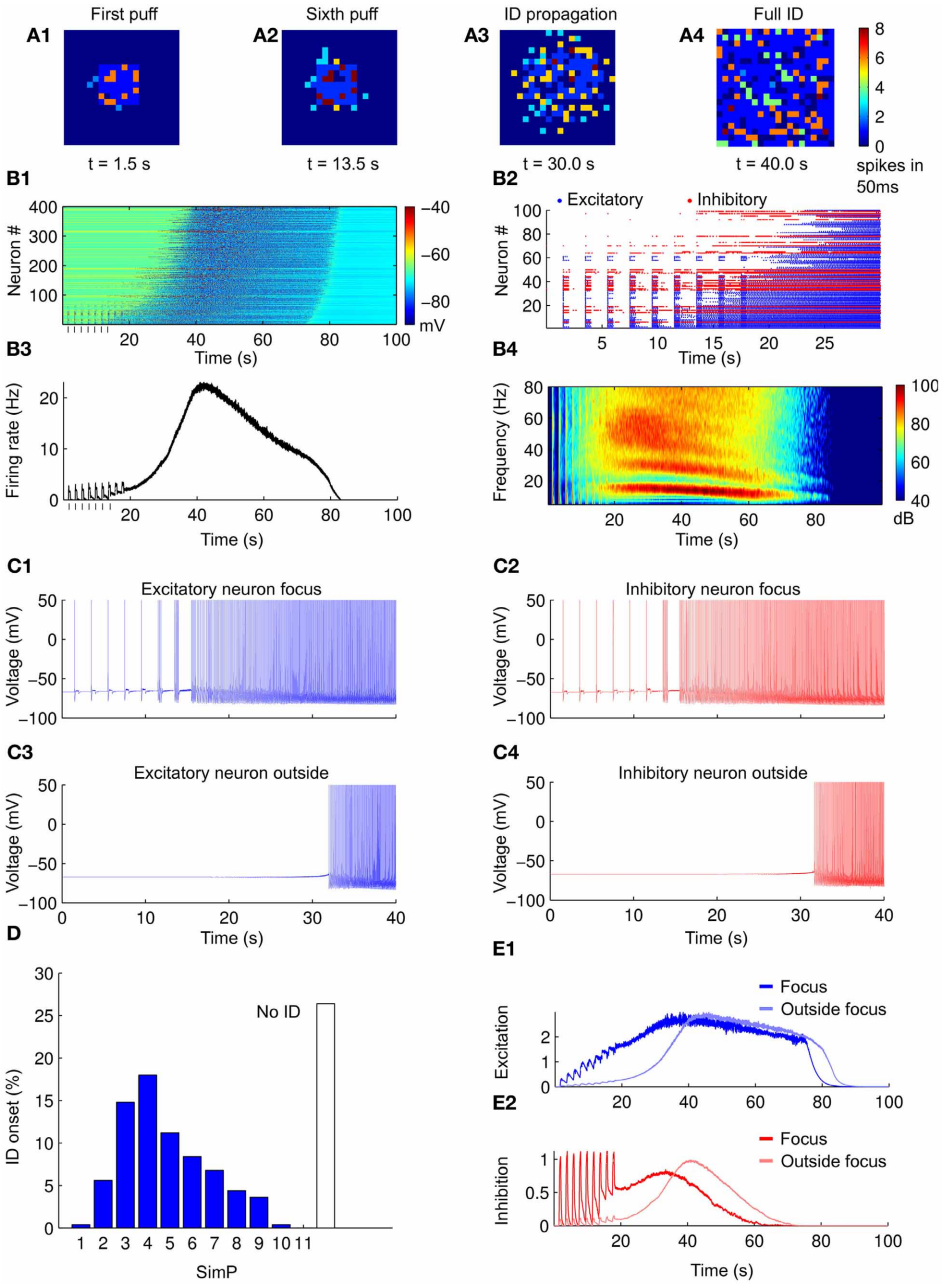

FIGURE 3 | Simulated IDs from a purely neuronal network. **(A1–A4)** Pseudocolor plot of the number of action potentials calculated in 50 ms time windows at different times of the simulation. **(B1)** Membrane voltage (considered from −90 to −40 mV) for all the neurons in the network during a simulated ID. **(B2)** Raster plot of the spiking activity of a subpopulation of neurons in the network in the first 30 s of simulation (excitatory in blue and inhibitory in red). **(B3)** Average firing rate of the neurons in the network during a simulated ID. **(B4)** Spectrogam of the average firing rates for 40 simulations. Note the high frequency component corresponding to the firing of inhibitory neurons and the low one corresponding to excitatory neurons. **(C1–C4)** Examples of neuronal voltage traces of excitatory and inhibitory neurons within and outside the focus. **(D)** Histogram of ID threshold for 250 simulations. **(E1,E2)** Average value of excitation/inhibition (average post-synaptic excitatory currents) at the focus (blue/red), outside the focus (light blue/light red). Note that, differently from excitation, the inhibition does not accumulate after subsequent SimPs.

during the ID (**Figure 3B3**) and its spectrogram clearly revealed two main components corresponding to the different activity in excitatory and inhibitory neurons that fire at about 15 and 60 Hz, respectively (**Figure 3B4**). This pattern of activity in the two neuronal populations is consistent with experimental observations (Ziburkus et al., 2006). While both excitatory and inhibitory neurons at the focus were activated upon the initial stimulation (representative traces in **Figures 3C1**,**C2**), neurons outside the focus were recruited into the propagating ID with some delay (representative traces in **Figures 3C3**,**C4**).

ID GENERATION THRESHOLD

We consider as ID threshold the number of NMDA pulses that are needed to evoke an ID. This value is constant for a given slice (Gomez-Gonzalo et al., 2010), but it can vary for different slices. Simulations with different parameters (see "Methods") showed that an ID could be generated in average by five SimPs and in more than 25% of cases no IDs could be evoked regardless the number of applied SimPs (**Figure 3D**, $n = 250$ simulations). The successive SimPs induced excitatory responses at the focus with increasing amplitude (**Figure 3E1**). The excitatory and inhibitory neurons outside the focus were not directly activated by the SimPs and increased their firing activity simultaneously, but with a marked delay (**Figures 3E1**,**E2**).

DYNAMICS OF EXCITATION AND INHIBITION AT THE FOCUS EXPLAIN ID GENERATION

We next investigated the interplay between excitation and inhibition in the genesis of the ID. We compared the simulations which successfully evoked an ID with those that failed to evoke an ID (in the different simulations excitatory and inhibitory neurons were randomly located within or outside the focus while maintaining their total number). For the cases in which an ID was successfully generated, we find that the ratios between the number of excitatory and inhibitory neurons, the average inhibitory and excitatory currents during the first SimP and the firing rate of inhibitory and excitatory neurons in the same time interval were lower compared to cases where an ID was not successfully generated (**Figures 4A1–A3**).

We next tested whether a different strength in either excitation or inhibition at the focus changed the efficacy of the SimP in generating an ID. We analyzed the time course of excitation and inhibition at the focus and the average firing rate in the whole network. We examined three sets of network parameters chosen at random, but leading to different ID thresholds, i.e., no ID generation, high ID threshold (five SimPs) and low ID threshold (three SimPs) (**Figures 4B–D** respectively). In all cases, excitatory and inhibitory drive increased the firing rate (**Figures 4B2**,**B3**,**C2**,**C3**,**D2**,**D3**). A detailed analysis of the dynamics revealed that after each SimP both excitation and inhibition were strongly but transiently activated (**Figures 4B1**,**C1**,**D1**). An important difference is that, in contrast to inhibition, excitation failed to recover the initial basal conditions, including the simulations in which no ID is generated (**Figure 4B**). An additional striking difference between the three examples is the maximal inhibition level provided by the inhibitory neurons (the dynamic range). Inhibition reached its highest value in the high ID threshold condition. These results support the view that inhibition strength is a critical factor for focal ID onset. Notably, excitation rose faster than inhibition (slope > 1) driving the growth in firing rate forward. However, the ID occurred only after inhibition reached its maximal value (all inhibitory neurons were active). Therefore the ratio of excitatory versus inhibitory drive and the limiting dynamic range of inhibition are the two critical factors in ID generation. As a summary of results obtained, we report the distribution of points in the excitation-inhibition plane at the focus during the first seven SimPs in 250 Monte-Carlo simulations for the cases that evoked or failed to evoke an ID (normalized by the total area; **Figures 4E1**,**E2**). When inhibition at the focus reached high values, no IDs were generated and the ratio between excitation and inhibition remained low. This stands in contrast to the cases which lead to IDs, further supporting the notion that the relationship between excitation and inhibition determines not only the threshold for ID generation, but also whether or not an ID could be evoked. Data obtained from 250 runs also showed a clear correlation between the ID threshold and the average excitation and inhibition in the network during the first SimP (**Figures 4F1**,**F2**). This indicates that the overall response of the network, in terms of excitation and inhibition levels, is a good predictor of ID threshold: an increased excitation results in the lowering of the ID threshold and an opposite relationship holds for inhibition.

ASTROCYTE-TO-NEURON SIGNALING DECREASES THE ID THRESHOLD

The model of the single astrocyte (see "Methods") was incorporated into the network to test how astrocytes may affect ID threshold. Specifically, 400 astrocytes were added to the network model in a parallel 2D sheet of cells (see "Methods"). Astrocytes provide an excitatory feedback to neuronal activity in a Ca^{2+}-dependent way (**Figure 5A**). As illustrated in **Figure 5B**, in the presence of the astrocyte feedback signal, the ID was evoked by two SimPs, while in its absence a more intense stimulation of neurons was necessary. As illustrated in **Figures 5F1**,**F2** the Ca^{2+} change from a representative astrocyte at the focus was observed to follow rapidly the spiking activity in neurons (**Figures 5C1**,**F1**), and astrocytic glutamate release occurred upon the second SimP (**Figure 5C2**). The average astrocytic Ca^{2+} follow the neuronal activity (example in **Figure 5D1**) while the average glutamate release occurs transiently (**Figure 5D2**). The Ca^{2+} change and the release of glutamate from astrocytes outside the focus failed to affect focal ID threshold. The results from 250 Monte-Carlo runs show that the ID threshold was lowered after including the astrocytic feedback signal to neurons (**Figure 5E**). Once the ID was fully evolved, both the Ca^{2+} change and the release of glutamate from astrocytes within and outside of the focus did not differ significantly (**Figures 5F1**,**F2**). However, the initially dominant activity of astrocytes at the focus was followed by an activity of the astrocytes outside the focus that became dominant immediately after the ID onset. These results are consistent with those from slice experiments which showed that when Ca^{2+} elevation in astrocytes from the focus were selectively blocked (by the Ca^{2+} chelator BAPTA) or stimulated (by TFLLR, a peptide agonist of thrombin PAR-1 receptors), the threshold of ID generation increased or decreased, respectively (Gomez-Gonzalo et al., 2010).

DOES AN ASTROCYTE INHIBITORY FEEDBACK SIGNAL TO NEURONS AFFECT ID THRESHOLD?

The ID threshold is mainly affected by the interplay between excitation and inhibition. Indeed, as we reported above, ID threshold can be increased by increasing the overall value of the inhibitory activity. The bar graph in **Figure 6A** reports the results from 250 simulations in different simulation settings, with and without an astrocytic contribution (mean and errors represent the results of a Poisson fit to the ID threshold distributions), while **Figure 6B**

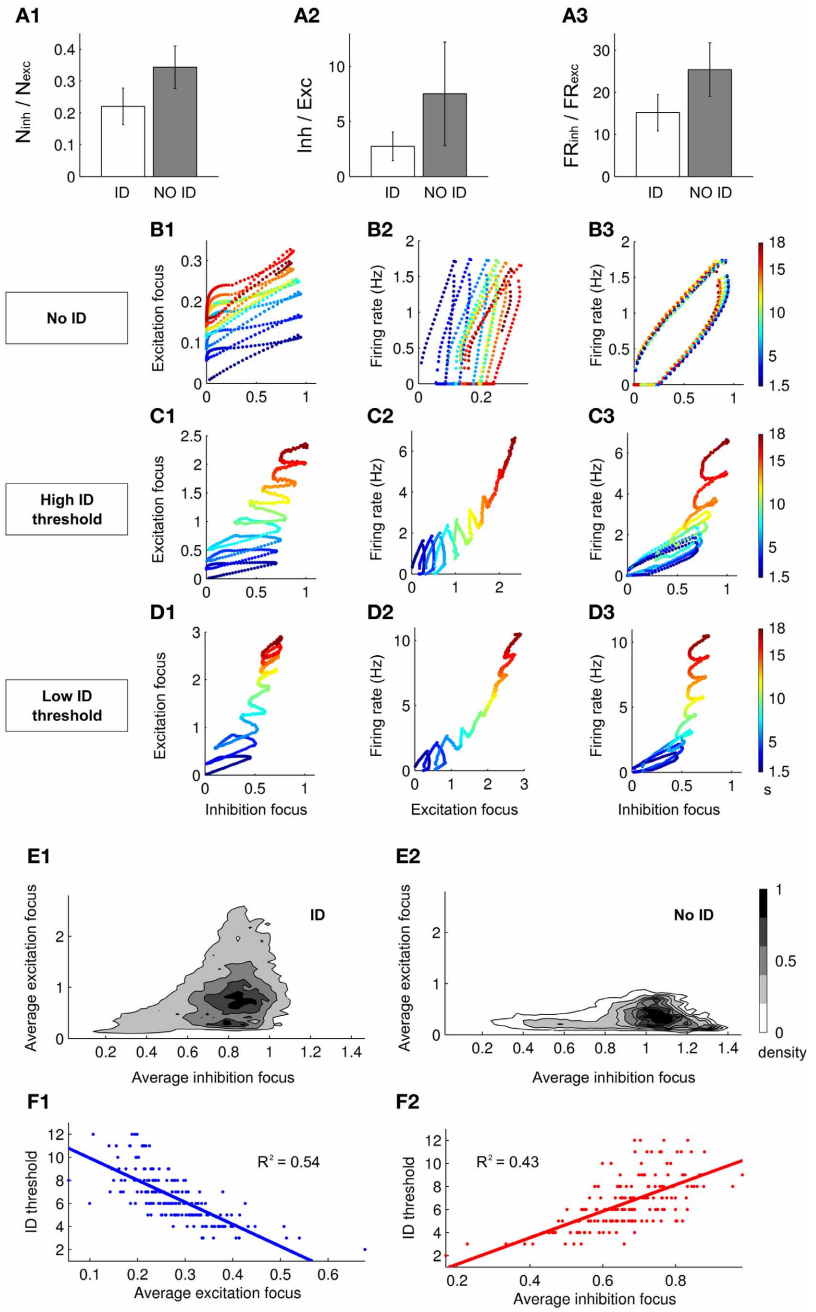

FIGURE 4 | Excitation–inhibition interplay during ID generation. **(A1–A3)** Histograms showing the difference between the ratio of the number **(A1)**, the average synaptic currents **(A2)** and the firing rate **(A3)** between inhibitory and excitatory neurons when an ID is or is not generated (**A2** and **A3** are relative to the response to the first SimP). **(B1–B3,C1–C3,D1–D3)** Dynamic representation of the first 18 s of simulations in which an ID: **(B)** is not generated, **(C)** is evoked with a high threshold (five SimPs) and **(D)** is evoked with a low threshold (three SimPs). The dynamics of the network is represented as a point in the plane representing excitation and inhibition (in the focus, **B–D1**), the average firing rate of the network and excitation (at the focus **B–D2**) and the average firing rate of the network and inhibition (at the focus, **B–D2**). **(E1,E2)** Normalized density of dynamical points in the excitation/inhibition plane during the first seven SimPs (250 simulations) when an ID is generated **(E1)** or it is not generated **(E2)**. **(F1,F2)** Linear regression showing the correlation between the ID threshold and, respectively, the average excitation **(F1)** and inhibition **(F2)** in the focus.

is the cumulative sum of the ID threshold distributions corresponding to the analyzed cases. Blue and red bars show that the ID threshold can be increased by increasing the overall strength of inhibitory connections (in this case from 0.01 to 0.015) in a purely neuronal network (no astrocytes). With higher inhibition, the simulated stimulation failed to induce an ID in 40% of simulations (**Figure 6B**). As already shown, the introduction of an astrocytic excitatory feedback lowers the ID threshold (green

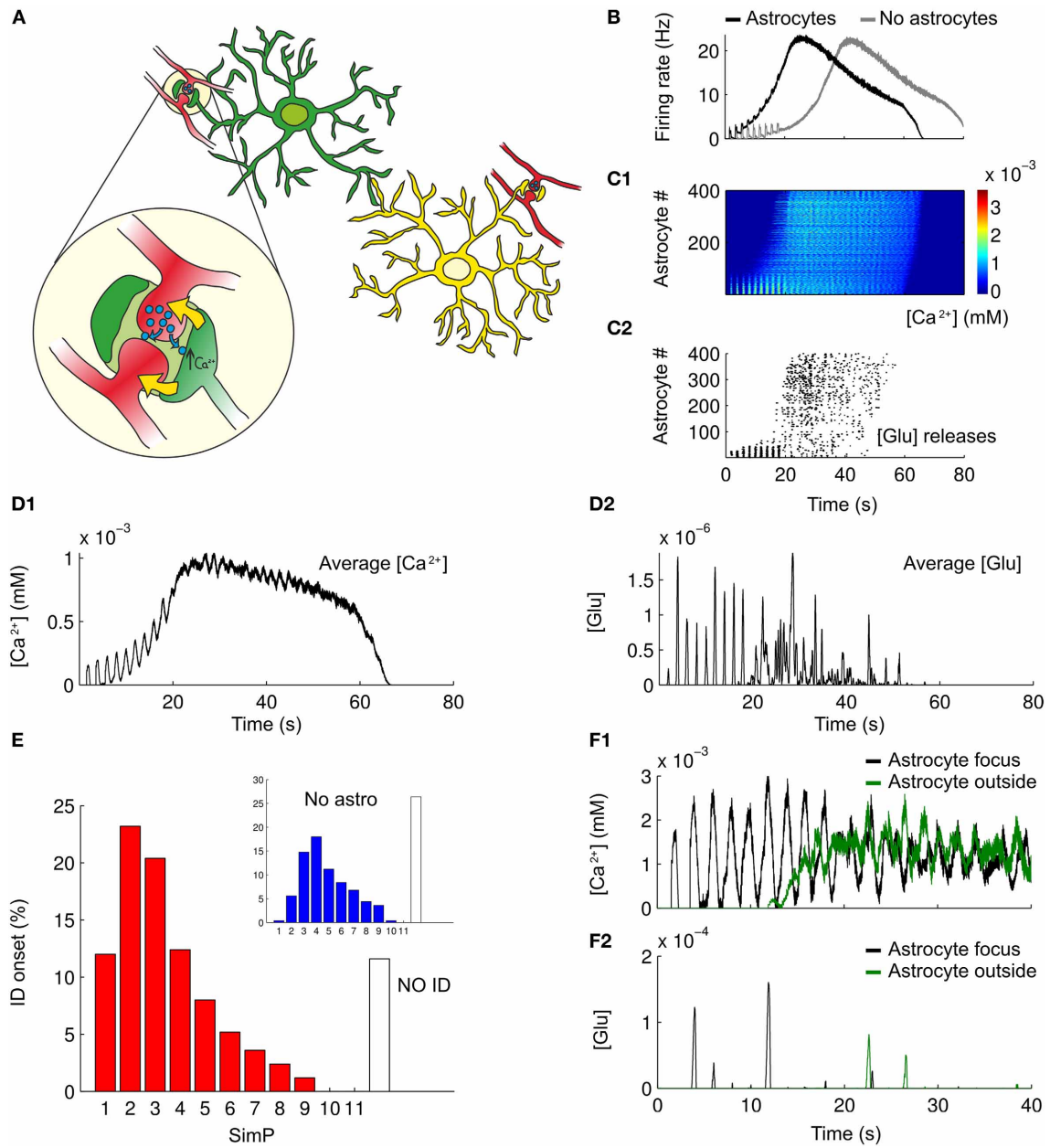

FIGURE 5 | Astrocytes modulate ID threshold. (A) A schematic representation of the tripartite synapse and the interplay between astrocytes and neurons in the network. **(B)** Representative average firing rates during simulated IDs with or without astrocytic contribution (black and gray traces respectively). **(C1)** Pseudocolor plot of the Ca^{2+} changes in the astrocytes during a simulated ID. **(C2)** Raster plot of the glutamate released by astrocytes caused by the Ca^{2+} increases as in **C1**. **(D1,D2)** Average trace of Ca^{2+} changes and glutamate released from astrocytes during a simulated ID. **(E)** ID threshold for 250 simulations in a network composed by both neurons and astrocytes. For comparison, panel **D** from **Figure 3** has been reproduced as an inset. **(F1,F2)** Representative traces of Ca^{2+} changes and glutamate released from a single astrocytes at the focus (black line) and outside the focus (green line) during a simulated ID.

bar) and decreases the number of failures to about 10%. In slice experiments we observed that the inhibition of Ca^{2+} signals in astrocytes at the focus, but not outside the focus, increased the threshold of ID generation. These observations were fully reproduced in the computational model (dark blue and magenta bars) without further manipulations of the model over the results from the previous section. Astrocytic excitatory feedback in a network with stronger inhibitory connections (0.015 as for the red bar) brings back to baseline the ID threshold (yellow).

To explore other factors that may affect ID threshold, we considered the possibility that the activation of astrocytes, or of a subpopulation of astrocytes, results in a release of GABA that can lead to an overall increase of the inhibition strength in the neuronal network. Astrocytes can, indeed, release GABA

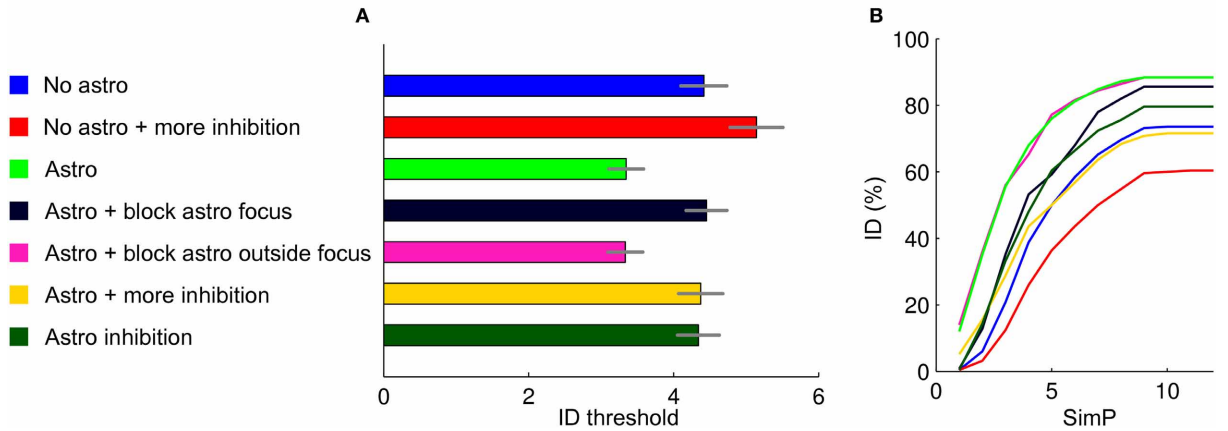

FIGURE 6 | Summary of the results. (A) ID threshold histogram in the different conditions. The distributions were described by fitting with Poisson distributions and determining the average values and relative errors. **(B)** Cumulative sum of the ID threshold histograms to show more in details how, for the different conditions tested, the threshold and the number of failures in generating an ID change.

(Kozlov et al., 2006; Lee et al., 2011; Le Meur et al., 2012). As expected, when we included an astrocytic GABA release in the model, the threshold for ID generation increased (**Figure 6**, dark green). Note that this was an inhibitory-only feedback involving only GABA release and no glutamate. Surprisingly, however, the threshold for ID generation did not rise over the baseline condition with no astrocytic feedback (blue). A possible explanation for this could be the synchronizing action of an inhibitory GABA signal. Alternatively, the inhibitory feedback signal from astrocytes could be more effective in suppressing inhibitory than excitatory neurons. This action may ultimately generate a new level of complexity in the mechanism that governs the inhibition/excitation balance in the neuron-astrocyte network.

DISCUSSION

Increasing experimental evidence highlights the physiological significance of the tripartite synapse in which the astrocyte senses neurotransmitter release and, in turn, releases through a Ca^{2+}-dependent mechanism gliotransmitters that have feedback modulatory actions on neurons. A number of recent studies *in vitro* and *in vivo* showed that the release of glutamate from astrocytes can, indeed, control both the basal excitability of neurons and some forms of long-term potentiation of synaptic strength (Serrano et al., 2006; Jourdain et al., 2007; Navarrete and Araque, 2010; Santello et al., 2011; Min and Nevian, 2012) and long-term depression (Zhang et al., 2003; Serrano et al., 2006; Han et al., 2012; Min and Nevian, 2012). The contribution of gliotransmission to brain dysfunctions remains, however, poorly understood. A model composed of a network of interacting neurons and astrocytes represents a useful tool in which the spatial-temporal features of focal seizure generation observed in slice models can be replicated and new mechanistic hypotheses can be tested.

Over the last 10 years, different models have been advanced to describe the Ca^{2+} dynamics of astrocytes in response to neuronal signals (Nadkarni and Jung, 2003, 2007; Silchenko and Tass, 2008; Di Garbo, 2009). These biophysical approaches described not only the astrocytic Ca^{2+} response (Li and Rinzel, 1994), but also the possible feedback to neurons. More recently, the possible contribution of astrocytes in events related to the plasticity of synaptic transmission were also included in models (Nadkarni et al., 2008; De Pittà et al., 2011; Wade et al., 2011). While biophysical models are very useful to simulate basic units, like the tripartite synapse, they are hardly suitable for large scale simulations. In contrast, simplified models that include only the basic features of neuron-astrocyte interactions (Postnov et al., 2009) appear more appropriate to describe network dynamics and to investigate the role of astrocytes in epilepsy (Amiri et al., 2012). In our model we did not include any distinct biophysical features that characterize the physiological actions of either neurons or astrocytes. We rather describe the activity of a single astrocyte in terms of the specific input-output signals with which astrocyte and neurons interact. This simplified astrocyte model was then embed in a neuronal network model of IDs based on the Izhikevich's single neuron model.

The slow kinetics of the astrocyte Ca^{2+} response to neuronal activity in the model reflect those of the mGluR-mediated Ca^{2+} elevations that were evoked in astrocytes by axonal afferent stimulation in young rat hippocampal slices (Porter and McCarthy, 1995; Pasti et al., 1997; Perea and Araque, 2005). Indeed, the intracellular Ca^{2+} variations in astrocytes depend primarily, although not exclusively, on activation of metabotropic receptors, phospholipase C-dependent inositol(1,4,5)-trisphosphate (IP3) production and, finally, stimulation of Ca^{2+} release from IP3-sensitive internal Ca^{2+} stores (Kawabata et al., 1996). Glutamate release at the synapse triggers a Ca^{2+} response in astrocytes that increases in both amplitude and frequency of oscillations according to increased levels of the neuronal activity (Pasti et al., 1997). These Ca^{2+} changes trigger a SNARE-dependent exocytosis of glutamate that signals back to affect the excitability of neurons (Araque et al., 2000; Pasti et al., 2001; Parpura et al., 2004). Accordingly, in the model we reproduced the most essential features of glutamate release in response to Ca^{2+} elevations in astrocytes. The release of glutamate is pulsatile and depends on the frequency of Ca^{2+} oscillations (Pasti et al., 2001), while its

efficacy is controlled by the amplitude of the Ca^{2+} increase (Parpura and Haydon, 2000). In addition, a steady state Ca^{2+} elevation may trigger only a single episode of release (Pasti et al., 2001).

Some approximations were applied to describe two features that characterize astrocyte signaling in our model. Firstly, we restricted our analysis to somatic Ca^{2+} signals. These Ca^{2+} increases can not be intended to fully represent the synapse-to-astrocyte signaling occurring fundamentally at the proximal and the distal processes that are in contact with the synapse. Indeed, somatic Ca^{2+} increases exhibit a lower frequency and slower kinetics with respect to those at the processes (Di Castro et al., 2011; Panatier et al., 2011). While these recent studies also showed that Ca^{2+} elevations at the astrocytic processes can have a distinct functional role, it is noteworthy that the Ca^{2+} elevation at the soma may represent a response that integrates the signals from the processes where astrocytes sense neurotransmitter release. Accordingly, Ca^{2+} signals at the soma may adequately reflect the overall firing activity of surrounding neurons. Amplitude, frequency and general pattern of somatic Ca^{2+} changes are, indeed, observed to vary according to different levels of neuronal activity (Pasti et al., 1997; Porter and McCarthy, 1997). Secondly, while glutamate (Parpura et al., 1994; Pasti et al., 1997; Bezzi et al., 1998), D-serine (Mothet et al., 2005; Henneberger et al., 2010), ATP (Arcuino et al., 2002; Serrano et al., 2006; Bowser and Khakh, 2007), and GABA (Kozlov et al., 2006; Lee et al., 2011) [for a review see Haydon and Carmignoto (2006)] are the main gliotransmitters mediating astrocyte-to-neuron signaling, in our model we fundamentally focused on glutamate because a large body of information is available about its modulatory action on both basal synaptic transmission (Fellin et al., 2004; Di Castro et al., 2011; Panatier et al., 2011) and long-term plasticity (Zhang et al., 2003; Panatier et al., 2006; Serrano et al., 2006; Jourdain et al., 2007; Navarrete and Araque, 2010; Santello et al., 2011; Han et al., 2012; Min and Nevian, 2012). In addition, the contribution of astrocytic glutamate in some forms of long-term potentiation of synaptic transmission has been recently confirmed in *in vivo* experiments (Takata et al., 2011; Navarrete et al., 2012). The potential role in focal seizure generation of ATP, D-serine and GABA will be the subject of future investigations. It is worth mentioning here that D-serine, and not glycine, is most likely the physiological co-agonist of the synaptic NMDA receptor in the brain (Mothet et al., 2000; Panatier et al., 2006; Fossat et al., 2012; Papouin et al., 2012). Given that D-serine is mainly, although not exclusively (Ding et al., 2011), synthesized in astrocytes and released through a Ca^{2+}-dependent mechanism (Wolosker et al., 1999; Wolosker, 2011), astrocytic D-serine may cooperate with glutamate to enhance NMDA receptor openings and through this action favor neuronal excitability ultimately promoting epileptic discharges.

The pathological increase in brain network excitability that eventually leads to focal seizure generation is believed to derive from the activity of excitatory and inhibitory neurons as well as of astrocytes. The cellular events that favor or oppose seizure initiation and propagation remain, however, poorly defined. Our model offers the opportunity to study ID generation in simulated networks composed by either only neurons or interactive astrocytes and neurons. The results that we obtained are summarized in **Figure 6** and can be, in our opinion, useful to understand how distinct signaling pathways may govern focal ID generation. **Figure 6** plots the average threshold for ID generation in the different conditions (mean ± SD, **Figure 6A**) and the cumulative sum of the threshold histograms (**Figure 6B**) showing failures. We found that in a network composed exclusively of neurons an ID can be generated by applying an intense stimulation of a group of neurons. The introduction of astrocytes into the network lowered ID threshold, while the inhibition of astrocyte signaling to neurons within, but not outside the focus, increased ID threshold. These results are fully consistent with those obtained in slice experiments (Gomez-Gonzalo et al., 2010) and demonstrate that focal IDs can be faithfully reproduced in our computational model. Accordingly, our model can be used to make predictions on the distinct contribution of different signaling pathways to ID generation. We present here some results regarding inhibitory signaling pathways. The ID threshold was observed to increase upon procedures that increase the strength of inhibition onto the principal neurons. This was achieved by either increasing the strength of the inhibitory transmission or by including in the model a distinct inhibitory feedback signal from astrocytes to neurons via $GABA_A$ receptors. These observations will be useful when addressing in future slice experiments the role of inhibitory signaling in ID generation.

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A computational model to investigate astrocytic glutamate uptake influence on synaptic transmission and neuronal spiking

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Over the past decades, our view of astrocytes has switched from passive support cells to active processing elements in the brain. The current view is that astrocytes shape neuronal communication and also play an important role in many neurodegenerative diseases. Despite the growing awareness of the importance of astrocytes, the exact mechanisms underlying neuron-astrocyte communication and the physiological consequences of astrocytic-neuronal interactions remain largely unclear. In this work, we define a modeling framework that will permit to address unanswered questions regarding the role of astrocytes. Our computational model of a detailed glutamatergic synapse facilitates the analysis of neural system responses to various stimuli and conditions that are otherwise difficult to obtain experimentally, in particular the readouts at the sub-cellular level. In this paper, we extend a detailed glutamatergic synaptic model, to include astrocytic glutamate transporters. We demonstrate how these glial transporters, responsible for the majority of glutamate uptake, modulate synaptic transmission mediated by ionotropic AMPA and NMDA receptors at glutamatergic synapses. Furthermore, we investigate how these local signaling effects at the synaptic level are translated into varying spatio-temporal patterns of neuron firing. Paired pulse stimulation results reveal that the effect of astrocytic glutamate uptake is more apparent when the input inter-spike interval is sufficiently long to allow the receptors to recover from desensitization. These results suggest an important functional role of astrocytes in spike timing dependent processes and demand further investigation of the molecular basis of certain neurological diseases specifically related to alterations in astrocytic glutamate uptake, such as epilepsy.

Keywords: astrocyte, glutamate uptake, glutamatergic synapse, computational model, neuron spiking

INTRODUCTION

Until a few decades ago the quest to better understand high level brain functions, such as learning, memory, and cognition, mainly focused on investigating the rapid, spike-based information processing performed by neurons. Glial cells, and among them astrocytes, were largely regarded as passive support cells, providing neurons with nutrition and structural support without directly participating in information processing functions (Kandel et al., 1991). Over the past 20 years, however, a growing body of evidence has demonstrated that astrocytes do participate in bi-directional signaling with neurons and, therefore, possibly play an important role in shaping communication in the brain (Volterra and Steinhauser, 2004; Perea and Araque, 2005). These findings demand a revision of the traditional neuron-centric model used to explain higher order brain functions to include astrocytes as part of a neuron-glia network model. Within this new framework, signaling includes both, fast spike-based processing and slower modulation mediated by astrocytic elements (Nedergaard and Verkhratsky, 2012).

In this paper, we present a computational modeling framework that spans across several hierarchical layers of the central nervous system (CNS), from the molecular to the synaptic, dendritic, and neuronal levels. The structure of this framework allows us to investigate the influence of glial cells at each of these levels and how they can modulate neuronal communication. Traditionally, high level brain processes are explained using the framework illustrated in red in **Figure 1A** where molecular dynamics at neuronal synapses are linked to system-level brain functions via synaptic neuronal signaling (Kandel et al., 1991). The new methodology we have chosen to explain and model these complex mechanisms is represented by the combination of the red, neural components, and the green, glial/astrocytic components, which have been added to account for the contribution and influence of astrocytes. Since glutamate is the most important neurotransmitter involved at excitatory synapses

and in astrocytic signaling, we focus on modeling the effect of astrocytic glutamate transporters on neuronal spiking within this new framework (**Figure 1A**).

In the original, neuron-centric framework shown in red, synapses, and neurons are the fundamental building blocks of a network within which information exchange is mediated by molecular mechanisms. The lowest level of hierarchy in **Figure 1A** comprises elements that interact at the molecular level, such as the three classes of glutamate receptors, ionotropic receptor-channels, AMPAR (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor) and NMDAR (*N*-Methyl-D-aspartate receptor), and mGluR (metabotropic glutamate receptor). At the synaptic level, ion fluxes through these receptor-channels give rise to synaptic responses of varying time courses and amplitudes depending on channel kinetics. These responses sum in a non-linear spatio-temporal manner and evoke spiking activity in neurons. A network of these neurons constitutes the system that emulates a physiological function.

The box outlined in green shows the inclusion of the previously underappreciated glial components (Nedergaard et al., 2003). One particular type of glial cell, the astrocyte, has thousands of processes and extensions that are often found in close proximity to hundreds of synapses, which it can potentially modulate (Halassa et al., 2007b).

Additionally, it has been discovered that astrocytes express large amounts of neurotransmitter receptors and transporters (Wang and Bordey, 2008). Since a neuron's pre- and postsynaptic nerve terminals are often ensheathed by astrocytes, which participate in synaptic signal processing, the term tripartite synapse was coined (Araque et al., 1999).

Within a tripartite synapse, biochemical, and morphological studies suggest that excitatory amino-acid transporters (EAATs) expressed on astrocytes are of the type EAAT2. These glutamate transporters maintain low extracellular glutamate concentration, which prevents neurotoxicity in spinal cord, striatum, and hippocampus (Rothstein et al., 1996), and might play a functional role in regulating synaptic currents by clearing glutamate after its synaptic release (Bergles and Jahr, 1998; Diamond, 2005). Neuronal transporters (EAAT3) also take up glutamate from the extracellular space, however, at a significantly lower rate than astrocytes due to their lower expression levels (Rothstein et al., 1996). The role of neuronal transporters could be to limit glutamate

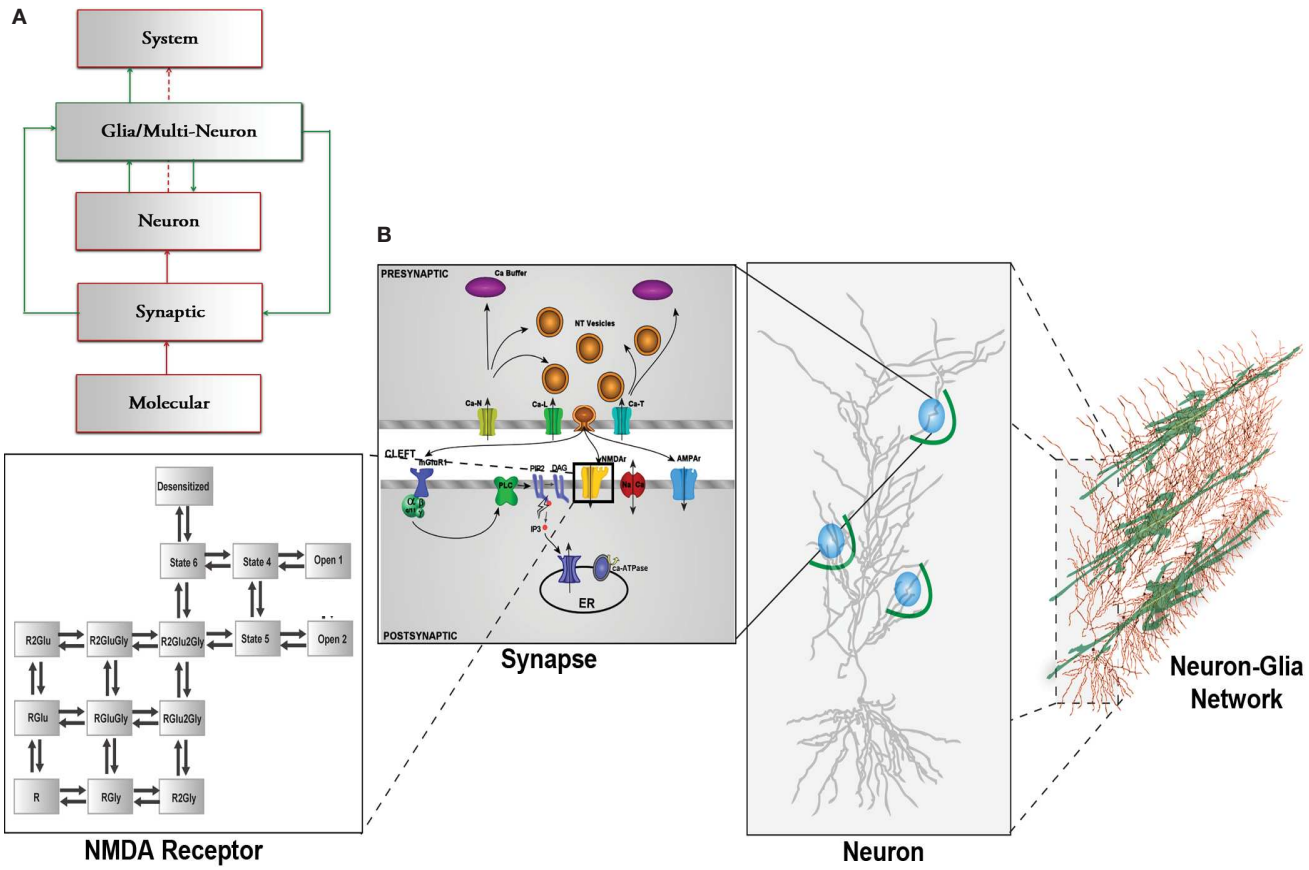

FIGURE 1 | (A) CNS hierarchy with the traditional neuron-centric framework in red and the revised neuron-glia framework including glial interactions in green. **(B)** Multi-scale framework of the CNS hierarchy including molecular, synaptic, neuronal, and network level including glial cells., modified from Bouteiller et al. (2011) The molecular level is represented with kinetic schema. The synaptic level includes several molecular elements and their spatio-temporal interaction. The neuron level comprises morphologically realistic neuron model with synapses (blue circles) randomly located on dendritic branches surrounded by astrocyte processes (green arcs). The network level takes into account the interaction between neurons and glial cells.

spill-over and to slow down glutamate clearance by glial transporters (Diamond, 2001; Scimemi et al., 2009). The importance of astrocytes in the regulation of glutamate uptake, the transformation of glutamate to glutamine (Uwechue et al., 2012) for re-usage in synaptic transmission, and epilepsy pathogenesis has recently been reviewed (Coulter and Eid, 2012).

Several studies demonstrated that astrocytes not only uptake glutamate inside a tripartite synapse, but under certain conditions, can also release glutamate (Araque et al., 1998), through a process termed gliotransmission (Halassa et al., 2007a). This term describes the process of glutamate release from astrocytes due to an increase in intracellular calcium via mGluR-mediated mechanisms in response to neural signaling inside a tripartite synapse (Parpura et al., 1994; Fiacco and McCarthy, 2006; Zur Nieden and Deitmer, 2006).

Because of the astrocyte' structural characteristics and biochemical signaling mechanisms, these cells may play an important role at the neuron and network levels of the CNS hierarchy (Volterra et al., 2002). However, the exact manner in which astrocytes communicate with neurons *in vivo* is still unclear. For example, the effect of glutamate uptake in synaptic transmission, and on neuronal spiking, is difficult to verify using state-of-the-art experimental procedures. Furthermore, several experimental and review articles have been published that challenge the tripartite synapse concept (Agulhon et al., 2010; Nedergaard and Verkhratsky, 2012), and raised a number of issue regarding the way astrocytes contribute to synaptic signaling by performing feedforward and/or feedback action through the uptake and release of neuro- and gliotransmitters (Smith, 2010).

One of the primary reasons why these disagreements cannot easily be resolved, is the lack of experimental techniques to directly study astrocytes and their biochemical signaling mechanisms (Nedergaard and Verkhratsky, 2012). Since astrocytes are generally not electrically excitable (do not generate action potentials), techniques for measuring glial cell activity mainly rely on imaging for *in vitro* studies or genetic manipulations for *in vivo* experiments. Hence, experiments are often performed under non-physiological conditions (Nedergaard and Verkhratsky, 2012), as *in vitro* experiments do not represent the natural environment of these cells and their responses might be drastically amplified. Disabling astrocytes through genetic manipulations is equally non-physiological and might result in compensatory effects *in vivo* (Smith, 2010).

One promising approach that can resolve these uncertainties is to use computational models. Such models provide opportunities to analyze the behavior of the system in response to various stimuli and conditions otherwise difficult to conduct experimentally, either due to the lack of the necessary technology, or difficulties in accessing readouts at the sub-cellular level.

In recent years, many computational modeling groups have demonstrated the glial influence within synaptic, neuronal, or network dynamics. Nadkarni and Jung (2007) have characterized astrocytic effects on spontaneous activity at the postsynaptic level. Silchenko and Tass (2008) have modeled the effects of glutamate release from astrocytes on neuronal depolarization and activity. Somjen et al. (2008) have used computer simulations to demonstrate that neuron-glia interactions are in part mediated by potassium ion fluxes between the two entities. Finally De Pitta et al. (2011) and Wade et al. (2011) have presented a modeling approach with bi-directional communication and synchrony between astrocytes and neuron clusters.

Our modeling approach integrates the dynamics from the molecular to the neuronal level and provides an original framework that allows for a better understanding of the effects of glia in a hierarchical manner. This modeling methodology mimics the structure of the CNS which spans several spatial and temporal scales and forms a hierarchical system from the bottom-up: molecular to synapse level, synapse to neuron level, and neuron to network level, with the inclusion of glial interactions. For example, the activation of receptors at single synapses, which are distributed along the dendritic tree of pyramidal neurons, sum in a non-linear fashion, which changes the membrane potential (Poirazi et al., 2003). These responses influence the network level, along with additional modulatory inputs from other pathways and inhibitory connections.

In this paper, we focus on the role of astrocytic glutamate uptake on synaptic responses and how it can modify neuronal spiking within the context of this modeling framework (**Figures 1A,B**). We observe that glial glutamate uptake is important to decrease desensitization of ionotropic glutamate receptors resulting in enhanced paired pulse facilitation for very short input intervals. Based on these observations, we propose that the time interval between input pulses and the interactions between receptors and transporters significantly contribute to neuron spiking patterns. Our model provides a unique method to assess critical parameters that can influence neural network behavior in relation to glutamate uptake.

METHODOLOGY

The synaptic modeling platform EONS/Rhenoms™ (Elementary Objects of the Nervous System) was developed to configure detailed synapses and define the distribution and arrangement of molecular elements within a synaptic environment (Bouteiller et al., 2008). The models and their descriptions are available online at http://synapticmodeling.com/. Each of the individual synaptic elements are described by kinetic schema that characterize the behavior of receptors using parameters fitting the model's responses to experimental data. The generic synapse model described here comprises presynaptic calcium buffers, voltage-dependent calcium channels, a single vesicular glutamate release site, glutamate diffusion in the synaptic cleft (Savtchenko and Rusakov, 2007), and binding of glutamate to postsynaptic ionotropic and metabotropic glutamate receptors (Ambert et al., 2010; Greget et al., 2011). Glutamate uptake is incorporated in the platform by including a model of a high-affinity glial glutamate transporter (EAAT2/GLT-1) adapted from Bergles et al. (2002), and a neuronal transporter (EAAT3) with parameters adapted from Larsson et al. (2004). Glutamate molecules released from presynaptic vesicles diffuse in the synaptic cleft. Glutamate molecules taken-up by these transporters are subtracted from those available at ionotropic and metabotropic receptors in the postsynaptic density. Numerous studies have quantified the tortuosity of the synaptic cleft and estimated the glutamate diffusion coefficient (Nielsen et al., 2004). The diffusion model used here has been

adapted from Savtchenko and Rusakov (2007) to calculate glutamate concentration inside the cleft as a function of the distance of the receptor from the release site using Eq. 1. The glutamate diffusion coefficient is 0.4 μm^2 ms^{-1}. The total number of transmitter molecules released from the vesicle in the model is 3,000 per release event. The concentration of glutamate inside the cleft is determined by the following equation:

$$\mathrm{Glu}\,(r, t, Q, D, \delta) = \frac{Q}{4\pi\delta Dt} e^{\frac{-r^2}{4Dt}} \quad (1)$$

Where Glu, r, and D represent the concentration of glutamate inside the cleft, the radial distance, and the diffusion coefficient, respectively. Q represents the number of glutamate molecules released instantaneously. "δ" stands for the height of the cleft and is maintained constant throughout the simulations at 20 nm. The glutamate profile seen by postsynaptic receptors is shown in **Figure 2B**.

The concentration levels of glutamate available at AMPAR and NMDAR is determined by their spatial location on the post synaptic density (PSD). The astrocytic glutamate uptake is calculated by the following rate equation for glutamate flux:

$$\frac{d\mathrm{Gluo}}{dt} = k_6 \times \mathrm{Na2ToH} \times \mathrm{Glu} - k_{-6} \times \mathrm{Na2ToGH} + k_3 \times \mathrm{Glu} \times \mathrm{Na2To} - k_{-3} \times \mathrm{Na2ToG} \quad (2)$$

where Na2ToH, Na2ToGH, Na2To, Na2ToG are the intermediate states that determine glutamate bound and transported by the transporter (see **Figure A3** in Appendix). The values for parameters k_6, k_3 is 6 mM^{-1} ms^{-1} and for k_{-6}, k_{-3}, is 0.5 ms^{-1}. All the other rate constants and ion concentrations are the same as reported in Bergles et al. (2002). The uptake rate in the equation above is for a single transporter channel. The diameter of the postsynaptic disk is 200 nm (Takumi et al., 1999) the height of the synaptic cleft is fixed at 20 nm (Savtchenko and Rusakov, 2007). The astrocyte membrane and its transporters are set at a distance of 400 nm from the release site. The distance of the transporter from the release site does not influence its uptake rate (Diamond, 2005). Transporters are expressed on astrocytes surrounding synapses with densities within a range of 6,500–13,000 μm^{-2} (Lehre and Danbolt, 1998; Diamond, 2005). The astrocyte is modeled as a cylindrical surface of height 20 nm and radius 400 nm as depicted by the green ensheathment around the synapse in **Figure 2A**. Assuming the maximum transporter density (13,000 μm^{-2}) and a 50% astrocytic coverage surrounding the synapse, we calculate the number of glutamate transporters per synapse to be 650. For the same conditions but with 50% density, we calculate the number of transporters to be 325. Glutamate uptake from neuronal transporters is calculated in a similar manner to that by glial transporters (Eq. 2) with the kinetic rate constants adapted from Larsson et al. (2004) and a transporter density of 90 μm^{-2} (Holmseth et al., 2012). The total glutamate cleared from the receptor vicinity is obtained by multiplying the cleared glutamate rate $d\mathrm{GluO}/dt$, integrating over the time steps of the simulation and multiplying by the number of transporters. This amount of cleared glutamate is then subtracted from the glutamate input concentration available at the receptors.

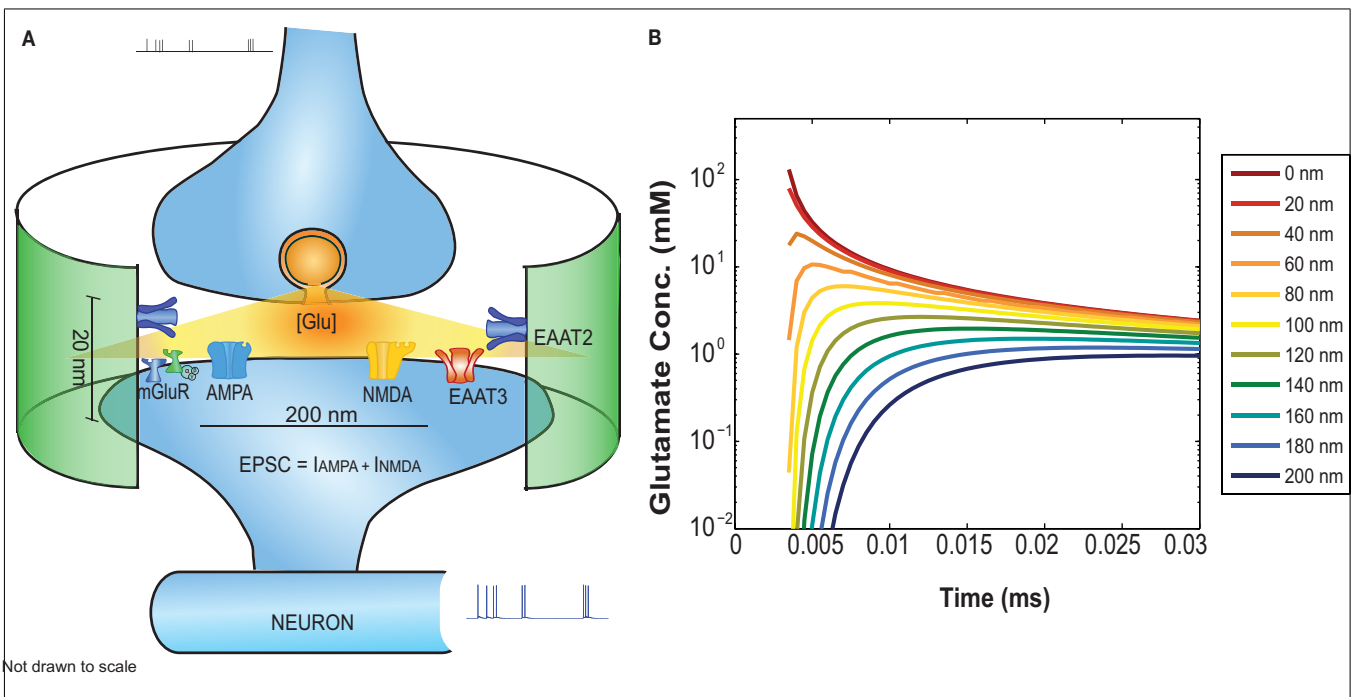

FIGURE 2 | (A) Functional block diagram of the EONS synapse model including: glutamate diffusion inside the cleft, AMPAR, NMDAR, mGluR, and glutamate uptake mediated by glial (EAAT2) and neuronal transporters (EAAT3). The green cylindrical ensheathment represents the astroglial process on which EAAT2s are expressed. **(B)** The glutamate concentration profile as a function of time for receptors located at different distances relative to the glutamate release side.

We used the AMPA receptor model described in detail in Robert and Howe (2003) which represents a 16 states model describing the receptor transitions between resting, desensitized, and conducting open states. Successive binding of two, three, and four glutamate molecules produces conformational changes leading to fast opening and closing of the channel.

The current through the channel is calculated by:

$$I_{\mathrm{AMPA}} = \mathrm{nb}_{\mathrm{AMPA}} \times \left(g_2 O_2 + g_3 O_3 + g_4 O_4\right) \times (V - V_{\mathrm{rev}}) \quad (3)$$

Where the open conducting states evolve as:

$$\dot{O}_i = [M\,(\mathrm{Glu})] \cdot O_i \quad (4)$$

where I_{AMPA} is the current mediated by AMPA receptors, $\mathrm{nb}_{\mathrm{AMPA}}$ is the number of AMPA receptors (in this study $\mathrm{nb}_{\mathrm{AMPA}}$ is 80), consistent with reported AMPAR numbers between 46 and 147 at CA1 hippocampal synapses (Matsuzaki et al., 2001). g_2, g_3, g_4 are unitary conductances with values 9, 15, and 21 pS associated with the channel in open states when 2, 3, and 4 glutamate molecules are bound respectively. The probabilities for the O_2, O_3, O_4 states are calculated based on ODEs simulated using solvers in SBML™. The derivatives of open states $\dot{O}_i$ (where $i = 2, 3, 4$) are calculated as a product of matrix M containing other states transition rate constants with input Glu and vector of currents states O_i. V_{rev} is the reversal potential of the AMPAR (V_{rev} is 0 mV) and V is the membrane potential that changes dynamically during the simulation. More details on the model can be obtained from Robert and Howe (2003) and for kinetic rate parameters, please, see Section "Appendix."

Glutamate concentration available at NMDAR after glutamate uptake is provided as input to the NMDAR model represented in a 15-state kinetic scheme, which includes agonist (glutamate) and co-agonist (glycine) binding sites, channel blockers (memantine and magnesium), as well as several antagonist sites. The kinetics of this model are borrowed from Ambert et al. (2010). For validation of the NMDAR model, various protocols were tested. For a single short pulse of glutamate, experimental results reported by Schorge et al. (2005) were used to validate the model. For long or repetitive glutamate inputs to the model, the kinetic parameters were adjusted to properly capture effects of desensitization and to match experimental data from Zhang et al. (2008). The equations to calculate NMDAR-mediated synaptic current are:

$$I_{\mathrm{NMDA}} = \mathrm{nb}_{\mathrm{NMDA}} \frac{I_o}{1 + \left(\frac{Mg_0^{2+}}{K_0}\right) \mathrm{e}^{-\delta z F \Psi m / RT}} \quad (5)$$

$$I_o = g\,(V - V_{\mathrm{rev}})\,O\,(t)$$

$$g = g_1 + \frac{g_z - g_1}{1 + \mathrm{e}^{\alpha \Psi m}}$$

where I_{NMDA} is the current mediated by NMDA receptors. $\mathrm{nb}_{\mathrm{NMDA}}$ is set at 20 for this study consistent with observations made in Takumi et al. (1999) and Racca et al. (2000). I_{o} is the current associated with the open conducting state $O(t)$ calculated using ODEs solved with kinetics described in Ambert et al. (2010). The magnesium concentration in the external solution is set to 1 mM; Ψm is the electrical distance of the magnesium binding site from the outside of the membrane (set at 0.8); R, the molar gas constant (8.31434 J mol^{-1} K^{-1}); F, the Faraday constant (9.64867.104 C mol^{-1}); T, the absolute temperature (273.15 K); g_1 and g_2 are the conductances associated with the open states when one or two glutamate molecules are bound and are 40 and 247 pS respectively; $\alpha = 0.01$ is the steepness of the voltage-dependent transition from g_1 to g_2.

The total synaptic current is calculated from the sum of AMPAR and NMDAR currents.

$$I_{\mathrm{syn}} = I_{\mathrm{AMPA}} + I_{\mathrm{NMDA}} \quad (6)$$

The synaptic currents calculated using Eq. (6) drive the neuron membrane potential and synapses are now approximately acting as current sources (Jaffe and Carnevale, 1999). A CA1 pyramidal cell model with active sodium and potassium channels and a morphology described in Jarsky et al. (2005) was used within the NEURON simulation environment (Hines and Carnevale, 1997). Synapses were placed at 16 random locations in stratum radiatum (middle one-third of the cell, 100–200 μm). The synaptic strength was tuned by a factor of 6 to reach threshold levels for neuronal spiking such that spiking probability was 1.

RESULTS

EFFECT OF ASTROCYTIC GLUTAMATE UPTAKE ON POSTSYNAPTIC CURRENTS

The EONS/Rhenoms™ modeling platform allows the investigation of critical parameters that modulate synaptic transmission and neuronal spiking. In this paper we study the effects of EAATs within the astrocyte membranes surrounding CA1 hippocampal neurons. The result section begins with a demonstration of the model's fidelity in replicating the influence of astrocytic glutamate uptake at the synapse level, as previously shown (Sarantis et al., 1993; Bergles and Jahr, 1998; Diamond, 2005). We then build on these results by investigating how astrocytic glutamate uptake influences ionotropic elements' responses for different input stimulation protocols such as paired pulse and random interval trains (RITs). The molecular level effects, such as receptor desensitization and transporter saturation, arising due to the relative timing between the inputs (glutamate release from single vesicular sites) are demonstrated. We believe that understanding the interactions between these molecular level elements through simulation studies provide insights into synaptic level responses that are difficult to explore experimentally. In this work, we demonstrate how sub-cellular responses can affect neuronal spiking.

Astrocytic glutamate uptake decreases peak amplitudes of AMPAR-mediated EPSCs

AMPARs are known for their crucial role in mediating fast excitatory synaptic transmission. AMPARs and NMDARs co-exist at many central glutamatergic synapses (Bekkers and Stevens, 1989) and have very distinct kinetics contributing to the fast and slow components of the EPSCs respectively (Umemiya et al., 1999). It was previously shown that astrocytic glutamate transporters do not shape the decay of non-NMDA receptor-mediated synaptic responses (Sarantis et al., 1993).

We proposed to test if our model yielded the same effects and studied the role of glutamate uptake on current going through the AMPAR channels in response to a single vesicular release. We simulated AMPAR-mediated EPSCs for a single pulse input with 50, 100% glutamate transporter densities and without glutamate transporters. These three cases were chosen to demonstrate the effect of glutamate transporters at moderate and extreme cases of transporter density on astrocytes. **Figure 3A** shows the values of peak amplitude responses of AMPAR-mediated EPSCs plotted against varying density of glutamate transporters. AMPAR currents obtained here are in response to a single presynaptic input pulse eliciting a single vesicular release of glutamate as a function of the number of surrounding astrocytic glutamate transporters. Due to increased density of transporters and hence more glutamate uptake, glutamate input to AMPARs is decreased. As expected, the amplitude of AMPAR-mediated synaptic responses decreased with increase in the number of transporters. The red line in **Figure 3A** is a linear regression fit to the data (in black) with a correlation coefficient of $r^2 = 0.69$. **Figure 3B** shows the normalized AMPAR-mediated EPSCs in response to a single release event with (50 and 100% density) and without transporters. These results indicate that the decay of the time course remains the same with 50% transporter density and without the transporters. However, at 100% density of transporters, the peak itself is shifted, which could be due to elevated glutamate uptake, but the time course decay is very similar. Since AMPARs are sensitive to glutamate concentration, removal of glutamate from the cleft due to buffering or uptake by EAATs modifies this concentration and thus affects the AMPAR-mediated responses.

Astrocytic glutamate uptake influences decay phase of NMDAR-mediated EPSC time course

Unlike for AMPARs, the responses of NMDARs are slower (Lester et al., 1990). Therefore, it is important to study the role of glutamate uptake on NMDARs. Numerous experimental studies report that glutamate uptake mediated by glia and neuronal transporters significantly influences the decay phase of NMDAR-mediated EPSCs (Bergles and Jahr, 1998; Bergles et al., 2002; Diamond, 2005).

Figure 3C shows the simulated responses of normalized NMDAR-mediated postsynaptic currents to a single release event with 50 and 100% densities, and without transporters. Our simulations confirm previously reported results suggesting that an increased number of EAATs leads to an increase in glutamate uptake, which causes a faster decay of NMDAR-mediated EPSC. Thus, increased expression and density of glutamate transporters could possibly account for the developmental changes that occur with age in NMDAR-mediated EPSCs between P14 and adult rat pyramidal cells (Diamond, 2005).

We also explored the role of the neuronal transporters, which are located within perisynaptic regions (He et al., 2000) on NMDAR-mediated EPSCs. Neuronal transporters at a density of 90 μm^{-2} (Holmseth et al., 2012) were placed within the presynaptic annulus of radius 100–400 nm. **Figure 3D** shows NMDAR-mediated EPSCs elicited in response to a single pulse taking into account glutamate uptake mediated by glial transporters (EAAT2) shown in red and the combined effect of glial and neuronal transporters (EAAT3) shown in blue. Our simulation results showed that uptake by neuronal transporters did not significantly affect EPSC responses, in contrast to glia-mediated glutamate uptake, which showed a stronger effect NMDAR-mediated EPSCs.

Astrocytic glutamate uptake effect on paired pulse responses is different for small and large input time intervals

In this section we investigate the influence of astrocytic glutamate uptake on EPSCs mediated by both AMPARs and NMDARs for a paired pulse input. The fast component of the EPSC is mediated by AMPARs and the slow component is mediated by NMDARs (Umemiya et al., 1999). The quantal value of EPSCs may vary with developmental stage or age of the animals (Bellingham et al., 1998), as differential expression and density of AMPA and NMDA receptors will result in different values of AMPAR- and NMDAR-mediated components. For this study, we use AMPA to NMDA receptor ratio of 80–20 (see Methodology). The paired pulse protocol is commonly used for testing presynaptic effects on EPSCs (Debanne et al., 1996) by changing the timing between two release events. **Figure 4A** shows the composite EPSCs to paired pulse inputs for different input intervals from 10 to 500 ms with (dark gray traces) and without glutamate transporters (light gray traces). The asterisks highlight the peak amplitudes of the EPSC with (red) and without (blue) glutamate uptake. To better demonstrate the effects of glutamate uptake for shorter input intervals, the results are plotted on a logarithmic time scale. In **Figure 4A**, the difference in the peak amplitude of the response to the first pulse with and without glutamate uptake is significant. For input intervals up to 100 ms, we observe paired pulse facilitation (peak amplitude of the second pulse is larger, as compared to that to the initial pulse) with glutamate transporters, and paired pulse depression (peak amplitude of the second pulse smaller, as compared to that to the initial pulse) when there are no transporters. For larger intervals, however, this facilitation/depression effect becomes less prominent in both cases, with and without glutamate transporters.

To better explain these effects, we take advantage of the modeling platform features to access the individual desensitization states (probability of receptors being desensitized) of AMPARs and NMDARs.

The probability of the desensitized state of AMPARs with two glutamate molecules bound is plotted as a function of paired pulse intervals in **Figure 4B**. At smaller input time intervals, the AMPAR enters into a desensitized state more rapidly, thus reducing its probability of being open or more responsive to the second pulse of glutamate, thereby decreasing the current through its channel. As shown in **Figure 4C**, the NMDA receptor also enters into a desensitized state and the probability of being in this state increases with increase in the time intervals between input pulses, as the receptor takes a much longer time to return to its original resting state. This desensitization property reduces the receptor's ability to be in the open state in response to subsequent pulses for almost all interval ranges examined here.

Figure 4D shows the dynamics of the EAAT2 transporter in its $HGN3T_0$ state (when glutamate, Na^+, and H^+ ions are bound and the transporter is facing outward). These results show that the glutamate transporter has to go through a recovery phase

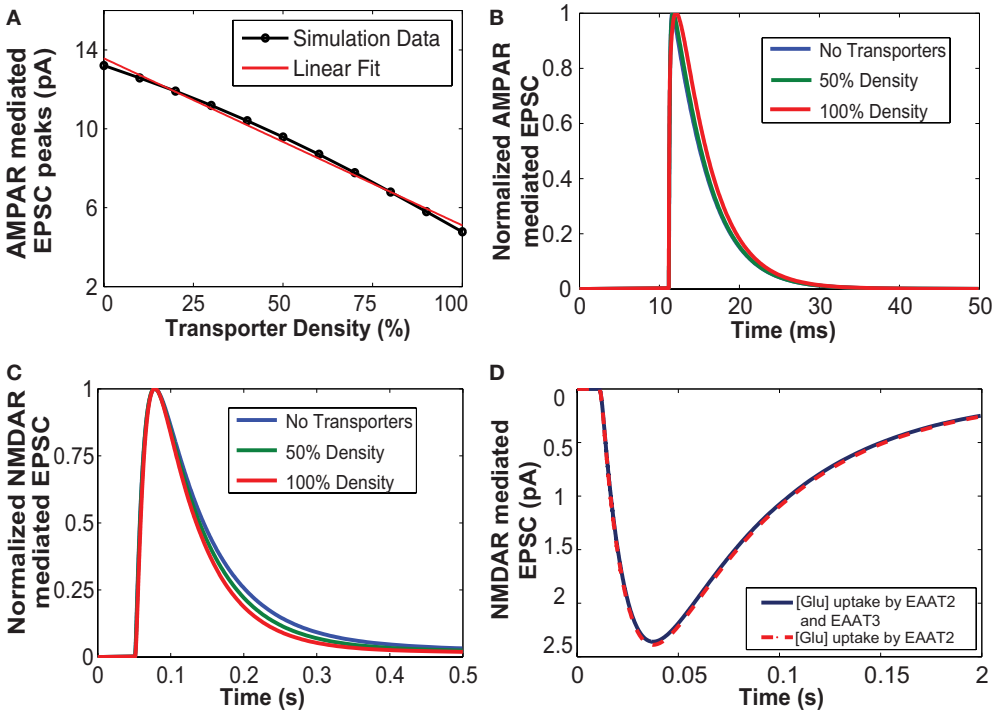

FIGURE 3 | (A) The increased number of glutamate transporters affects the peak amplitude of AMPAR-mediated current due to uptake of glutamate. **(B)** Normalized responses of AMPA mediated EPSCs elicited from a single input pulse for cases with no transporters, 50% density and 100% density of astrocytic glutamate transporters. The decay time course of normalized AMPAR currents with 50% density and without any transporters did not show any change. **(C)** Glutamate uptake by the glial transporters affects the decay time course of NMDA receptor-mediated EPSC. An increase in the density of transporters results in an increase in the rate of uptake thus decreasing the time of decay of NMDA receptor-mediated EPSC. **(D)** NMDAR-mediated EPSCs with glial glutamate uptake (red), and with both glial and neuronal uptake (blue). The uptake mediated by neuronal transporters (EAAT3) is not significant.

during which the clearance rate is slowed down. This indicates that the transporter may not be in its full capacity shortly after an initial pulse, thereby reducing its responsiveness to glutamate released on subsequent pulses. Supporting evidence for changes in glutamate transporter's uptake rate and its effects have been reported for different input pulse intervals (Otis and Jahr, 1998; Diamond, 2005). In these studies, glutamate uptake rate was assessed through synaptically activated transporter–mediated anion currents (STCs).

In **Figure 4A**, the light gray traces show EPSCs responses elicited by paired pulse inputs. Blue asterisks mark the peak amplitude of the responses. The peak amplitudes on the second pulse elicited after intervals of 10 ms and up to 300 ms are much lower than the peak amplitude of the first pulse. This effect is known as paired pulse depression and it is due to the desensitization of the receptors during these input intervals, as described above. The peaks elicited by the second pulse slowly recover toward the first peak, as receptors return to the original responsive state. However, when glutamate uptake occurs, there is paired pulse facilitation, indicated by the red asterisks on the peaks of the dark gray traces for the shorter input intervals. This effect could be due to (i) a significant reduction in the peak amplitude of AMPARs because of glutamate uptake, as shown in **Figure 3A** for single pulse, as well as (ii) transporter's recovery time to uptake glutamate with the same efficiency. As seen from these simulation studies, we hypothesize that inter-play between receptor desensitization and transporter recovery explains the clear differences in paired pulse responses in the presence (facilitation) or absence (depression) of astrocytic glutamate uptake. These differences disappear for longer intervals, as the transporter recovers to its initial state and receptors recover from desensitization. Thus, these simulation results highlight the importance of understanding the interactions between glutamate receptors and transporters at the molecular level. They also demonstrate the power of such computational modeling to facilitate understanding of these mechanisms.

ASTROCYTIC GLUTAMATE UPTAKE INFLUENCES NEURONAL SPIKING

The results presented in the previous sections demonstrate that glutamate uptake influences EPSC kinetics at the synaptic level. Given the hierarchical organization of the nervous system, a critical question we propose to address in this section is whether the local effects at the synaptic level can significantly affect neuronal signaling. The changes in input glutamate concentration profile due to EAATs affect ionotropic receptors and modify the kinetics of excitatory synaptic currents. These currents subsequently change membrane potential in dendrites and thus influence neuronal spiking activity. In order to effectively model these aforementioned phenomena, we simulated a neuron model using the NEURON

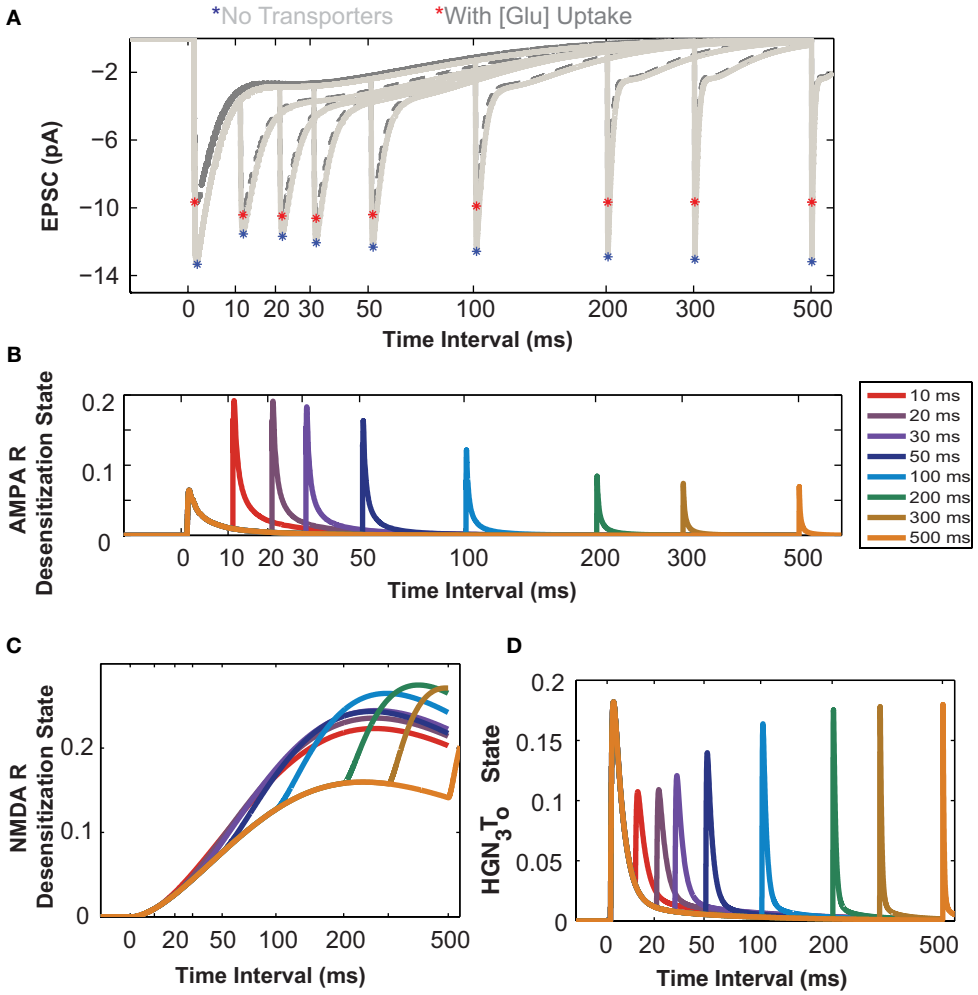

FIGURE 4 | Astrocytic glutamate uptake effect on paired pulse responses is distinct for small and large input time intervals. (A) Composite EPSCs elicited by paired pulse stimulation plotted against input time intervals separated by 10–500 ms (Time axis in log scale to zoom into the effects at shorter input time intervals for all plots). Paired pulse depression (PPD) effect is observed for responses when no transporters are present (light gray, peaks marked by red asterisks). With the presence of transporters and astrocytic glutamate uptake (dark gray, peaks marked with blue asterisks), there is a paired pulse facilitation (PPF) effect observed for responses when the input time intervals are short. This reversal of effect from PPD to PPF is only apparent for shorter input time intervals. **(B)** The probability of the AMPARs in desensitization state as a function of input time intervals. These receptors are highly desensitized for shorter input time intervals. **(C)** The probability of NMDARs in desensitization state. The NMDARs are highly desensitized and this increases with increasing input time intervals. **(D)** HGN_3T_o state probability of the glutamate transporter, when H^+, Glu, 3 Na^+ are bound to the transporter. The transporter recovers to this same state only after longer input time intervals (>200 ms).

simulation software that incorporate our detailed synaptic models, as described in the Section "Methodology."

The neuron model used here (Jarsky et al., 2005) has a realistic CA1 pyramidal cell morphology with synapses distributed at random locations within 100–200 μm from the soma. The input to the system is a RIT (average rates at 2 and 5 Hz) to mimic low frequency spiking activities of the CA3 inputs to CA1 under physiological conditions. The synaptic input strength was chosen such that a spike was elicited by synchronous firing of all synapses. This condition makes the neuron very sensitive to threshold levels for firing. The neuron configuration described here is a specific case, and can be configured in multiple ways with respect to synaptic distributions and locations, strengths, and ion channel distribution. Previous work has shown how the rate of change of membrane potential contributes to neuronal firing of by modifying spiking threshold. In addition to these parameters, the synchrony of excitatory synaptic inputs and previous occurrence of an action potential can also determine the probability of occurrence of the next action potential (Azouz and Gray, 2000; Henze and Buzsaki, 2001).

Figure 5A illustrates the spiking activity of a CA1 pyramidal neuron elicited by a 2-Hz RIT input in the absence (blue) or presence (green) of EAATs. The average number of spikes elicited in the presence of EAATs is always smaller, as compared to that in the absence of EAATs. **Figure 5C** shows the results for a 5-Hz RIT input. In addition to spiking activity, the Figure shows two

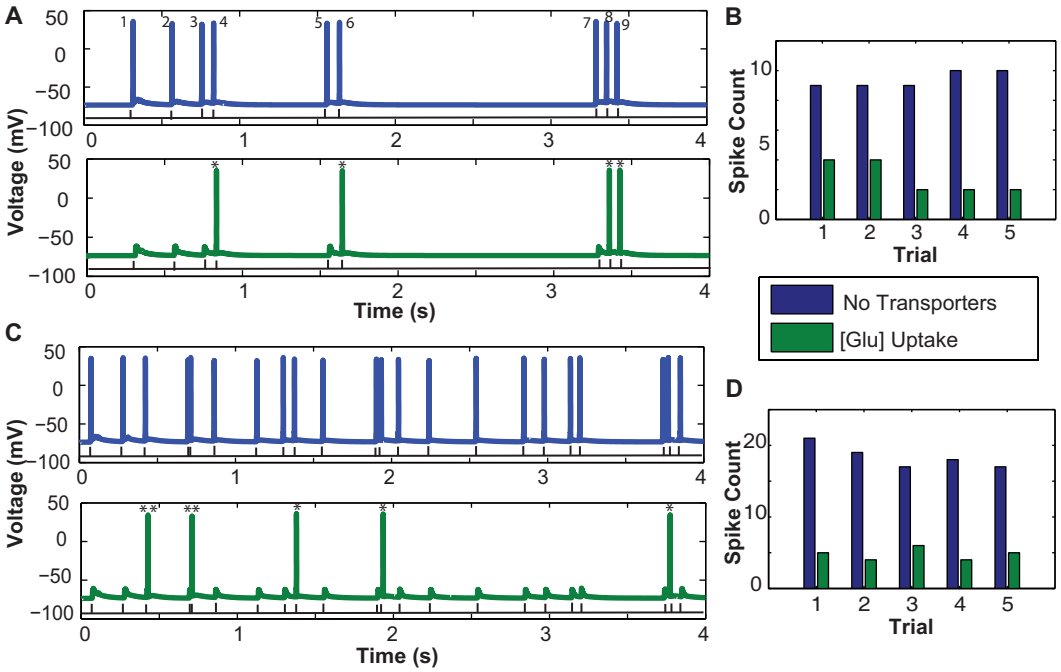

FIGURE 5 | Influence of astrocytic glutamate uptake on spiking activity of a CA1 pyramidal neurons at different input frequencies. (A) Neuronal spiking activity elicited by a random input interval train with mean frequency of 2 Hz. The number of spikes occurring in the presence of glutamate uptake are much less (green) vs. when there are no transporters in the vicinity of synapses. **(B)** Number of spikes per trial within a span of 4 s without any transporters (blue) and with transporters and glutamate uptake (green). Across trials we observe a consistent decrease in the spike count. **(C)** Neuronal spiking activity elicited by a random input interval train with a mean frequency of 5 Hz. Similar effects of spike failure as seen in 2 Hz are observed. **(D)** Number of spikes per trial across five trials show the consistent failure of spikes due to increased glutamate uptake in the presence of transporters. Two critical events marked by * show that the spikes elicited without glutamate uptake (no transporters) and with glutamate uptake have a timing difference between 1 and 2 ms and ** indicates when timing lies in between 3 and 8 ms.

critical events marked by ∗ when spikes elicited without glutamate uptake and those with glutamate uptake have a timing difference between 1 and 2 ms and by ∗∗ when timing differences are between 3 and 8 ms. Spiking properties of neurons are dependent on multiple parameters; they could be due either to intrinsic neuronal properties, such as non-homogenous distribution of various ion channels, or to the diversity of synaptic inputs.

In **Figure 5A**, a RIT with mean frequency of 2 Hz containing nine pulses within a span of 4 s (black trace) is the input to the presynaptic terminals of all synapses. In the case without glutamate uptake or when no transporters were present, the neuron evoked nine output spikes at the soma, but only four spikes were elicited with glutamate uptake. Based on the paired pulse effects on EPSCs with and without glutamate transporters presented in **Figure 4A**, we expected to observe these effects on the number of spikes elicited at the neuron level. Between the two cases in **Figure 5A**, without (blue) and with (green) glutamate transporters, spike numbers 1, 2, 3, 5, and 7 failed to appear when there is glutamate uptake. Spike numbers 4, 6, 8, and 9 are not suppressed but arrive with a small delay. Looking at the time intervals between spikes 3 and 4, 5 and 6, and 8 and 9, which are all less than 200 ms, we can, see from the results in **Figures 5A,C** that the probability of a spike being suppressed due to glutamate uptake is more when the input inter-spike interval is longer than 200–300 ms. For longer inter-spike intervals, glutamate transporters should have recovered to their full potential for efficient uptake. Also, the subtle timing differences that occur between the two cases could be potentially due to the relative changes in NMDAR-mediated EPSC time course decay. The change in peak amplitude responses, mainly mediated by AMPARs, due to glutamate uptake may or may not drive the neuron's membrane potential to spiking threshold values. Moreover it is the inter-play between the kinetics of AMPARs, NMDARs, and the transporters that can potentially lead to varying spiking patterns in neurons. We repeated a similar experiment, but with a higher input frequency of 5 Hz RITs (**Figure 5C**), which elicited between 19 and 22 spikes in the span of 4 s. Our results indicate that less spikes were elicited in the presence of glutamate uptake, in agreement with the observations with 2 Hz RIT responses. However, the spiking failure is comparatively less as there is a lower probability of spikes occurring with intervals longer than 200–300 ms. Five Hertz RIT contains inputs which are separated by intervals most likely shorter than 200 ms and all those paired pulse effects observed in **Figure 4A** at these intervals could possibly explain the spiking effects seen here.

To test the robustness of these simulations, several trials with RIT with the same mean frequency were run. It appears that the spike failure across trials elicited by 5 Hz was relatively consistent. However, as shown in **Figure 5B**, across trials elicited by

2 Hz RITs, there was more spike failure in some trials (such as 3 and 4). When we analyzed the simulation data, we observed that these trains had spikes separated by intervals longer than 300 ms. These interesting behaviors in spike failure may be attributed to the hypothesis described above, that the transporter would have recovered its uptake capacity thereby reducing amplitude and time course of AMPAR-mediated EPSCs.

These results are preliminary and were meant to demonstrate how simulation studies can be used to show that subtle changes in synaptic currents induced by glutamate uptake contribute to distinct neuronal spiking and temporal patterns. Changes in amplitude and time course of AMPAR- and NMDAR-mediated EPSCs, as shown in **Figure 4A**, were translated into subtle changes in spike arrival timings (**Figures 5A,C**) and spike failure (**Figures 5B,D**) with and without glutamate uptake. We built a model that could take into account receptor dynamics at elaborate synapses. Some of these dynamics, such as time course decay and amplitudes, were influenced by glutamate uptake mediated by glutamate transporters present on the astrocytic membrane surrounding these synapses. These results thus show the relevance of astrocytic mediated glutamate flux interactions between synapses and thus their effects on neurons.

DISCUSSION

In this paper we focused to study the role of glutamate uptake mediated by glutamate transporters present on astrocytic membranes surrounding CA1 hippocampal synapses on synaptic transmission and neuronal spiking. First we showed that the model was able to reproduce previously observed phenomena regarding the role of glutamate uptake on synaptic transmission by modulating responses mediated by AMPA and NMDA receptors. This was achieved by removing glutamate molecules that are bound to or transported by glutamate transporters from those available at the levels of the receptors. Previous studies have emphasized the importance of taking into account both glutamate diffusion and binding to transporters to determine changes in the decay kinetics of synaptic glutamate concentration (Wadiche et al., 1995). In agreement with this study, glutamate is removed from synaptic cleft by diffusion, and binding to and transport by glutamate transporters.

Some of the phenomena demonstrated here under the conditions we used are summarized:

(i) An increase in the density of astrocytic glutamate transporters results in a decrease in AMPAR-mediated EPSC's peak amplitude. This effect is due to the rapid decrease in glutamate concentration mediated by the transporters. However, for a certain density of transporters and under the assumption that there is 50% ensheathment surrounding these synapses, the time course of AMPAR-mediated EPSCs is not influenced, as previously reported (Sarantis et al., 1993). Some experimental and simulation studies however show that there is no effect on AMPAR peak amplitudes (Zheng et al., 2008). This lack of effect could be due to a much higher density of transporters as compared to receptors and also to Monte Carlo diffusion studies that assumed a different configuration of glutamate diffusion, receptors kinetics, and transporter arrangement.

(ii) Astrocytic glutamate uptake has a more predominant effect on the decay phase of NMDAR-mediated EPSCs. This result is consistent with previously reported experimental results (Diamond, 2005). Our simulations allowed us to closely examine the effect of glutamate uptake on the desensitization properties of these receptors as well.

(iii) These two effects on amplitude and time course on EPSCs, combined with the transporter's recovery behavior following paired pulse stimuli separated by short time intervals give rise to interesting dynamics. When glutamate uptake is not considered, there is paired pulse depression for responses to stimuli delivered at short intervals. However in the presence of glutamate uptake, paired pulse facilitation is observed. The transporter's recovery kinetics are often neglected based on the assumption that, at physiological temperature, they have large capacity and respond to high frequency stimuli in a similar way (Wadiche and Kavanaugh, 1998; Auger and Attwell, 2000). However, modifying their kinetics as a function of temperature and pH may give rise to different outcomes. The importance of neuronal transporters was shown in studies where reduced expression of neuronal transporters (EAAT3) can lead to behavioral abnormalities (Sepkuty et al., 2002). In the current work, we included the EAAT3 kinetic model described in Larsson et al. (2004). EAAT3 type transporters are localized at dendrites and soma, and especially at perisynaptic regions (He et al., 2000). However, their role in mediating glutamate uptake is debated because of their low expression density, 1%, as compared to other types of EAATs expressed mainly by glia with densities of 20% for GLAST and 80% for GLT-1 (Holmseth et al., 2012). Experimental and simulation studies by Scimemi et al. (2009) show that neuronal transporters may slow down glutamate clearance time by astrocyte transporters and that they can influence NMDAR-mediated synaptic transmission. Glutamate molecules bound to efficient neuronal transporters are more likely to be transported once bound, than to be unbound (Otis and Jahr, 1998). Studies using knock out models of EAAC1/EAAT3 showed no significant changes in AMPAR- and NMDAR-mediated EPSCs for single vesicle release (Scimemi et al., 2009), which was the basic assumption of the synapse model used here (see Methodology). From other simulation studies by Diamond (2001) it is hypothesized that neuronal transporters might influence perisynaptic NMDA receptors, as they are more likely to be activated by glutamate spill-over from neighboring synapses. We tested the influence of neuronal transporters on NMDAR-mediated EPSCs at 90 μm^{-2} density and found that they had no insignificant effect on EPSC profile. Higher densities of neuronal transporters might induce a greater influence on EPSCs, but given the experimental findings mentioned above, it is generally accepted that they exhibit a low density of expression. Since the effects contributed by neuronal transporters were negligible, we focused in this study on understanding the changes in neuron spiking behavior induced by glutamate uptake mediated by astrocytic transporters. This focus was also a modeling choice to reduce the computational load in simulating synapses with too many elements. Exploring the influence of neuronal transporters with different kinetic

parameters and density may or may not have an effect, as previously discussed and explored in previous studies.

(iv) All the above effects at the molecular and synaptic levels are translated into conductance changes with varying amplitudes and time courses that impact the temporal coding and spiking of neurons. Blocking glial glutamate uptake may have serious consequences on raising glutamate concentration to neurotoxic levels and causing epileptic conditions (Rothstein et al., 1996). These effects have been shown in both experimental and simulation studies (Oyehaug et al., 2012). In our simulations, we examined the effects of glutamate uptake on neuron spiking behavior elicited by 2 and 5 Hz RIT stimuli. Neurons with blocked astrocytic glutamate uptake showed higher spike counts. We attribute the failure of spikes in the presence of glutamate uptake mainly to the reduced levels of glutamate (i) that decrease synaptic amplitudes mediated by AMPARs and (ii) time course decay mediated by NMDARs, which also cause subtle differences in spike arrival. A closer look at the pattern of spike generation between the two cases without and with glutamate uptake shows that, spike usually occurs even after glutamate uptake, when the timing between the input pulses is less than 200–300 ms, implying that sometimes, the spike could be evoked because the transporters have not cleared glutamate levels up to optimal levels required for suppressing spike generation. Note that in this model the strength and number of synapses were chosen such that the neuron membrane potential reached threshold values easily. These parameters were selected in order to test the influence of glutamate uptake on spike activity around the membrane potential threshold for spike generation. Under our assumed conditions, the simulation studies show that glutamate uptake mediated by astrocytic transporters have a significant impact on neuronal spiking. To test the robustness of these results we ran several trials and found that the spike failure rates were more predominant for 2 Hz RITs, because there seems to be a higher probability of input trains with inter-spike intervals separated by more than 500 ms, when transporters and receptors have completely recovered for efficient glutamate uptake and responses, respectively.

We also underscore the diversity of synapses that arises from the variability in spatial location of the receptors, which we are investigating in a separate study. It is interesting to note that most of the receptors, such as NMDARs and mGluRs, have many modulatory sites, which consequently increase the number of parameters needed in the simulation. Our highly configurable geometric synapse model allows for the exploration of various parameters that influence sub-cellular/molecular level interactions and their direct or indirect influence on the synapse and neuron levels. By linking such complex unified model of a synapse to morphologically realistic models of neuron within the NEURON simulation environment, we can investigate this complexity in an orderly and hierarchical fashion. This modeling effort allows for the investigation of key phenomena that are otherwise difficult to explore through mainstream reductionist modeling approaches. The main technical drawback of this kind of approach is the computational overhead involved. The modeling paradigm itself is complex in its nature due to (i) the level of parametric details and (ii) the time scale of processing of some elementary models, which in some cases takes place within tenths of microseconds, thereby slowing down the entire system.

The astrocyte model presented here is not a complete model, and astrocytes are known for their role in influencing synaptic transmission beyond glutamate uptake and clearance. This model needs to be expanded to incorporate other important features, such as direct neurotransmitter release from astrocytes and signaling to neurons. All synapses are wrapped differently by astrocyte processes, covering smaller or larger areas with different levels of transporter expression.

We have assumed for simplicity a constant wrapping for each individual synapses, which may not be necessarily true *in vivo*. Parametric models, where the model behavior is explained by a set of parameters are in general limited by the scope of available experimental evidence. However, with a parametric modeling paradigm, we can test the reliability and sensitivity of these parameters. The observations described through our simulation studies are still preliminary and the modeling architecture established here will enable us to further investigate the effects caused by changing the amount of ensheathment around the synapses, as the density of EAATs in both glia and neurons appears to play an influential role in shaping synaptic glutamate concentration profile and its functional consequences. Including other details of a tortuous path for glutamate and including extrasynaptic NMDARs, may also affect synaptic responses. Future modeling efforts will be directed toward investigating the hierarchical effects of astrocytes on sub-populations of neurons and synapses contacted by astrocyte processes, by incorporating geometry-related considerations. Here, we demonstrated the hierarchical link between synaptic currents to spike generation while also taking into account astrocytic glutamate uptake effects on molecular elements. This is a novel approach and one of the few times such a link has been shown. These results may have significant implications for understanding glial cell effects on nerve cell membrane potential and thus, nerve cell spiking, i.e., neuronal information flow. These results also are important because they strongly suggest that glial cell uptake of synaptic glutamate during neuron-to-neuron synaptic transmission should influence spike-dependent processes that are relevant to secondary messenger pathways and other long-term effects. This comprehensive framework will allow investigating complex mechanisms within large neuron/glia networks, including neurodegenerative diseases and their underlying processes. We can identify the most sensitive parameters in neuron glial interactions and develop different testing paradigms to understand the molecular basis of diseases associated with astrocytic dysfunction.

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APPENDIX

EONS (Elementary Objects of the Nervous System) synaptic modeling platform is available online at http://synapticmodeling.com

The user guide is described here: http://www.synapticmodeling.com/eons_v1_user_guide.html

This software is implemented using JAVA. A library of elementary models is available within this platform.

Each elementary model within this is developed using Cell Designer™ platform. After testing with several pulse protocols, we integrated these into the EONS software, where elementary models interact with each other, i.e., one model's output represents an input to another model.

The elementary model represents the receptor behavior in various intermediate states such as resting to inactive to open states. The time-dependent evolution of the states are calculated using ODEs and the number of equations depends on the number of states modeled.

$$\dot{X} = M.X$$

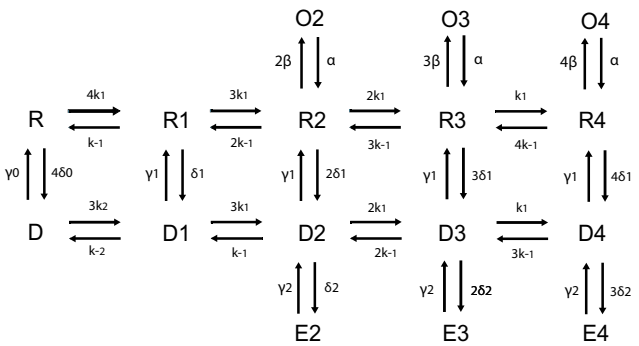


FIGURE A1 | Schematic of 16 states AMPA receptor model adapted from Robert and Howe (2003) with kinetic rate constants provided in the table below.

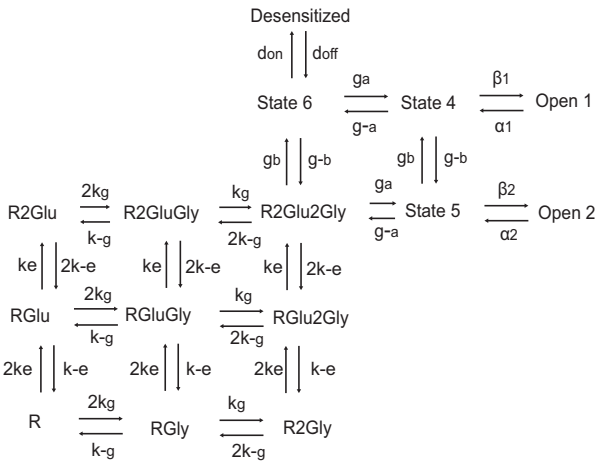


FIGURE A2 | Schematic of 15 states NMDA receptor model with kinetic rate constants adapted from Schorge et al. (2005) provided in the table below.

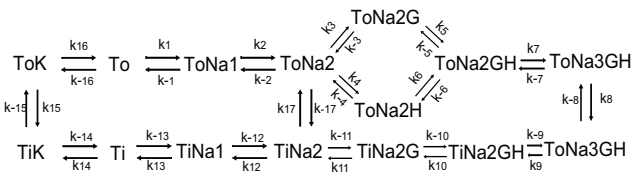


FIGURE A3 | Schematic of glutamate transporter receptor model with kinetic rate constants for glial glutamate transporter adapted from Bergles et al. (2002) and provided in the table below.

Model	Parameters	Descriptions	Values
Glutamate diffusion: $\mathrm{Glu}(r, t, Q, D, \delta) = \frac{Q}{4\pi\delta Dt} e^{\frac{-r^2}{4Dt}}$	Q	Number of glutamate molecules	3,000
	δ	Cleft height	20 nm
	D	Glutamate diffusion coefficient	0.4 μm^2 ms^{-1}
	R	Distance between the release site and the receptors or transporters	0–400 nm
	t	Time	ms
Glutamate uptake rate per transporter: $\frac{d\mathrm{Gluo}}{dt} = k_6 \times \mathrm{Na2ToH} \times \mathrm{Glu} - k_{-6} \times \mathrm{Na2ToGH} + k_3 \times \mathrm{Glu} \times \mathrm{Na2To} - k_{-3} \times \mathrm{Na2ToG}$	k_6, k_3	Forward rate constants	6 mM^{-1} ms^{-1}
	k_{-6}, k_{-3}	Backward rate constants	0.5 ms^{-1}
Glutamate at receptor after uptake: $\mathrm{Glu} - \int d\mathrm{Gluo}$	N2ToH, N2ToGH, N2To, N2ToG	Intermediate states of the glutamate transporter	Dynamically change during simulation
		Number of glial glutamate transporters	325
		Number of neuronal glutamate transporters	42
AMPAR-mediated current: $I_{\mathrm{AMPA}} = \mathrm{nb}_{\mathrm{AMPA}} \times (g_2 O_2 + g_3 O_3 + g_4 O_4) \times (V - V_{\mathrm{rev}})$ Robert and Howe (2003)	$\mathrm{nb}_{\mathrm{AMPA}}$	Number of AMPA receptors	80
	g_2, g_3, g_4	Unitary channel conductances associated with open states O_2, O_3, O_4 updated during simulation	9,15, 21 pS respectively
	V	Membrane potential	Dynamically changes during simulation
	V_{rev}	Reversal potential	0 mV
NMDAR-mediated current: $I_{\mathrm{NMDA}} = \mathrm{nb}_{\mathrm{NMDA}} \frac{Io}{1+\left(\frac{Mg_0^{2+}}{k_0}\right) e^{-\delta z F \Psi m / RT}}$ $I_o = g(V - V_{\mathrm{rev}}) O(t)$ $g = g_1 + \frac{g_2 - g_1}{1+e^{\alpha \Psi m}}$	nb_{NMDA}	Number of NMDA receptors	20
	IO	current associated with the open conducting state O	Dynamically changes during simulation
	V	Membrane potential	Dynamically changes during simulation
	V_{rev}	Reversal potential	0.7 mV
	Mg^{2+}	External Mg concentration	1 mM
	α	is the steepness of the voltage-dependent transition from $g1$ to $g2$	0.01
	R	molar gas constant	8.31434 $J\,mol^{-1}\,K^{-1}$
	F	Faraday constant	9.64867.104 $C\,mol^{-1}$
	T	absolute temperature	273.15° K
Synaptic current: $I_{\mathrm{syn}} = I_{\mathrm{AMPA}} + I_{\mathrm{NMDA}}$	I_{AMPA}	Current through AMPARs	Dynamically changes during simulation
	I_{NMDA}	Current through NMDARs	Dynamically changes during simulation
Neuron model please, see Jarsky et al. (2005)	Simulation archive available from http://dendrites.esam.northwestern.edu/		

ADDITIONAL RATE CONSTANTS

AMPAR model

Parameter	Values
k_1	$10\ mM^{-1}\ ms^{-1}$
k^{-1}	$7\ ms^{-1}$
k_2	$10\ mM^{-1}\ ms^{-1}$
k^{-2}	$4.1e^{-4}\ ms^{-1}$
γ_o	$0.001\ ms^{-1}$
δ_o	$3.3e^{-6}\ ms^{-1}$
γ_1	$0.42\ ms^{-1}$
δ_1	$0.017\ ms^{-1}$
γ_2	$0.2\ ms^{-1}$
δ_2	$0.035\ ms^{-1}$
β	$0.55\ ms^{-1}$
α	$0.3\ ms^{-1}$

NMDAR model

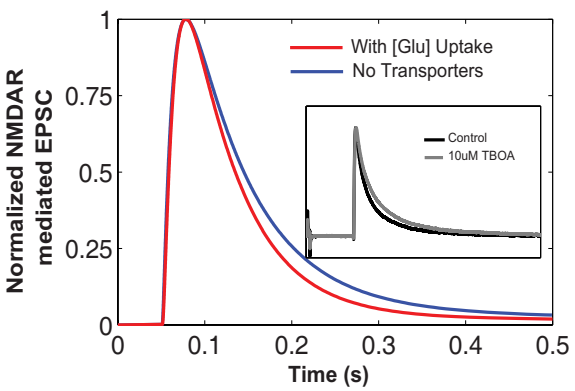
Parameter	Values
k_e	$8.3\ Mm^{-1}\ ms^{-1}$
k_{-e}	$0.0263\ ms^{-1}$
k_g	$10\ mM^{-1}\ ms^{-1}$
k_{-g}	$0.0291\ ms^{-1}$
g_b	$0.0671\ ms^{-1}$
g_{-b}	$0.15\ ms^{-1}$
g_a	$2.03\ ms^{-1}$
g_{-a}	$22.8\ ms^{-1}$
β_1	$35.2\ ms^{-1}$
α_1	$0.728\ ms^{-1}$
β_2	$0.787\ ms^{-1}$
α_2	$11.2\ ms^{-1}$
d_{on}	$0.03\ ms^{-1}$
d_{off}	$9.5e^{-4}\ ms^{-1}$

Glial transporters

Parameter	Values
$k_1; k_{-1}$	$0.01\ mM^{-1}\ ms^{-1}; 0.1\ ms^{-1}$
$k_2; k_{-2}$	$0.01\ mM^{-1}\ ms^{-1}; 0.5\ ms^{-1}$
$k_3; k_{-3}$	$6\ mM^{-1}\ ms^{-1}; 0.5\ ms^{-1}$
$k_4; k_{-4}$	$60{,}000\ mM^{-1}\ ms^{-1}; 0.7\ ms^{-1}$
$k_5; k_{-5}$	$60{,}000\ mM^{-1}\ ms^{-1}; 0.7\ ms^{-1}$
$k_6; k_{-6}$	$6\ mM^{-1}\ ms^{-1}; 0.5\ ms^{-1}$
$k_7; k_{-7}$	$0.01\ mM^{-1}\ ms^{-1}; 1\ ms^{-1}$
$k_8; k_{-8}$	$2\ ms^{-1}; 1.9\ ms^{-1}$
$k_9; k_{-9}$	$1\ ms^{-1}; 0.04\ mM^{-1}\ ms^{-1}$
$k_{10}; k_{-10}$	$3\ ms^{-1}; 90{,}000\ mM^{-1}\ ms^{-1}$
$k_{11}; k_{-11}$	$3\ ms^{-1}; 0.1\ mM^{-1}\ ms^{-1}$
$k_{12}; k_{-12}$	$100\ ms^{-1}; 20\ mM^{-1}\ ms^{-1}$
$k_{13}; k_{-13}$	$100\ ms^{-1}; 100\ mM^{-1}\ ms^{-1}$
$k_{14}; k_{-14}$	$1\ mM^{-1}; 1\ ms^{-1}$
$k_{15}; k_{-15}$	$0.04\ ms^{-1}; 0.01\ ms^{-1}$
$k_{16}; k_{-16}$	$20\ ms^{-1}; 1\ mM^{-1}$
$k_{17}; k_{-17}$	$0.0014\ ms^{-1}; 1.0e^{-5}\ ms^{-1}$

Neuronal transporters

Parameter	Values
$k_1; k_{-1}$	$0.01\ mM^{-1}\ ms^{-1}; 2.5\ ms^{-1}$
$k_2; k_{-2}$	$0.01\ mM^{-1}\ ms^{-1}; 2.5\ ms^{-1}$
$k_3; k_{-3}$	$6.8\ mM^{-1}\ ms^{-1}; 0.3\ ms^{-1}$
$k_4; k_{-4}$	$60{,}000\ mM^{-1}\ ms^{-1}; 0.7\ ms^{-1}$
$k_5; k_{-5}$	$60{,}000\ mM^{-1}\ ms^{-1}; 0.7\ ms^{-1}$
$k_6; k_{-6}$	$6\ mM^{-1}\ ms^{-1}; 0.5\ ms^{-1}$
$k_7; k_{-7}$	$0.01\ mM^{-1}\ ms^{-1}; 1\ ms^{-1}$
$k_8; k_{-8}$	$0.5\ ms^{-1}; 0.55\ ms^{-1}$
$k_9; k_{-9}$	$0.8\ ms^{-1}; 0.04\ mM^{-1}\ ms^{-1}$
$k_{10}; k_{-10}$	$6\ ms^{-1}; 90{,}000\ mM^{-1}\ ms^{-1}$
$k_{11}; k_{-11}$	$3\ ms^{-1}; 1\ Mm^{-1}\ ms^{-1}$
$k_{12}; k_{-12}$	$0.5\ ms^{-1}; 2\ mM^{-1}\ ms^{-1}$
$k_{13}; k_{-13}$	$4\ ms^{-1}; 1\ mM^{-1}\ ms^{-1}$
$k_{14}; k_{-14}$	$0.1\ mM^{-1}\ ms^{-1}; 10\ ms^{-1}$
$k_{15}; k_{-15}$	$0.05\ ms^{-1}; 0.005\ ms^{-1}$
$k_{16}; k_{-16}$	$0.8\ ms^{-1}; 0.01\ mM^{-1}\ ms^{-1}$
$k_{17}; k_{-17}$	$0.008\ ms^{-1}; 1.0\ e^{-5}\ ms^{-1}$



Comparison between results showing NMDAR-mediated EPSCs simulated in EONS synaptic modeling platform. The blue trace represents the normalized EPSC waveform when there are no transporters. In the presence of glutamate transporters and due to glutamate uptake, we observed the decay phase shift inward indicating a faster decay time (red trace). This is qualitatively compared to the experimental findings (shown in inset) from Diamond (2005). The normalized NMDAR-mediated EPSCs shown in the inset, control condition (black trace), and with the addition of TBOA which blocks the transporter's uptake activity. This is shown in gray trace. Note that with transporters and increased glutamate uptake, the decay phase of the time course decreases.

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The computational power of astrocyte mediated synaptic plasticity

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Research in the last two decades has made clear that astrocytes play a crucial role in the brain beyond their functions in energy metabolism and homeostasis. Many studies have shown that astrocytes can dynamically modulate neuronal excitability and synaptic plasticity, and might participate in higher brain functions like learning and memory. With the plethora of astrocyte mediated signaling processes described in the literature today, the current challenge is to identify, which of these processes happen under what physiological condition, and how this shapes information processing and, ultimately, behavior. To answer these questions will require a combination of advanced physiological, genetical, and behavioral experiments. Additionally, mathematical modeling will prove crucial for testing predictions on the possible functions of astrocytes in neuronal networks, and to generate novel ideas as to how astrocytes can contribute to the complexity of the brain. Here, we aim to provide an outline of how astrocytes can interact with neurons. We do this by reviewing recent experimental literature on astrocyte-neuron interactions, discussing the dynamic effects of astrocytes on neuronal excitability and short- and long-term synaptic plasticity. Finally, we will outline the potential computational functions that astrocyte-neuron interactions can serve in the brain. We will discuss how astrocytes could govern metaplasticity in the brain, how they might organize the clustering of synaptic inputs, and how they could function as memory elements for neuronal activity. We conclude that astrocytes can enhance the computational power of neuronal networks in previously unexpected ways.

Keywords: astrocytes, synaptic plasticity, spike-timing-dependent plasticity, STDP, metaplasticity, heterosynaptic plasticity, computation, calcium

Astrocytes represent the largest cellular population in the human brain. It has even been suggested that the increased complexity of human astrocytes strongly adds to the computational power of the human brain (Oberheim et al., 2006). However, the precise function of astrocytes in brain signaling is only partially understood. Astrocytes play a crucial role in brain homeostasis (for review see Simard and Nedergaard, 2004). For example, they take up and redistribute excess extracellular potassium (K^+) that is released upon high neuronal activity (Chever et al., 2010; Bay and Butt, 2012), and they maintain the brain water balance by taking up or releasing water through water channels (Haj-Yasein et al., 2012). However, in addition to these vital homeostatic functions, recent studies have shown that astrocytes actually engage in active bidirectional interactions with neurons. Research in rodents in the last two decades has highlighted numerous ways in which astrocytes can interact with neurons. In this review, we aim to give an overview of experimental studies on astrocyte-neuron interactions. We focus on the role that astrocytes play in different forms of synaptic plasticity in the healthy brain.

HOW ARE ASTROCYTES ACTIVATED?

Astrocytes are strategically positioned to sense ongoing brain activity, i.e., a single astrocyte tightly ensheathes tens of thousands of synapses, hundreds of axons and dendrites and several neuronal somata (Bushong et al., 2002; Halassa et al., 2007). The fine processes of astrocytes make tight associations with pre- and post synaptic neuronal elements, thereby forming the so-called tripartite synapse (Araque et al., 1999). Because astrocytes are electrically passive, their signaling is fundamentally different from neuronal signaling. Instead of integrating membrane depolarization and hyperpolarization into action potential output, like neurons do, astrocytes sense and integrate information mainly through the generation of intracellular calcium (Ca^{2+}) signals (**Figure 1**). It is now well-established that astrocytes are able to sense transmitters released by neurons and other glial cells (either astrocytes or microglia), displaying a wide variety of intracellular responses ultimately leading to a physiological feed-back. Indeed, receptors for all major neurotransmitters seem to be present on the astrocytic plasma membrane *in situ* (Volterra and Meldolesi, 2005), and they can be activated during brain activity *in vivo* (Wang et al., 2006). Activation of these receptors leads to an intracellular Ca^{2+} increase and subsequent release of transmitters from the glial cell (called gliotransmitters), a feedback that is believed to be important for several forms of synaptic plasticity and memory storage (Perea et al., 2009).

The main classes of transmitter receptors that can be found on astrocytic processes are G-protein coupled receptors (GPCRs). Their activation leads to the intracellular activation

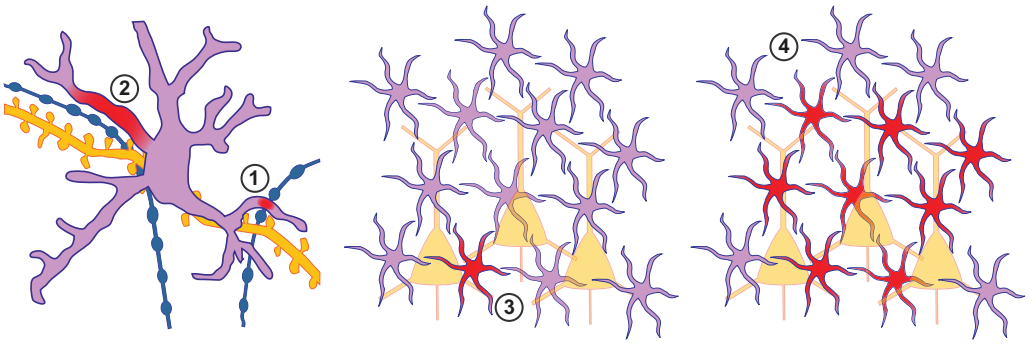

FIGURE 1 | Spatial properties of astrocytic Ca^{2+} signals. Left: close-up view of a single astrocyte (pink) together with presynaptic (blue) and postsynaptic (yellow) neuronal elements. (1) Local Ca^{2+} signals (spread ~4 μm) can occur upon activity at a putative single neighboring synapse. (2) More robust regional events (spread ~12 μm) occur presumably when an astrocyte process senses release at several neighboring sites simultaneously. **Middle, Right:** zoomed out view of a network of astrocytes (pink) and neurons (yellow). Upon stronger stimulation an astrocyte Ca^{2+} signal can be generalized to the entire astrocyte (3), or spread through a network of neighboring astrocytes (4).

of phospholipase C (PLC), followed by the production of the messenger molecule inositol trisphosphate (IP_3) which subsequently induces release of Ca^{2+} from the astrocyte endoplasmic reticulum (ER). Thanks to those receptors astrocytes are not only capable to respond to the classical neurotransmitters glutamate and γ-aminobutyric acid (GABA) released from synapses [through metabotropic glutamate receptors (mGluRs) mGluR1-5 and $GABA_B$ receptors respectively; Pasti et al., 1997; Kang et al., 1998] but also to neuromodulators like acetylcholine (Takata et al., 2011; Navarrete et al., 2012) and dopamine (Khan et al., 2001). Moreover, purines like adenosine-5′-triphosphate (ATP) and adenosine (co-)released from synapses or other astrocytes can be sensed by astrocytes (Jourdain et al., 2007; Santello et al., 2011) and play a role in the propagation/amplification of Ca^{2+} signals through the astrocytic syncytium (Poskanzer and Yuste, 2011) or serve as a signal for microglial reaction (Haynes et al., 2006). In addition to sensing axonal release of transmitters, astrocytes have been shown to respond to so-called retrograde signals: transmitters of postsynaptic or dendritic origin (Bernardinelli et al., 2011). For example, strong evidence exists for astrocytes being stimulated by endocannabinoids acting on astrocytic cannabinoid CB_1 receptors (CB_1Rs; Navarrete and Araque, 2008, 2010; Min and Nevian, 2012).

In addition to GPCRs, other pathways have been shown to lead to astrocyte Ca^{2+} signals. Astrocytes can possess ligand-gated ion channels whose activation can lead to direct Ca^{2+} influx. For instance, specialized cerebellar astrocytes called Bergmann glia possess Ca^{2+} permeable AMPA type glutamate receptors (AMPARs; Burnashev et al., 1992) which sense ectopic release of glutamate from glutamatergic fibers (Matsui et al., 2005). Other channels that have been shown to mediate astrocyte signals are purinergic P2X channels (Lalo et al., 2008) and transient receptor potential (TRP) channels (TRPC) (Malarkey et al., 2008), and TRPA (Shigetomi et al., 2011). Finally, GABA and glutamate transporter activity linked to Na^+/Ca^{2+} exchangers (Rojas et al., 2007; Doengi et al., 2009) as well as connexin hemichannel opening (Torres et al., 2012) have been shown to play a role in astrocytic Ca^{2+} signaling.

It should be noted that Ca^{2+} signaling is not the only form of "excitability" displayed by astrocytes. For example, astrocytes also show dynamic sodium (Na^+) transients mediated by channels, exchangers, and transporters (for recent review see Kirischuk et al., 2012). An important source of activity dependent Na^+ influx into astrocytes is the Na^+ that is cotransported by the astrocytic glutamate transporters when astrocytes clear away glutamate from the extracellular space (Chatton et al., 2000).

THE SPATIOTEMPORAL CHARACTERISTICS OF ASTROCYTIC Ca^{2+} SIGNALS

When stimulated with specific metabotropic receptor agonists, astrocytes display prominent and extremely slow (up to 10 s of seconds) whole-cell Ca^{2+} responses. This is also true for *in vivo* experiments, where sensory stimulation reliably induces astroglial slow Ca^{2+} transients (Wang et al., 2006) sometimes related to vascular responses (Petzold et al., 2008). The recorded Ca^{2+} signal can remain restricted to a single or few astrocytes responding to specific sensory stimuli (Wang et al., 2006; Schummers et al., 2008). Additionally, since astrocytes form complex networks through gap-junctional coupling with neighboring astrocytes (for review see Giaume, 2010; Giaume et al., 2010) Ca^{2+} signals can spread like a wave through the astrocyte network (Nimmerjahn et al., 2009; Kuga et al., 2011). Although the mechanisms underlying the propagation of such Ca^{2+} waves are not fully understood, transport of either IP_3 or Ca^{2+} itself through gap-junctions may play an important role (Venance et al., 1997). Furthermore, regenerative activity through astrocytic release of signaling molecules like ATP, which in turn activate Ca^{2+} signals in neighboring astrocytes, can be involved in Ca^{2+} wave propagation (Guthrie et al., 1999).

The temporal characteristics of astrocytic Ca^{2+} transients have led to the idea that unlike neurons, astrocytes display exclusively particularly slow responses, and that their signals are not suited to be restricted to small cellular compartments, as happens for example, in dendritic spines. However, *in vivo* experiments have shown that faster local responses of astrocytes in the somatosensory cortex can occur upon hind limb stimulation (time scale

of hundreds of ms; Winship et al., 2007). Recently, localized Ca^{2+} activity has been thoroughly studied *in vitro*. Two parallel studies have indeed identified small and relatively fast Ca^{2+} signals that are restricted to the astrocyte process (Di Castro et al., 2011; Panatier et al., 2011). Two main classes of local calcium events have been identified: focal highly confined transients (about 4 μm) and more robust regional events (about 12 μm; **Figure 1**; Di Castro et al., 2011). The more local events have been proposed to be generated by spontaneous single vesicle release at individual synapses whereas the expanded events seem to be generated by single action potentials activating several neighboring synapses in the astrocyte domain. Remarkably, only the latter have been proposed to have an influence on synaptic transmission, giving, for the first time, some clues to decipher the language the astrocyte employs to communicate with synapses (Di Castro et al., 2011; Panatier et al., 2011).

Another recent study has described an additional form of localized Ca^{2+} events in hippocampal astrocytes. These so-called spotty Ca^{2+} signals spread with a half width of about 5 μm, and last for several seconds (Shigetomi et al., 2011). Unlike most other described astrocyte Ca^{2+} signals, spotty Ca^{2+} signals are mediated by spontaneous openings of a Ca^{2+} permeable channel on the astrocyte membrane, the transient receptor potential A1 (TRPA1) channel. TRPA1 mediated spotty Ca^{2+} signals seem to play an important role in setting the basal intracellular Ca^{2+} concentration of hippocampal astrocytes (Shigetomi et al., 2011).

Because astrocytic Ca^{2+} signals can be very localized, this raises the question whether the distribution of receptors over the astrocyte membrane is homogeneous, or whether astrocytes show hot-spots for certain receptors. This question remains largely unanswered so far, but some interesting results have recently been obtained. For example, hippocampal astrocytes can be activated by agonists of both PAR-1 as well as $P2Y_1$ receptors. Both agonists induce somatic Ca^{2+} transients in the astrocyte which look similar, albeit with a different time-course. But surprisingly only astrocytic PAR-1 receptor activation, and not $P2Y_1$ receptor activation, leads to astrocyte mediated slow inward currents through NMDA receptors in neighboring neurons (Shigetomi et al., 2008). This suggests that the coupling of these two receptors to intracellular signaling cascades is different, and argues for subcellular differentiation of receptors in astrocytes. Furthermore, a recent study on the distribution of mGluRs on the astrocyte membrane shows that mGluRs show an increased density on astrocyte processes as compared to the soma (Arizono et al., 2012), while another recent study used immunocytochemistry and glutamate uncaging to show that metabotropic glutamate receptors cluster together at discrete locations along astrocytic processes (Panatier et al., 2011).

As mentioned earlier, astrocytic Ca^{2+} elevations can be extended and generalized (involving one entire astrocyte or spreading through several of them; **Figure 1**). In this way, activity in one astrocyte could spread through a network of neighboring astrocytes. However, either trains of sustained stimulation of synaptic activity (Grosche et al., 1999; Matyash et al., 2001) or a large number of activated fibers (Honsek et al., 2012) are necessary to induce this type of astrocytic Ca^{2+} activity. *In vivo* it has been suggested that astrocytes can synchronize their activity in clusters of 2–5 astrocytes (Hirase et al., 2004; Sasaki et al., 2011a) or spread through a network consisting of dozens to hundreds of astrocytes (Hoogland et al., 2009; Nimmerjahn et al., 2009; Kuga et al., 2011).

Understanding Ca^{2+} signaling in astrocytes has very much been aided by the development of mathematical models. Such models offer a good description of intrinsic and neurotransmitter evoked Ca^{2+} dynamics in astrocytes (De Pittà et al., 2009), as well as the contribution of both gap-junctions (Venance et al., 1997; Kazantsev, 2009; Goldberg et al., 2010; Matrosov and Kazantsev, 2011) and intercellular ATP signaling (Macdonald et al., 2008) to the spread of Ca^{2+} waves through the astrocyte network. However, the experimental data upon which many of these models are based often stems from studies on cultured astrocytes, which have a very simplified morphology compared to the *in vivo* situation. Furthermore, the majority of Ca^{2+} imaging studies performed to date have focused on Ca^{2+} dynamics in the astrocyte soma. As described in the previous paragraphs, high-resolution Ca^{2+} imaging data obtained recently has shown the complicated subcellular interplay between synaptic activity and localized astrocytic Ca^{2+} signals (Di Castro et al., 2011; Panatier et al., 2011). A challenge for the future will be to use a combination of high-resolution imaging and mathematical modeling to understand how such localized signals arise, and how they relate to the more global Ca^{2+} signals occurring in astrocyte networks.

HOW DO ASTROCYTES SIGNAL BACK TO NEURONS?

As discussed in the previous section, astrocytes can sense a wide variety of neurotransmitters and signaling molecules, and respond with increased Ca^{2+} signaling. But how do astrocytes signal back to neurons? Broadly speaking, astrocytes can do this through three separate mechanisms. Firstly, because astrocytes are crucial for ion homeostasis, they can influence neurons by dynamically altering the ionic balance. Secondly, astrocytes can alter neuronal functioning by modulating the uptake of neurotransmitter molecules from the extracellular space (Theodosis et al., 2008). Thirdly, astrocytes can release transmitters themselves (Araque et al., 2001). We will briefly describe these three mechanisms.

As mentioned earlier, astrocytes are crucial for the passive homeostatic regulation of extracellular ions like potassium. This astrocytic function is essential for brain homeostasis. Furthermore, if astrocytes would actively modulate the uptake of extracellular ions this would endow them with a powerful way to modulate neuronal excitability. A recent study has shown that hippocampal astrocytes indeed actively control the extracellular ion concentration (Wang et al., 2012a). In this study, it was found that G-protein mediated increases in astrocyte Ca^{2+} lead to an increase in uptake of K^+ into astrocytes, which in turn leads to a slight neuronal hyperpolarization (Wang et al., 2012a). Similar results have been obtained in cerebellum (Wang et al., 2012b), suggesting that active regulation of the extracellular K^+ concentration is a general mechanism by which astrocytes signal to neurons.

Another important function of astrocytes is the uptake of released neurotransmitters from the extracellular space. Through uptake, astrocytes limit the spread of neurotransmitters from the

release site, thereby determining the spatiotemporal spread of transmitters (Tzingounis and Wadiche, 2007). Although it has been suggested that under control conditions astrocytes can efficiently clear synaptically released transmitters even upon high frequency stimulation (Diamond and Jahr, 2000), modulation of this uptake could results in changes in neuronal excitability and synaptic transmission. The efficacy of neurotransmitter clearance by astrocytes can be modulated either if astrocytes modulate their structural relationship with neurons, or if astrocytes change the amount or efficacy of their neurotransmitter transporters. Both mechanisms have been shown to occur under physiological conditions. An extreme example of a structural change occurs in the hypothalamus, where astrocytes dramatically retract their processes during lactation, parturition or chronic dehydration (for review see Theodosis et al., 2008). This retraction leads to an increase in glutamate spillover, and enables synaptically released glutamate to activate presynaptic mGluRs either on the same terminal (Oliet et al., 2001) or at neighboring synapses (Piet et al., 2004). Although the hypothalamus provides an extreme example of astrocytic reorganization, similar changes might occur in a more subtle way in other brain regions. For example, similar, albeit less dramatic, reorganization of astrocytic coverage has been shown to occur in the barrel cortex. Here, prolonged sensory activation leads to both a 2-fold increase in glial glutamate transporter levels, as well as an increased coverage of dendritic spines by astrocytic processes (Genoud et al., 2006). Through this mechanism active synapses would be equipped with a more efficient means of preventing crosstalk.

Thirdly, astrocytes can modulate neurons by releasing transmitters themselves. These so-called gliotransmitters are very diverse, including conventional transmitters like GABA and glutamate, as well as signaling molecules like purines, D-serine, taurine, cytokines, peptides, and metabolites like lactate (Volterra and Meldolesi, 2005). Astrocytes can release transmitters through two mechanisms. Firstly, they can release transmitter containing vesicles through SNARE mediated exocytosis. Astrocytes contain the necessary proteins for SNARE mediated exocytosis (Araque et al., 2000; Bezzi et al., 2004; Parpura and Zorec, 2010; Schubert et al., 2011), and genetic or pharmacological interference with proteins of the SNARE-complex in astrocytes inhibits numerous forms of astrocyte-neuron signaling (Pascual et al., 2005; Jourdain et al., 2007; Halassa et al., 2009; Henneberger et al., 2010; Min and Nevian, 2012). Secondly, transmitter can be released through reverse transport (Héja et al., 2009), or through membrane channels (Kozlov et al., 2006; Lee et al., 2010).

In conclusion, astrocytes can influence neurons by modulating the extracellular ionic composition, by changing their structural properties, or by releasing signaling molecules (gliotransmitters). But what is the impact of this on neuronal functioning? Numerous studies in different brain regions have uncovered a multitude of ways in which astrocytes can modulate neuronal excitability and synaptic transmission. In the following section we will review several of these different pathways.

ASTROCYTE MODULATION OF NETWORK EXCITABILITY

The excitability of neurons is one of their most fundamental properties. Dynamic modulation of excitability is a powerful way of implementing state-dependent changes in neuronal computation. Several studies have shown that astrocytes can regulate neuronal excitability. Astrocytes can achieve this through several mechanisms: by regulation of the extracellular ionic composition, by maintaining a tonic extracellular transmitter concentration, by regulation of basal synaptic transmission, and by the induction of phasic events in neighboring neurons. We will shortly discuss these different mechanisms.

As described in the previous section, astrocytes can dynamically control the concentration of extracellular K^+. Both hippocampal astrocytes (Wang et al., 2012a) as well as cerebellar Bermann glia translate an astrocytic Ca^{2+} signal into a transient decrease in the extracellular K^+ concentration (Wang et al., 2012b). This decrease in extracellular K^+ concentration leads to a transient hyperpolarization of adjacent neurons, thereby changing neuronal excitability. In hippocampus, this change in excitability is translated into a decrease in frequency and an increase in fidelity of excitatory synaptic transmission (Wang et al., 2012a). In cerebellum, paradoxically, the decrease in excitability is translated into an increase in the duration of Purkinje neuron upstates (Wang et al., 2012b). Therefore, astrocytes can dynamically modulate excitability through active modulation of extracellular ions.

Because astrocytes are responsible for the clearance of released neurotransmitters, they control the accumulation of these transmitters in the extracellular space. Therefore, astrocytes can regulate tonic neurotransmitter receptor mediated currents. For example, interfering with astrocyte uptake of GABA in the paraventricular nucleus leads to an increased tonic GABA(A) receptor mediated current, and a decrease in neuronal excitability (Park et al., 2009). Conversely, interfering with astrocytic glutamate uptake leads to increased excitability (Jabaudon et al., 1999; Pannasch et al., 2011). Interestingly, astrocytes can also regulate tonic transmitter concentration by releasing transmitter themselves. It was recently shown that tonic GABA mediated inhibition in the cerebellum is completely mediated by Bestrophin channel mediated GABA release from Bergman glia, and that interfering with these channels abolishes tonic inhibition (Lee et al., 2010). Furthermore, tonic GABA mediated inhibition in the hippocampus has been suggested to be mediated by reverse transport of GABA from astrocytes, and this mechanism was hypothesized to be boosted by astrocytic glutamate uptake (Héja et al., 2009). In this way, an increase in tonic inhibition would counterbalance increased excitatory neurotransmission. Therefore, astrocytes are in a prime position to control tonic inhibition and excitation of neurons, although it remains unclear whether this process is dynamically modulated.

In addition to controlling tonic currents, astrocytes are crucial for maintaining basal synaptic transmission. The astrocyte network provides energy to neurons in the form of lactate, and this energy supply is necessary to keep synaptic transmission intact (Rouach et al., 2008). Interestingly, the supply of energy is activity dependent, with lactate or its precursor glucose being able to travel through gap-junctions between astrocytes to reach the location where it is required (Rouach et al., 2008). In addition, astrocytes also set the level of basal synaptic strength. Two recent studies have shown that astrocytes are responsible for

maintaining the basal release probability of excitatory synapses in the hippocampus at an elevated level (Di Castro et al., 2011; Panatier et al., 2011). In these studies, it was found that interfering with basal astrocyte signaling caused a reduction in synaptic efficacy in neighboring neurons in either the CA1 region (Panatier et al., 2011) or the dentate gyrus (Di Castro et al., 2011). The mechanism by which this regulation of basal transmission comes about seems to differ between the two brain regions. In CA1, astrocytes sense basal synaptic activity through mGluR5 activation, and maintain basal activity through activation of presynaptic adenosine A_{2A} receptors, which facilitate synaptic release (Panatier et al., 2011). In the dentate gyrus, astrocytes presumably sense synaptic corelease of ATP through P2Y1 receptors, and increase release through activation of presynaptic NMDA type glutamate receptors (NMDARs; Di Castro et al., 2011). However, it should be mentioned that two other studies have shown that selective stimulation of astrocytes through transgenic expression and activation of an exogenous receptor (Fiacco et al., 2007) or by genetically interfering with astrocyte Ca^{2+} signaling (Petravicz et al., 2008) has no effect on basal synaptic transmission in the CA1 region, thereby calling into question the importance of astrocytes for regulating basal synaptic transmission.

As a final mechanism by which astrocytes can regulate the excitable state of the network, astrocytes can physically release transmitters, leading to depolarization or hyperpolarization of neighboring neurons. Astrocytic glutamate release in the hippocampus can activate postsynaptic NMDARs, giving rise to so-called slow inward currents (SICs; Angulo et al., 2004; Fellin et al., 2004; Perea and Araque, 2005). SICs are NMDAR mediated slow events, typically lasting several hundreds of milliseconds. It is thought that one astrocytic glutamate release event can induce SICs simultaneously in several neighboring pyramidal neurons, which has led to the assumption that they play a role in the local synchronization of neurons (Angulo et al., 2004; Fellin et al., 2004). SICs have also been described in other brain regions, including the thalamus (Parri et al., 2001), spinal cord (Bardoni et al., 2010), and nucleus accumbens (D'Ascenzo et al., 2007). Interestingly, it was recently shown that, while activation of single astrocytes gives rise to SICs in neighboring neurons, simultaneous activation of a cluster (3–5) of astrocytes gives rise to a slower (>3 s) depolarization of neighboring neurons by 1–2 mV (Sasaki et al., 2011a). In contrast to SICs, this depolarization is mediated by non-NMDA glutamate receptors.

Analogous to the NMDAR mediated SICs that occur upon astrocytic glutamate release, astrocytic release of GABA can induce so-called slow outward currents (SOCs; Kozlov et al., 2006). These events have only been described in the olfactory bulb (Kozlov et al., 2006) and in the thalamus (Jiménez-González et al., 2011). Like SICs, SOCs occur simultaneously in neighboring neurons, so they might also play a role in neuronal synchronization. Interestingly, the source of GABA responsible for SOCs seems to be GABA flowing through a volume regulated anion channel, rather than vesicular GABA release (Kozlov et al., 2006).

Evidence for a role of astrocytically released neurotransmitters in the synchronization of neurons comes from experiments on slow cortical oscillations, which are formed by neuronal up- and down- states. Interfering with astrocyte signaling reduces the power of slow oscillations *in vivo* (Fellin et al., 2009), presumably through modulation of NMDARs and adenosine A1 receptors. Furthermore, *in vitro* experiments have shown that astrocytes control the frequency of cortical up-states (Poskanzer and Yuste, 2011).

In conclusion, astrocytes can modulate neuronal excitability by controlling the concentration of ions and neurotransmitters in the extracellular space, by regulating basal synaptic transmission, and by synchronously activating groups of neurons.

ASTROCYTES AND SHORT-TERM SYNAPTIC PLASTICITY

In addition to modulating neuronal excitability and basal synaptic transmission, astrocytes play a role in the specific strengthening or weakening of synaptic connections, either transiently (short-term plasticity), or long-lasting (long-term plasticity). Short-term plasticity (a change in synaptic strength lasting up to 10s of seconds) is thought to underlie critical computational functions of neuronal networks (Abbott and Regehr, 2004), whereas long-term plasticity (a change in synaptic strength lasting hours or longer) is involved in development, learning and memory formation. Short-term plasticity can be induced at three different levels: the conduction of the presynaptic action potential, the presynaptic release probability and the postsynaptic receptors. Strikingly, astrocytes have the ability to interfere with synaptic transmission at all these levels. Here we will review what is known about astrocyte involvement in short-term plasticity.

Recently, it was shown that glutamate release from astrocytes can activate axonal AMPARs (Sasaki et al., 2011b), and that this causes an axonal depolarization. The axonal depolarization leads to inactivation of axonal potassium channels, which causes a local broadening of the action potential waveform and an increase in presynaptic release probability (Alle and Geiger, 2006; Kole et al., 2007; Sasaki et al., 2011b). Through this mechanism, astrocytes can cause a transient increase in release probability in a population of neighboring synapses originating from the same axon.

As mentioned before, astrocytes can modulate basal synaptic transmission through tonic activation of presynaptic receptors (Di Castro et al., 2011; Panatier et al., 2011). However, if the activation of presynaptic receptors is transient, this leads to a short-term enhancement or depression of synaptic release probability. For example, phasic release of glutamatergic vesicles from astrocyte processes activates presynaptic glutamate receptors at several synapses. At excitatory CA3 to CA1 pyramidal neuron synapses in hippocampus, astrocyte activation by uncaging of Ca^{2+} or IP_3 leads to astrocytic glutamate release, which in turn activates presynaptic mGluRs, leading to a transient increase in release probability (Fiacco and McCarthy, 2004; Perea and Araque, 2007). Importantly, this signaling cascade can also be activated by physiological astrocyte stimulation: a recent study has shown that activation of astrocytic CB_1Rs by postsynaptically synthesized endocannabinoids leads to astrocytic glutamate release followed by mGluR mediated transient potentiation (Navarrete and Araque, 2010).

Astrocytic glutamate release can not only modulate synaptic transmission through activation of metabotropic receptors. There is also evidence that astrocytic glutamate release can activate presynaptically located ionotropic receptors. For example, in the hippocampal dentate gyrus, either electrical stimulation-induced or P2Y1 receptor mediated astrocyte activation leads to Ca^{2+} mediated exocytosis of glutamate, which activates presynaptic NMDARs (Jourdain et al., 2007; Santello et al., 2011). These receptors in turn induce a transient increase in release probability, as indicated by an increase in miniature excitatory postsynaptic current (mEPSC) frequency and an increased release probability at perforant-path synapses onto dentate granule cells (GCs). Interestingly, the astrocytic capacity to release glutamate is under tight regulation of tumor necrosis factor alpha (TNFα; Santello et al., 2011). In the absence of TNFα, altered vesicle docking dramatically slows down astrocytic glutamate release, leading to ineffective presynaptic NMDAR activation.

Another presynaptic ionotropic receptor that can be activated by astrocytic release of glutamate is the kainate receptor. At inhibitory synapses onto CA1 pyramidal neurons, presynaptic kainate receptors can increase release probability (Jiang et al., 2001). Ca^{2+} uncaging in astrocytes causes glutamate release which activates these presynaptic kainate receptors (Liu et al., 2004). More physiologically, repetitive interneuron firing activates this same pathway by activating astrocytic GABA(B) receptors through spillover of synaptically released GABA (Kang et al., 1998).

As mentioned before, astrocytes also release purines as gliotransmitters. Astrocyte derived ATP and its breakdown product adenosine mediate various forms of short-term synaptic plasticity. ATP released from astrocytes is quickly degraded to adenosine, and activation of presynaptic adenosine receptors can either lead to an increase or decrease in release probability, through activation of presynaptic A_{2A} and A_1 receptors respectively. As mentioned before, astrocytic activation of presynaptic A_{2A} receptors maintains high basal release probability in the hippocampal CA1 region (Panatier et al., 2011). However, other studies have shown that astrocyte derived adenosine transiently decreases excitatory inputs to CA1 neurons through activation of presynaptic A_1 receptors (Zhang et al., 2003; Pascual et al., 2005). The accumulation of astrocyte-derived adenosine during wakefulness (Schmitt et al., 2012) gradually increases this tonic presynaptic A_1 receptor mediated suppression of synaptic transmission at these same synapses through activation of presynaptic A_1 receptors, and this process is necessary for the buildup of sleep pressure (Halassa et al., 2009). Additionally, activity dependent release of astrocyte-derived purines mediates heterosynaptic short-term depression in CA1 neurons (Zhang et al., 2003; Pascual et al., 2005; Serrano et al., 2006). However, in this respect it is important to note that a recent publication disputes the glial origin of adenosine, arguing that heterosynaptic depression is not due to astrocyte derived ATP that is metabolized to adenosine, and instead suggests that adenosine is directly released from neurons (Lovatt et al., 2012).

In conclusion, several mechanisms have been described by which astrocytes can mediate short-term potentiation or depression. Interestingly, many of these processes have been studied at the same synapse: the excitatory Schaffer collateral synapse onto CA1 neurons of the hippocampus. From this, it can be concluded that Schaffer collateral synapses can either show short-term potentiation (Fiacco and McCarthy, 2004; Perea and Araque, 2007; Navarrete and Araque, 2010) or short-term depression (Zhang et al., 2003; Pascual et al., 2005; Serrano et al., 2006) upon astrocyte activation. Furthermore, it has also been suggested that astrocyte activation has no effect at all on synaptic transmission at Schaffer collateral synapses (Agulhon et al., 2010). Because the mentioned studies all differ in the way in which astrocytic activity was induced, a key question for future research is which physiological astrocyte stimuli lead to what kind of synaptic modulation. However, it is clear that astrocytes have a broad potential for modulating short-term synaptic plasticity.

ASTROCYTES AND LONG-TERM SYNAPTIC PLASTICITY

Long-term synaptic plasticity is thought to be the cellular correlate of learning and memory (Bliss and Collingridge, 1993). Long-term plasticity can be divided into long-term potentiation (LTP), a long lasting increase in synaptic strength, and long-term depression (LTD), a long-lasting decrease in synaptic strength. LTP and LTD can be induced by a variety of protocols, including repetitive presynaptic stimulation (Bliss and Lomo, 1973; Kirkwood et al., 1993), precise timing of pre- and post-synaptic activity (spike-timing-dependent plasticity; STDP; Markram et al., 1997; Feldman, 2000; Sjöström et al., 2001) and application of pharmacological agents (Hosokawa et al., 1995; Lee et al., 1998). Furthermore, some forms of long-term plasticity can be homeostatic, meaning that the change in synaptic strength occurs at all synapses onto the neuron (Turrigiano and Nelson, 2004). Recent studies have shown that astrocytes are involved in many different forms of long-term plasticity. In the next section, we will review the literature on astrocyte involvement in long-term plasticity.

Most forms of long-term synaptic plasticity depend on activation of postsynaptic NMDARs. The NMDAR classically is described as a coincidence detector of pre- and post synaptic neuronal activity. This is because for activation it needs both binding of presynaptically released glutamate as well as a post-synaptic depolarization to relieve it from block by magnesium (Mg^{2+}) ions (Dingledine et al., 1999). Upon opening, NMDARs cause a synapse specific influx of Ca^{2+} into the postsynaptic neuron, which can activate second messenger cascades leading to either LTP or LTD (Malenka and Bear, 2004). However, a long ignored third requirement for NMDAR activation is the binding of a co-agonist to the receptor. NMDARs have a co-agonist binding site called the glycine binding site. The ion channel opens only when the glutamate binding site and the glycine binding site of the NMDAR are both occupied (Johnson and Ascher, 1987; Kleckner and Dingledine, 1988). The glycine binding site derives its name from the fact that glycine was first identified as an agonist at this site. However, recent reports have shown that D-serine is a more likely co-agonist for the NMDAR then glycine (Mothet et al., 2000). Interestingly, the source of D-serine in the brain seems to be the astrocyte. Astrocytes release D-serine into the extracellular space through vesicular fusion, and this astrocytic D-serine release is necessary to obtain sufficient NMDAR activation for induction of LTP in hippocampus (Yang et al., 2003; Henneberger et al., 2010) and prefrontal cortex (Fossat et al.,

2011). Hippocampal LTD, like LTP, also requires activation of postsynaptic NMDARs. However, it has been suggested that while LTP requires high-frequency stimulation and therefore a strong activation of postsynaptic NMDARs, LTD comes about upon lower-frequency stimulation and a milder activation of postsynaptic NMDARs (Bienenstock et al., 1982; Artola and Singer, 1993; **Figure 2B**). Because LTD requires NMDAR activation, it also requires astrocytic D-serine release (Zhang et al., 2008).

A similar dependence of NMDAR mediated long-term plasticity on astrocyte-derived D-serine as shown in hippocampus was described in the hypothalamic supraoptic nucleus (SON; Panatier et al., 2006). In the SON, astrocytic coverage changes with behavioral state: during parturition and lactation astrocyte processes retract from the synapses. Under this condition the availability of astrocyte-derived D-serine for postsynaptic NMDARs is greatly reduced, leading to a shift in the activity dependence of LTP and LTD (**Figure 2B**).

Interestingly, a recent study showed that astrocytic release of D-serine can actively gate LTP in the rat barrel cortex *in vivo* (Takata et al., 2011). In this study, it was shown that stimulation of the nucleus basalis of Meynert, which provides the major cholinergic input to the barrel cortex, activates cortical astrocytes (**Figure 2A**). If this stimulation coincides with sensory stimulation, this leads to a potentiation of the sensory evoked potential. The authors show that this LTP of the sensory evoked potential depends on D-serine release from the astrocytes upon activation. These findings highlight the importance of D-serine as a modulator of plasticity both *in vitro* and *in vivo*. Furthermore, since cholinergic input into the cortex has been suggested to gate cortical plasticity (Bakin and Weinberger, 1996), these findings reveal astrocytes as a possible candidate mediating this gating.

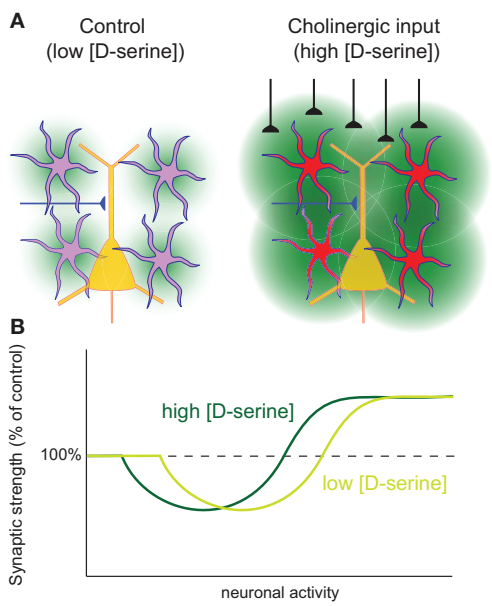

FIGURE 2 | Astrocyte mediated metaplasticity. (A) Left: under basal conditions astrocytes release a basal tone of D-serine (green) which is sensed by neuronal NMDARs. Right: when astrocytes are stimulated, for example by the activation of cholinergic fibers, this leads to an increased release of D-serine from the astrocytes, which leads to an increased availability of neuronal NMDARs for activation. **(B)** Hypothetical BCM curve for the induction of LTP and LTD. Depending on the concentration of astrocyte-derived D-serine, the neuronal activity requirements for induction of LTP and LTD will shift.

Apart from acting as a NMDAR co-agonist, a recent study has shown that astrocytic release of D-serine can also induce synaptic plasticity independent of its role at the NMDAR (Kakegawa et al., 2011). In the immature cerebellum, burst stimulation of parallel fiber to Purkinje neuron synapses induced release of D-serine from Bergmann glia. This D-serine subsequently activated postsynaptic δ2 glutamate receptors, which in turn caused internalization of postsynaptic AMPARs. Interestingly, interfering with this form of LTD disrupted motor coordination *in vivo*, showing its relevance for cerebellar development. From the study by Kakegawa et al. (2011) it is not clear how burst stimulation of the parallel fiber synapses leads to activation of Bergmann glia. However, one exciting hypothesis that we would like to put forward is that endocannabinoids might mediate this process. Burst stimulation of parallel fiber synapses is known to cause the production and release of endocannabinoids from Purkinje neurons (Brenowitz and Regehr, 2005). Furthermore, LTD in the immature cerebellum requires endocannabinoid signaling (Safo and Regehr, 2005). Therefore, it is tempting to speculate that the activation of Bergmann glia by postsynaptically synthesized endocannabinoids could underlie the induction of LTD, which would be similar to recent findings on LTD in neocortex (Min and Nevian, 2012) and hippocampus (Han et al., 2012; see below). However, this hypothesis awaits further testing.

Another gliotransmitter that can mediate long-term plasticity is glutamate. Previously, we mentioned that astrocyte stimulation at hippocampal Schaffer collateral synapses can induce short-term potentiation through astrocytic glutamate release followed by activation of presynaptic mGluRs (Fiacco and McCarthy, 2004; Perea and Araque, 2007). Interestingly, this short-term potentiation can be transformed into LTP if the astrocyte activation is paired with a postsynaptic depolarization (Perea and Araque, 2007). It was recently demonstrated that this LTP mediated by coincident astrocytic and neuronal activity can be induced physiologically when excitatory transmission coincides with cholinergic input coming from the septal nucleus *in vivo* (Navarrete et al., 2012).

Additionally, a recent study showed that pharmacological activation of astrocytes *in vivo*, by application of the cannabinoid receptor agonist Δ^9-THC, leads to LTD of Schaffer collateral synapses instead of LTP. This LTD also requires astrocytic glutamate release, but is mediated by activation of postsynaptic NMDARs, followed by endocytosis of postsynaptic AMPARs (Han et al., 2012). Therefore, depending on the type of stimulus, astrocyte activation can induce bidirectional hippocampal long-term plasticity.

In the neocortex, we have recently shown that astrocytic release of glutamate is necessary for the induction of spike-timing-dependent depression (t-LTD; Min and Nevian, 2012), a form of long-term plasticity that is indispensable for sensory development (Li et al., 2009). In the developing sensory neocortex, excitatory synapses between L5 neurons and between L4 and L2/3 neurons

contain presynaptic NMDARs (Sjöström et al., 2003; Bender et al., 2006; Nevian and Sakmann, 2006; Corlew et al., 2007; Rodríguez-Moreno and Paulsen, 2008). These receptors can modulate release probability directly (Sjöström et al., 2003; Brasier and Feldman, 2008), but additionally they are necessary for the induction of t-LTD of this synapse (Corlew et al., 2008). Until recently it was unclear whether these presynaptic NMDARs are autoreceptors for synaptically released glutamate, or if they are activated by glutamate coming from another source. Furthermore, while t-LTD was known to require activation of cannabinoid CB_1Rs (Sjöström et al., 2003; Bender et al., 2006; Nevian and Sakmann, 2006), it was unclear how presynaptic NMDARs and CB_1Rs together governed the induction of t-LTD. We showed that activation of presynaptic NMDARs during induction of t-LTD is mediated by astrocytic glutamate release (Min and Nevian, 2012; **Figure 3A**). Additionally, we found that postsynaptically synthesized endocannabinoids are the trigger for astrocyte activation during t-LTD induction. These endocannabinoids are only produced when a postsynaptic action potential is rapidly (within 10s of ms) followed by a presynaptic action potential. The reason for this narrow time window is the postsynaptic protein PLC, which acts as a coincidence detector (Hashimotodani et al., 2005) and is necessary for endocannabinoid synthesis (Nevian and Sakmann, 2006). Therefore, the precise timing window for the induction of t-LTD can be explained by the neuronal activity and coincidence detection upstream of the astrocyte activation. Subsequently, the synthesis of endocannabinoids leads to an increase in astrocytic Ca^{2+} signaling. This in turn leads to glutamate release from the astrocyte, which activates presynaptic NMDARs. Activation of the presynaptic NMDARs induces a long-lasting decrease in synaptic release probability, although the signaling cascade downstream from presynaptic NMDAR activation is still unclear (**Figure 3A**).

One interesting question rising from these results is how the presynaptic NMDARs are efficiently activated. Because the timing of the astrocytic Ca^{2+} signals, and therefore presumably the astrocytic glutamate release, is not correlated with respect to the pre- and post-synaptic action potentials (Min and Nevian, 2012), one would expect the Mg^{2+} block of the presynaptic NMDARs to hamper their efficient recruitment. However, it was recently shown that presynaptic NMDARs in the developing sensory cortex incorporate the NR3A subunit, which renders the receptor insensitive to Mg^{2+} block (Larsen et al., 2011). This modification would make presynaptic NMDARs ideal for sensing the temporally diffuse release of glutamate from astrocytes. However, it should also be noted that astrocyte stimulation by itself is not sufficient for the induction of t-LTD. Presynaptic action potential firing provides an additional requirement, although the mechanism behind this is currently unknown (Min and Nevian, 2012).

These results show that astrocytes form a crucial part of the retrograde signaling cascade for induction of t-LTD. Since endocannabinoid mediated forms of LTD occur in numerous brain regions and serve important functions (Heifets and Castillo, 2009), future studies will hopefully show whether astrocyte mediated retrograde signaling is a general principle for endocannabinoid mediated LTD.

Astrocytes also play a role in homeostatic control of synaptic transmission. This was first shown in cultured neurons, where glial release of the cytokine TNFα increases the synaptic expression of AMPARs, thereby controlling the weight of all excitatory synapses (Beattie et al., 2002). Another form of "multiplicative scaling" mediated by astrocytes was found in the hypothalamus.

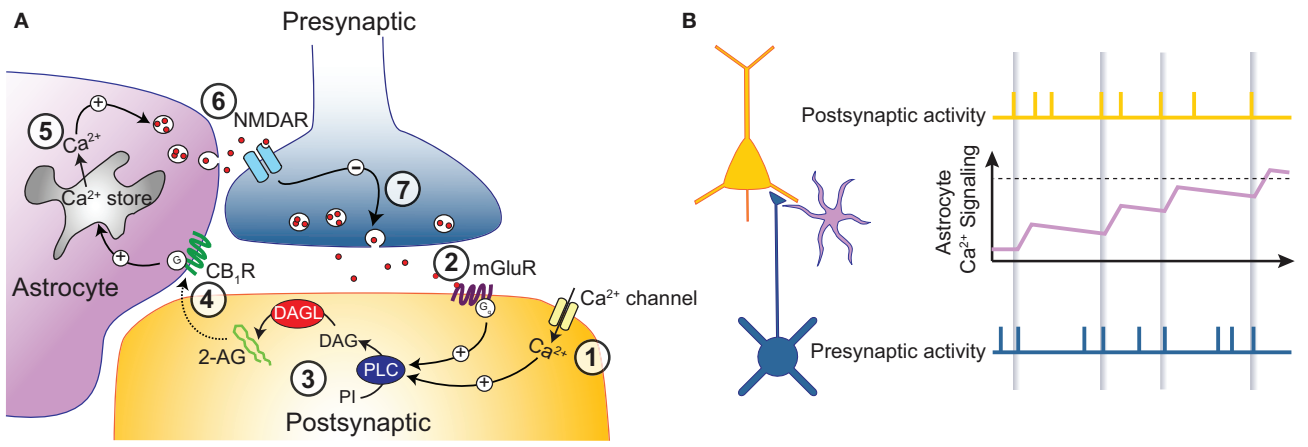

FIGURE 3 | Astrocytes as memory elements. (A) Schematic representation of the pathway leading to t-LTD at developing neocortical synapses. A postsynaptic action potential leads to Ca^{2+} influx into the postsynaptic neuron (1). When this is followed by a presynaptic action potential, leading to activation of mGluRs (2), PLC is strongly activated (3), leading to the synthesis of endocannabinoids. These endocannabinoids leave the postsynaptic neuron to activate astrocytic CB_1Rs (4), which cause release of Ca^{2+} from intracellular stores (5). The resultant Ca^{2+} transients trigger vesicular release of glutamate from the astrocyte, which in turn activates presynaptic NMDARs (6). This leads to a long-lasting depression in presynaptic release probability (7). **(B)** Astrocytes could act as memory elements. Schematic showing the action potential activity of a presynaptic (bottom, blue) and a postsynaptic (top, yellow) neuron, as well as the Ca^{2+} activity of a neighboring astrocyte (middle, pink). By sensing the buildup of endocannabinoids during subsequent pairings, astrocyte activity would gradually increase during the induction of t-LTD. t-LTD could then be induced only when astrocyte activity reaches a certain threshold (dotted line).

Here, exogenous stimulation of astrocytes by norepinephrine leads to astrocytic release of ATP, which subsequently activates postsynaptic $P2X_7$ receptors. Activation of these postsynaptic receptors leads to insertion of AMPARs, and thereby to a long-lasting postsynaptic increase in the efficacy of excitatory synapses (Gordon et al., 2005). Interestingly, a more physiological protocol of repetitive stimulation of hypothalamic excitatory synapses can activate astrocyte Ca^{2+} signaling and subsequent release of ATP through mGluR activation. This protocol can lead to a similar long-lasting postsynaptic upscaling of the synaptic efficacy (Gordon et al., 2009). Therefore, astrocytes can play a role in homeostatic plasticity by translating global network activity into a distributed plasticity signal to multiple synapses.

Finally, long-term synaptic plasticity is costly in terms of energy. As mentioned before, astrocytic supply of energy to neurons is essential for maintaining synaptic transmission (Rouach et al., 2008). In addition, a recent study has shown that astrocytic energy supply is essential for the long-term maintenance of hippocampal LTP, and thereby for memory formation (Suzuki et al., 2011). In this study, it was found that learning was accompanied by an increase in the extracellular concentration of the astrocyte derived metabolite lactate. Blocking transport of lactate from astrocytes into the extracellular space, as well as blocking transport of lactate from the extracellular space into neurons interfered with the maintenance phase of LTP as well as with long-term memory (Suzuki et al., 2011). A similar involvement of lactate in memory formation was found in another recent study (Newman et al., 2011). These results show that not only receptor mediated astrocyte-neuron signaling but also metabolic support are crucial for long-term plasticity.

In conclusion, astrocytes play a role in several forms of long-term plasticity. Astrocytes can either set the threshold for LTP/LTD induction by controlling the extracellular concentration of D-serine, they can directly induce synaptic plasticity by releasing glutamate or other gliotransmitters, and they can mediate homeostatic plasticity. In addition, dynamic regulation of metabolic support by astrocytes is crucial for synaptic plasticity.

THE COMPUTATIONAL POTENTIAL OF ASTROCYTES

In the previous section, we have discussed how astrocytes can modulate neuronal functioning. Based on the above described astrocyte-neuron interactions, we will summarize some possible roles that astrocytes could play in neuronal computation. We suggest that the functional role of astrocytes adds to the computational power of neuronal networks.

ASTROCYTE MEDIATED METAPLASTICITY

Metaplasticity is a higher-order form of synaptic plasticity, defined as a change in the ability to induce synaptic plasticity (Abraham and Bear, 1996). There are several examples of neuronal activity patterns that do not induce synaptic plasticity, but that affect the threshold for subsequent induction of synaptic plasticity. Such metaplasticity has been proposed to play a role in several forms of learning (Abraham, 2008). Many forms of metaplasticity involve a change in the functionality of the postsynaptic NMDAR. Through the release of D-serine, astrocytes are ideally positioned to mediate metaplasticity. The level of astrocytic D-serine release will determine the possible amount of NMDAR activation and therefore of NMDAR mediated postsynaptic Ca^{2+} influx upon synaptic activity. Since many forms of LTP and LTD require NMDAR mediated Ca^{2+} influx for their induction, astrocytes can shift the threshold for LTP and LTD induction (**Figure 2**). In this way astrocytes can slide the threshold of the Bienenstock-Cooper-Munro (BCM) learning rule (Bienenstock et al., 1982; Artola and Singer, 1993). A clear example of this sliding threshold occurs in the hypothalamus where, as mentioned earlier, the amount of astrocytic coverage determines the synaptic concentration of D-serine and therefore, the induction threshold for LTP and LTD (Panatier et al., 2006). Furthermore, astrocytic D-serine release upon astrocyte activation by cholinergic inputs opens the window for subsequent LTP induction in the *in vivo* neocortex (**Figure 2**; Takata et al., 2011). Therefore, astrocytic D-serine release is a physiologically important metaplasticity signal in the brain. It can translate the activity state of the neuronal network into a modulatory signal that determines the corresponding learning rule: during a high activity state due to arousal or heightened attention the neuronal and astrocyte networks are highly active supporting the induction of LTP. Future behavioral studies should determine what the importance of this astrocyte mediated metaplasticity is for learning and memory formation.

ASTROCYTE MEDIATED HETEROSYNAPTIC PLASTICITY/SYNAPTIC CLUSTERING

Single astrocytes form non-overlapping domains (Bushong et al., 2002), and cover an area containing 300–600 dendrites, contacting up to 36 spines per dendrite (Halassa et al., 2007). This makes astrocytes well suited to signal information to a population of neighboring synapses. This means that astrocytes could be involved in forms of heterosynaptic plasticity: a plasticity signal generated at a single synaptic site could travel to neighboring synaptic sites to influence synaptic plasticity there. Furthermore, because astrocyte mediated heterosynaptic plasticity might not be confined to a single dendrite, the plasticity could affect synapses on neighboring dendrites even if they do not belong to the same neuron (so-called heteroneuronal plasticity). This requires that the locally induced increase in astrocytic Ca^{2+} signaling spreads along an astrocytic process and results in the release of gliotransmitters at neighboring synapses. Heterosynaptic short-term plasticity could play a role in switching between synaptic ensembles during information processing. As described earlier, astrocytes mediate heterosynaptic short-term depression at excitatory synapses onto CA1 pyramidal neurons (Zhang et al., 2003; Pascual et al., 2005; Serrano et al., 2006). Furthermore, endocannabinoid mediated astrocyte activation by stimulation of a single neuron induces heteroneuronal short-term potentiation at synapses onto non-stimulated neurons (Navarrete and Araque, 2010).

Heterosynaptic long-term plasticity has been implicated in the homeostatic control of synaptic inputs to a neuron (Chistiakova and Volgushev, 2009). Furthermore, heterosynaptic forms of long-term plasticity could facilitate the formation of clusters of neighboring synaptic inputs on dendrites (Larkum and Nevian, 2008). Such clusters have been proposed to be essential for the

implementation of nonlinear synaptic integration in dendrites (Larkum et al., 2009; Legenstein and Maass, 2011). Several recent studies have shown the existence of clustered synaptic inputs (Kleindienst et al., 2011; Makino and Malinow, 2011; Takahashi et al., 2012). Whether astrocytes are able to fulfill a role in heterosynaptic long-term plasticity remains to be seen. However, it is interesting to note that several forms of endocannabinoid mediated LTD are heterosynaptic in nature (Chevaleyre and Castillo, 2003; Huang et al., 2008) and that astrocytes are implicated in some forms of endocannabinoid mediated LTD (Han et al., 2012; Min and Nevian, 2012).

It is important to note that not every activation of an astrocyte, manifested by increases in Ca^{2+} signaling, directly translates into synaptic plasticity. For example, we found that astrocyte activation with a voltage-clamp depolarization protocol results in increased Ca^{2+} signaling in the astrocyte, but that this activity alone does not change synaptic transmission strength at excitatory cortical synapses. An additional activation of the presynaptic axon was necessary in order to induce LTD (Min and Nevian, 2012). The astrocyte-stimulation-induced LTD (a-LTD) shares the same downstream signaling pathway with t-LTD, requiring activation of presynaptic NMDARs, but is independent of postsynaptic activity. This shows that additional factors have to be taken into account beyond astrocyte activation. A similar principle, where astrocyte activity is required but not sufficient for induction of plasticity, might explain the current controversy surrounding the involvement of astrocytes in hippocampal LTP (Agulhon et al., 2010; Henneberger et al., 2010; Smith, 2010). In this respect one further function that astrocytes might have in heterosynaptic modulation of synaptic transmission is that they lower the threshold for plasticity at neighboring synapses (heterosynaptic metaplasticity). In the case of a-LTD, release of glutamate onto presynaptic terminals that were previously not activated increases the propensity for LTD if these axons are activated within the time window of increased astrocyte activity. This can result in the above mentioned formation of clustered inputs onto neurons.

ASTROCYTES AS MEMORY ELEMENTS

The induction of many forms of synaptic plasticity requires a repeated presentation of a certain stimulus pattern (Petersen et al., 1998). An example of this is STDP, in which a single presentation of paired pre- and post-synaptic APs is insufficient for the induction of plasticity, but where repeated presentation of these stimuli over a period of several minutes can induce plasticity (O'Connor et al., 2005). This raises the question how a neuron can integrate transient coincident activity (on a millisecond timescale) over a period of minutes. Although it has been suggested that postsynaptic kinases or phosphatases could perform such integration (Lisman, 1989; Wang et al., 2005), astrocytes might also play an important role, since their basal timescale of signaling is several orders of magnitude slower than that of neurons. In support of this idea, we recently showed that the frequency of astrocytic Ca^{2+} transients gradually increases during the induction of t-LTD (Min and Nevian, 2012). This is achieved by the synthesis of endocannabinoids in the postsynaptic neuron during t-LTD induction. These endocannabinoids subsequently activate a neighboring astrocyte, and the astrocyte presumably senses the gradual buildup of the endocannabinoid concentration during repeated pairings (**Figure 3A**). The astrocyte then might act as a thresholding unit, releasing a retrograde t-LTD inducing signal onto the presynaptic neuron only when a sufficient level of astrocyte activation has been achieved (**Figure 3B**). In this way, astrocytes might act as memory elements for certain patterns of correlated neuronal activity.

How can an astrocyte act as a thresholding unit? The sophisticated Ca^{2+} signals in the processes of astrocytes can be used to perform chemical computations similar to linear and nonlinear Ca^{2+} signaling in neurons. Efficient Ca^{2+} buffering (Neher, 1998), receptor clustering (Panatier et al., 2011; Arizono et al., 2012) and extrusion/uptake mechanisms could create Ca^{2+} microdomains that limit the release of gliotransmitters in space and time. Only certain patterns of Ca^{2+} signals might cause a high enough concentration of Ca^{2+} in the astrocyte processes to trigger vesicular release. This could be accomplished by local Ca^{2+} buffer saturation and a specific spatial relationship between Ca^{2+} release sites in the ER and the vesicular Ca^{2+} sensor triggering exocytosis (Marchaland et al., 2008; Bergersen et al., 2011). Therefore, a better understanding of the excitation secretion coupling and of microdomain Ca^{2+} signaling in astrocytes is needed to understand their chemical computation.

HOW TO LINK ASTROCYTE MEDIATED SYNAPTIC PLASTICITY TO BEHAVIOR?

As described above, there is ample experimental evidence for an active role of astrocytes in neuronal plasticity. The most important question remaining is the functional relevance of the above described astrocyte-neuron interactions. This has proven very hard to address, since experimental approaches until recently were unable to specifically modify and study the role of astrocytes in neuronal computation and behavior *in vivo*. However, recent advances in genetics have made it possible to specifically alter astrocyte signaling *in vivo* while keeping neuronal signaling intact. This has led to some important insights into astrocyte involvement in information processing. For example, it is now clear that astrocyte derived adenosine modulates the build-up of sleep pressure as well as the cognitive effects of sleep deprivation (Halassa et al., 2009; Florian et al., 2011). Furthermore, using an astrocyte-specific knockout of the cannabinoid CB_1 receptor, Han et al. (2012) showed that astrocytes are responsible for cannabinoid-induced reduction in working memory performance. Using astrocyte specific pharmacology, it was shown that lactate transport from astrocytes to neurons is crucial for long-term memory formation (Newman et al., 2011; Suzuki et al., 2011). Finally, a recent study showed that AMPARs found on Bergmann glia in the cerebellum are crucial for maintaining the integrity of glutamatergic synapses and that their deletion impairs fine motor coordination *in vivo* (Saab et al., 2012). Undoubtedly, future studies will shed more light on the role of astrocytes in behavior. It does however remain difficult to link behavioral readouts as are often obtained from *in vivo* experiments with the subcellular astrocyte-neuron signaling mechanisms as described in this review.

One possible way to bridge the gap between cellular mechanisms observed with high-resolution *in vitro* experiments and behavioral readouts *in vivo* is the use of mathematical models. As mentioned earlier, progress has been made with mathematical models of astrocyte Ca^{2+} signaling. In addition, several models have included bidirectional signaling between astrocytes and neurons on the synaptic level, leading to new insights into the function of this bidirectional signaling. For example, a study by Nadkarni et al. (2008) suggests that bidirectional signaling between astrocytes and presynaptic terminals can optimize synaptic information content by tuning presynaptic release probability (Nadkarni et al., 2008). In another study it has been suggested that astrocyte signaling can transiently switch the direction of short-term synaptic plasticity between paired-pulse depression and facilitation (De Pittà et al., 2011). Such models are a starting point for the development of realistic neuron-glia networks.

An example of a recently developed neuron-glia network model comes from a recent study by Wade et al. (2011). Here, it was shown that astrocyte mediated SICs can synchronize pre- and post-synaptic neuronal responses. When combining this with a neuron-dependent STDP rule, the authors show that astrocyte mediated synchronization could act as a "learning signal" at remote synaptic sites (Wade et al., 2011). Another recent study shows the learning potential of neuron-glia networks (Porto-Pazos et al., 2011). In this study, the inclusion of astrocytes into a multi-layer feed forward neuronal network model designed to solve different classification tasks improved performance. Interestingly, increased performance seemed to come at the expense of learning speed. Astrocytes in this model were responding to highly active neuronal connections, and in turn bidirectionally regulated synaptic weights with a slow temporal time-course. The increase in performance could not be explained by the addition of extra elements to the neuronal network, since keeping the number of elements constant led to a similar increase in performance. However, it crucially depended on the response properties of the added astrocytes, with the astrocytes integrating neuronal activity over longer time-scales as well as being active for longer times then the neurons (Porto-Pazos et al., 2011). It should be noted that the astrocyte-neuron network implemented in this study has a simplified structure: each synapse in the network was controlled by a single-independent astrocyte. Therefore, the spread of activity through the astrocyte network, which might lead to heterosynaptic or homeostatic plasticity, was not included. Future work implementing more realistic astrocyte-neuron networks capturing features described in this review might lead to novel insights into how astrocytes contribute to neuronal computation. Based on the improvement seen with rudimentary astrocyte-neuron networks, we predict that network performance will be greatly enhanced when realistic astrocyte-neuron signaling is implemented.

In conclusion, research on the involvement of astrocytes in neuronal signaling in the last years has resulted in a rich array of possible astrocyte-neuron interactions. It is now evident that beyond their permissive role in synaptic and network function astrocytes can perform integration of neuronal signals by means of their Ca^{2+} dynamics, thereby potentially enhancing the computational power of neuronal networks. The challenge for the future is to understand how and when astrocyte mediated signaling processes are involved in computation in the brain. To answer this fascinating question will require a combination of state-of-the-art experimental techniques with advanced computational modeling.

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Bi-directional astrocytic regulation of neuronal activity within a network

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The concept of a tripartite synapse holds that astrocytes can affect both the pre- and post-synaptic compartments through the Ca^{2+}-dependent release of gliotransmitters. Because astrocytic Ca^{2+} transients usually last for a few seconds, we assumed that astrocytic regulation of synaptic transmission may also occur on the scale of seconds. Here, we considered the basic physiological functions of tripartite synapses and investigated astrocytic regulation at the level of neural network activity. The firing dynamics of individual neurons in a spontaneous firing network was described by the Hodgkin–Huxley model. The neurons received excitatory synaptic input driven by the Poisson spike train with variable frequency. The mean field concentration of the released neurotransmitter was used to describe the presynaptic dynamics. The amplitudes of the excitatory postsynaptic currents (PSCs) obeyed the gamma distribution law. In our model, astrocytes depressed the presynaptic release and enhanced the PSCs. As a result, low frequency synaptic input was suppressed while high frequency input was amplified. The analysis of the neuron spiking frequency as an indicator of network activity revealed that tripartite synaptic transmission dramatically changed the local network operation compared to bipartite synapses. Specifically, the astrocytes supported homeostatic regulation of the network activity by increasing or decreasing firing of the neurons. Thus, the astrocyte activation may modulate a transition of neural network into bistable regime of activity with two stable firing levels and spontaneous transitions between them.

Keywords: neuron, astrocyte, synaptic transmission, tripartite synapse, neuronal network, regulation

INTRODUCTION

Determining the principles of signal processing in brain networks has been one key challenge in modern neuroscience, which has thus far been unresolved. A central mechanism of signal propagation is synaptic transmission between neurons constituting networks. There is evidence that in addition to processes within the pre- and post-synaptic compartments, several extrasynaptic signaling pathways can affect this transmission (Semyanov, 2008; Dityatev and Rusakov, 2011), one of which is the influence of neighboring astrocytes modulating synaptic signaling. The idea of astrocytes being important in addition to the pre- and post-synaptic components of the synapse has led to the concept of a tripartite synapse (Araque et al., 1999; Haydon, 2001). A part of the neurotransmitter released from the presynaptic terminals (i.e., glutamate) can diffuse out of the synaptic cleft and bind to metabotropic glutamate receptors (mGluRs) on the astrocytic processes that are located near the neuronal synaptic compartments. The neurotransmitter activates G-protein mediated signaling cascades that result in phospholipase C (PLC) activation and insitol-1,4,5-trisphosphaste (IP3) production. The IP3 binds to IP3-receptors in the intracellular stores and triggers Ca^{2+} release into the cytoplasm. Such an increase in intracellular Ca^{2+} can trigger the release of gliotransmitters (Parpura and Zorec, 2010) [e.g., glutamate, adenosine triphosphate (ATP), D-serine, and GABA] into the extracellular space.

A gliotransmitter can affect both the pre- and post-synaptic parts of the neuron. By binding to presynaptic receptors it can either potentiate or depress presynaptic release probability. One of the key pathways in tripartite synapse is mediated by glutamate released by the astrocyte (Parri et al., 2001; Liu et al., 2004a,b; Perea and Araque, 2007). Such glutamate can potentially target presynaptic NMDA receptors which increase release probability (McGuinness et al., 2010), or presynaptic mGluRs which decrease it (Semyanov and Kullmann, 2000). Presynaptic kainate receptors exhibit a more complex modulation of synaptic transmission through both metabotropic and ionotropic effects (Semyanov and Kullmann, 2001; Contractor et al., 2011).

In addition to presynaptic feedback signaling through the activation of astrocytes, there is feedforward signaling that targets the postsynaptic neuron. Astrocytic glutamate induces slow inward postsynaptic currents (SICs) (Parpura and Haydon, 2000; Parri et al., 2001; Fellin et al., 2006). Their appearance is characterized by a high-degree of spatial and temporal correlation in different cells, thus producing a synchronization effect (Fellin et al.,

2006). Astrocytic release of D-serine is critical for the activation of postsynaptic NMDA receptors and the development of synaptic long-term potentiation (LTP) (Henneberger et al., 2010; Bergersen et al., 2011). In contrast, GABA released by astrocytes may be responsible for synchronous inhibition of postsynaptic neurons (Liu et al., 2000; Kozlov et al., 2006; Angulo et al., 2008). Another gliotransmitter, ATP, can also directly depress the postsynaptic neuron by activating purinergic receptors (Koizumi et al., 2003). Additionally, ATP can increase the spike generation probability in interneurons through activation of the P2Y1 receptors (Fellin et al., 2006; Torres et al., 2012). Thus, astrocytes may play a significant role in regulation of neuronal network signaling by forming local elevations of gliotransmitters that can guide excitation flow (Semyanov, 2008; Giaume et al., 2010). By integration of neuronal synaptic signals, astrocytes provide coordinated release of gliotransmitters affecting local groups of synapses from different neurons. This action may control the level of coherence in synaptic transmission in neuronal groups (for example, by means of above mentioned SICs). Moreover, different astrocytes are coupled by gap junctions and may be able propagate such effect even further by means intercellular IP3 and Ca^{2+} diffusion (Verkhratsky and Butt, 2007). Moreover the astrocytes can communicate to each other by extracellular ATP diffusion. Thus, theoretically the astrocytes may contribute in regulation of neuronal activity between distant network sites.

Several mathematical models have been proposed to understand the functional role of astrocytes in neuronal dynamics: a model of the "dressed neuron," which describes the astrocyte-mediated changes in neural excitability (Nadkarni and Jung, 2004, 2007), a model of the astrocyte serving as a frequency selective "gate keeper" (Volman et al., 2006), and a model of the astrocyte regulating presynaptic functions (De Pittà et al., 2011). It has been demonstrated that gliotransmitters can effectively control presynaptic facilitation and depression. The model of the tripartite synapse has recently been employed to demonstrate the functions of astrocytes in the coordination of neuronal network signaling, in particular, spike-timing-dependent plasticity and learning (Postnov et al., 2007; Amiri et al., 2011; Wade et al., 2011). In models of astrocytic networks, communication between astrocytes has been described as Ca^{2+} wave propagation and synchronization of Ca^{2+} waves (Ullah et al., 2006; Kazantsev, 2009). However, due to a variety of potential actions, that may be specific for brain regions and neuronal sub-types, the functional roles of astrocytes in network dynamics are still a subject of debate.

In this paper we illustrate how activations of local astrocytes may effectively control a network through combination of different actions of gliotransmitters (presynaptic depression and postsynaptic enhancement). We found bi-directional frequency dependent modulation of spike transmission frequency in a network neuron. A network function of the neuron implied the presence of correlation between neuron input and output reflecting feedback formed by synaptic transmission pathways. Surprisingly, the bi-directional astrocytic regulation, which may be negligibly small for local synaptic transmission, may induce significant changes in network firing states, including the appearance of rate-encoded bistable states.

MATERIALS AND METHODS

To study astrocytic regulation of neuronal activity, we introduced a computational model of synapses involved in spontaneous firing dynamics of a neuronal network using the mean field approach. We assumed that a spiking neuron is a member of a network, and the spikes of this neuron go through divergent/convergent connections of the network providing a certain level of correlation between neuron output and input. Because of complex network connectivity, it was impractical to follow the propagation of individual spikes, and thus we followed the evolution of the firing rates when the frequency was averaged in the time window of hundreds of milliseconds or seconds. We considered a postsynaptic neuron capable of spike generation when integrating the incoming postsynaptic currents (PSCs). These currents were treated as a mean field contribution of a large number of tripartite synapses. The presynaptic dynamics consisted of spontaneous glutamate release and the glutamate release induced by the network feedback. The presynaptic terminals were excited through different signaling pathways and thus were uncorrelated at the millisecond time scale. The astrocytic compartment represented a set of local processes that can independently modulate the transmission at particular synapses. Independent modulation of different synapses by the astrocyte is based on experimental observations that local Ca^{2+} sparks in astrocytic processes are independent from each other and different from the global Ca^{2+} transient that spread through the entire astrocyte (Nett et al., 2002). Thus, the feedback and feedforward actions of the particular astrocyte process were localized and related to a particular synapse in our model. The duration of Ca^{2+} sparks was a few seconds long, and each of them was associated with the local release of gliotransmitter. In the model, we assumed that these releases modulated synaptic transmission, but did not produced synaptic synchronization, which require whole astrocyte activation.

PRESYNAPTIC DYNAMICS

Each presynaptic event caused the release of a quantum of glutamate. Because the dynamics of the neurotransmitter was averaged from all the synaptic terminals at a relatively long time scale (up to seconds) we did not describe detailed presynaptic kinetics that operates in a short-time scale. The mean field amount of neurotransmitter, X, that diffused from synaptic cleft and reached the astrocyte was described by the following first-order equation,

$$\frac{dX}{dt} = -\alpha_x(X - k_{\text{pre}}H_x(I_{\text{pre}} - 0.5)),$$
$$I_{\text{pre}}(t) = \begin{cases} 1, & \text{if } t_i < t < t_i + \tau, \\ 0, & \text{otherwise,} \end{cases} \quad (1)$$

where $I_{\text{pre}}(t)$ is a pulse signal accounting for the release events, H_x is the Heviside step function, t_i is the event occurrence time at one of the presynaptic terminals satisfying Poisson distribution with average Poisson frequency f_{in} and τ is the pulse duration, $\tau = 1$ ms. Each presynaptic release event contributed to the concentration with the portion, $\Delta X_i \approx (k_{\text{pre}} - X)\alpha_x$, where k_{pre} is the efficacy of the release, and α_x is the neurotransmitter clearance constant. Thus, there was a temporal summation

of the neurotransmitter amount released with the time scale α_x. High frequency trains led to an increase in the mean field concentration. The amount of release varied with X to reflect the frequency-dependence of the release probability as demonstrated by Tsodyks and Markram in the short-term plasticity model (Tsodyks et al., 1998; De Pittà et al., 2011). We, however, did not include short-term plasticity in the model because the consequent pulses may indicate the release in spatially distinct synapses, which are averaged in Equation (1).

For sake of simplicity, our mean field model considers a set of independent (uncorrelated) events localized in different spatial sites as single averaged event. The diffusion processes are also accounted by effective average parameters α_x and k_{pre}.

POSTSYNAPTIC DYNAMICS

The release of neurotransmitter leads to a PSC. We assumed that a number of events occurred in different spatial sites of dendritic tree were integrated at soma and provided mean field synaptic input, I_{syn}, depolarizing the membrane that may lead to the response spike generation.

We focused on excitatory transmission and investigated excitatory postsynaptic currents (EPSCs), I_{EPSCs}, using the following equation:

$$\frac{dI_{\text{EPSCs}}}{dt} = \alpha_I(I_{\text{EPSCs}} - AH_x(I_{\text{pre}} - 0.5)), \quad (2)$$

where α_I is their rate constant and A is their amplitude. Following experimental observations, we assumed that the amplitude of the EPSCs satisfy the probability distribution, $P(A)$, in the following form:

$$P(A) = \frac{2A}{b^2}\exp\left(-A^2\Big/b^2\right), \quad \int_0^{+\infty} P(A)dA = \Gamma(1) = 1, \quad (3)$$

where Γ is the gamma function and b is the scaling factor that accounts for the effective strength of the synaptic input. Importantly, the synaptic events do not fully correlate with the consequent input pulses (action potentials) in the model because they can occur at different synaptic sites.

The postsynaptic events occurring at different sites of the dendritic tree are integrated and form synaptic current, I_{syn}. Because we consider $I_{\text{EPSCs}}(t)$ as a mean field contribution of all synapses, integrated synaptic current in the soma, I_{syn}, can be expressed as:

$$I_{\text{syn}} = I_{\text{EPSCs}}S(X), \quad (4)$$

where $S(X)$ is a dendrite integration function expressed in the form of a high-pass filter that reflects the fact that the postsynaptic spike generation requires a summation of several synaptic inputs, e.g., single synaptic events will be filtered.

$$S(X) = \frac{1}{1 + \exp\left(-\frac{(X - \theta_x)}{k_x}\right)} \quad (5)$$

where θ_x and k_x are the midpoint and the slope of the neuronal activation, respectively.

We modeled the spike generation with classical Hodgkin–Huxley equations (Hodgkin and Huxley, 1952). The membrane potential evolved according to the following current balance equation:

$$C\frac{dV}{dt} = -(I_{\text{mem}} + I_{\text{th}} + I_{\text{syn}}), \quad (6)$$

where $I_{\text{mem}} = I_{\text{Na}} + I_{\text{K}} + I_{\text{leak}}$ is the sum of the transmembrane currents responsible for the spike generation (for more details, see Izhikevich, 2007). **Figure 1** illustrates the dynamics of synaptic transmission in Equations (1–6) obtained in numerical simulations.

We analysed the frequency of the spike generation, f_{out}, depending on the input Poisson frequency, f_{in}. **Figure 2** illustrates the input–output characteristics of the spike transmission in Equations (1–6).

ASTROCYTIC DYNAMICS

We added an astrocytic component to Equations (1–4) and assumed that gliotransmitters are released and act on the synapses. In the mean field model, we described the concentration of gliotransmitter by the following equation:

$$\frac{dY_k}{dt} = -\alpha_k(Y_k - H_k(X)), \quad H_k(X) = \frac{1}{1 + \exp\left(-\frac{(X - \theta_k)}{k_k}\right)}. \quad (7)$$

We assumed that different types of gliotransmitter ($k = 1$ for glutamate and $k = 2$ for D-serine released from astrocyte) may have different clearance rate, α_k, and equilibrium activation function, $H_k(X)$, which accounts for the gliotransmitter amount released if the presynaptic activity exceeds a certain threshold (Perea and Araque, 2002) described here by the parameter θ_k. Note, that Equation (7) is functionally similar to the gliotransmitter model that was recently proposed in (De Pittà et al., 2011) by excluding the computation of intracellular Ca^{2+} dynamics and focusing on neurotransmission modulation. The dynamics of the gliotransmitter concentration is illustrated in **Figure 3**.

In the mean field approach we did not set a definite limit for the duration of the astrocyte action. In addition to the neurotransmitter concentration, X, which accounts for the mean field impact of a number of synapses was variable, Y_k, also represents an average of the local Ca^{2+} sparks that may independently occur at the different spatial sites (see, for example, Nett et al., 2002). In such way, the variable Y_k represents a tonic effect of astrocytic activation on the mean field synaptic dynamics. We further refer Y_1 as the concentration of astrocytic glutamate concentration modulating presynaptic release and Y_2 as D-serine concentration modulating postsynaptic response of NMDA receptors.

In the mean field model, we estimated astrocytic modulation of the average concentration of neurotransmitter released. Thus, the average amount released for each incoming pulse was scaled with factor $k_{\text{pre}} = k_0(1 + \gamma_1 Y_1)$, where $\gamma_1 > 0$ for the potentiation and $\gamma_1 < 0$ for the depression, respectively. Thus,

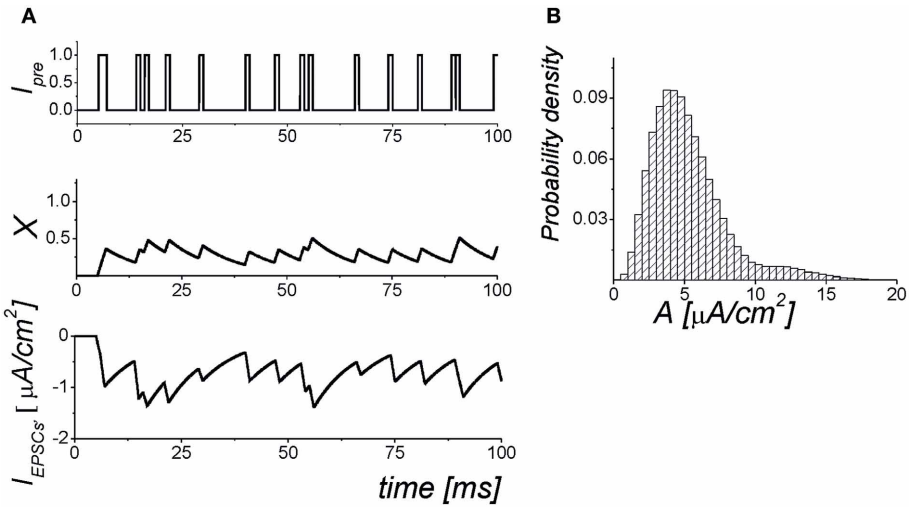

FIGURE 1 | The dynamics of the synaptic transmission model (1–6). **(A)** Signaling $I_{\text{pre}}(t)$ models of presynaptic events in the form of the Poisson pulse train. Each pulse has a fixed duration, $\tau = 1$ ms. The pulses exhibiting less than 1 ms intervals are considered as synchronized pulse events with a longer duration. The $X(t)$ is the mean field concentration of neurotransmitter released for each pulse. The I_{EPSC} is mean field postsynaptic current with amplitudes selected according to the probability distribution shown in panel **(B)**. The parameter values: $\alpha_x = 0.05\,\text{ms}^{-1}$, $k_{\text{pre}} = 1$, $b = 25$, $\theta_x = 0.35$.

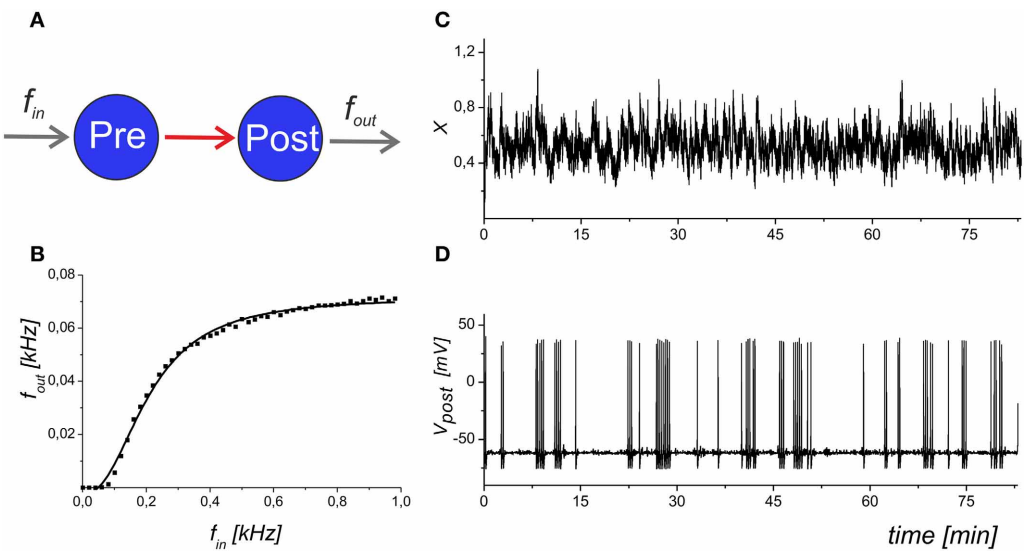

FIGURE 2 | The input–output dynamics of a neuron with synapses, but without astrocytic influence. **(A)** Schematic illustration of synaptically coupled neurons with input frequency f_{in} and output spiking rate f_{out}. **(B)** The dependence of the average firing rate, f_{out}, averaged for 1 s, on the presynaptic event frequency. The solid line shows the logistic curve fit of the model data. **(C)** The mean field concentration of the neurotransmitter, $X(t)$. **(D)** The output spike train that corresponds to the maximal slope of the frequency dependence. Parameter values: $f_{\text{in}} = 0.2$ kHz, $b = 5$.

Equation (1) for presynaptic dynamics can be re-written as:

$$\frac{dX}{dt} = -\alpha_x(X - k_0(1 + \gamma_1 Y_1)H_x(I_{\text{pre}} - 0.5)). \qquad (8)$$

In addition to the presynaptic effect release of D-serine modulated EPSC through postsynaptic NMDARs. In the model, it was accounted for by the increase of the amplitudes of PSCs, I_{EPSCs}, with:

$$b = b_0\,(1 + \gamma_2 Y_2)\,, \qquad (9)$$

where γ_2 is the gain of the D-serine effect.

Schematically, the mean field model of synaptic transmission is shown in **Figure 4A**.

THE FIRING RATE COORDINATION CIRCUIT

As a part of the neuronal network, the neuron is stimulated by signals generated by specific excitation transmission pathways and contributes to their sustainment by its own spikes. Because the synaptic architecture of even a simple neuronal network can be extremely complicated, it is very difficult to identify the precise spiking sequences generated by the network signaling circuits. Moreover, the same network may generate different sequences with variable interspike statistics. The repeatable spiking sequences found experimentally in both *in vivo* and *in vitro* conditions can serve as an example of such network behavior (Ikegaya et al., 2004). In the framework of the mean field approach, we followed the average frequency for the time scale up to seconds that is similar to a replay of the basic network signaling pathways. In such a consideration, a mean field neuron received a Poisson spike train input that fits the statistics of generally uncorrelated sources of input spikes, as we used in the synaptic transmission model. Next, we assumed that the mean field neuron contributes to the network activity by firing with a mean frequency, f_{out}. The output signal from the neuron further propagates through the network in divergent/convergent signaling pathways and returns to the neuron in the form of separate inputs. In spontaneous network dynamics, the homeostatic states should be characterized by a reproducible activation. Perhaps the simplest model of such a network impact could be the presence of a correlation between the output and input firing states:

$$f_{\text{in}} = f_0 + k_N f_{\text{out}}, \tag{10}$$

where k_N is the correlation coefficient determining the gain of the network control of a particular neuron, and f_0 is the rate of the input-independent spontaneous presynaptic release.

For our input–output correlation model very simple predictions can be immediately derived from Equation (10) in limit cases. If $k_N \ll 1$, then the neuron is out of network feedback and its activity goes at low level induced by spontaneous release,

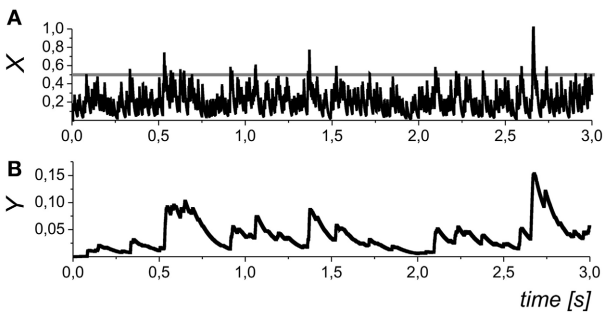

FIGURE 3 | The dynamics of the mean field concentration of neurotransmitter (A) and gliatransmitters (B) in Equations (5) and (10). The slow transients in the Y_k variables may be induced by different astrocytes (and/or different compartments of the same astrocytes), and, in general, they may have variable amplitudes. The gray line in the in panel **(A)** shows the midpoint of astrocyte activation function $H_k(X)$. Parameter values: $\theta_k = 0.5$, and $k_k = 0.01$.

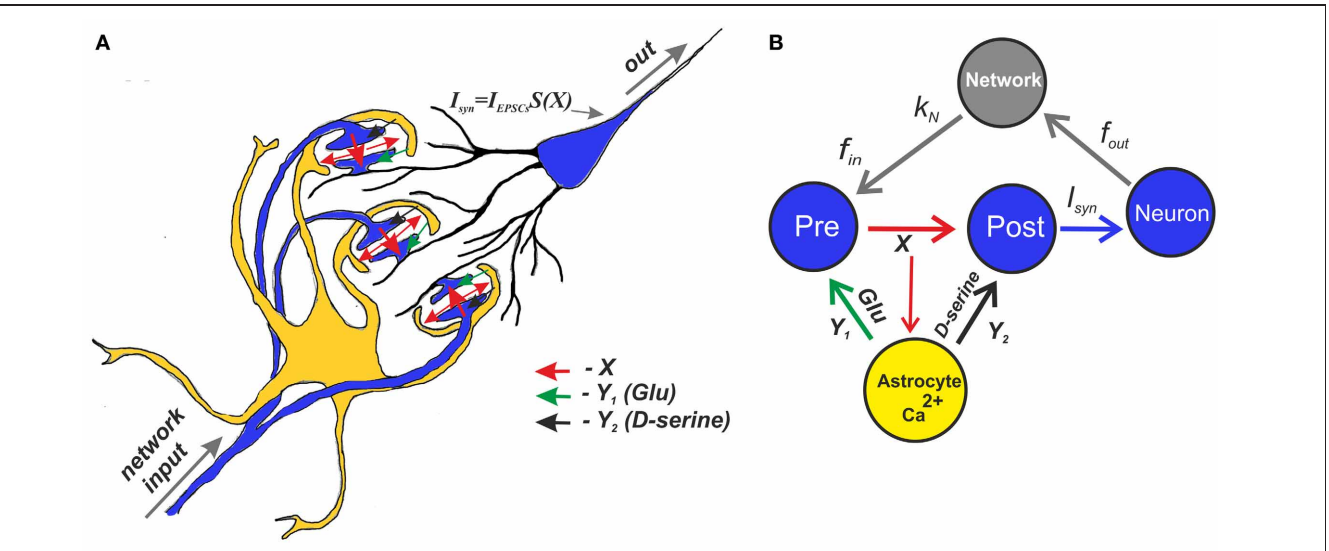

FIGURE 4 | A schematic view of the mean field model of synaptic transmission supplied with a network feedback. (A) A network neuron has a large number of synaptic contacts. The presynapses were described by the mean field concentration of neurotransmitter (e.g., glutamate released by presynaptic terminals), X. The postsynapses were described by postsynaptic currents, I_{EPSCs}. The local postsynaptic events were summarized and described by an integration function $S(X)$, which reflects single synaptic events that have been filtered below the threshold. Astrocytic activation was accounted for by the transient increase of the mean field concentration of gliatransmitter (e.g., glutamate and D-serine). **(B)** A tripartite synapse with astrocytic feedback mediated by glutamate and D-serine provides activation of the postsynaptic neuron shown here as the mean field network neuron. The synapse is activated by an input spike train, f_{in}. There is also spontaneous release rate, f_0, that is independent of the input rate. In addition, the output spikes go through the network and return as separate inputs. The network impact is accounted for by the presence of the correlation, k_N, between the output spiking rate and the input frequency.

f_0. If $k_N \gg 1$, then the excitation circulation circuits are rapidly stimulating the neuron to its maximal hyperexcited state and may be considered as seizure-like dynamics. In the framework of our modeling approach, we described the network feedback variable f by the time scale parameter, τ_N, and formulate the feedback using a first-order linear relaxation equation:

$$\frac{df}{dt} = (k_N f_{\text{out}} + f_0 - f)/\tau_N. \quad (11)$$

The solution of Equation (11) defines the input frequencies $f_{\text{in}} = f(t)$ for simulation of the evoked responses.

Figure 4B shows a schematic illustration of the mean field model of synaptic transmission with network feedback.

The central element of the circuit is a network neuron that integrates EPSCs coming from a mean field synapse defined by Equations (1–11). The presynaptic dynamics is defined by the mean field concentration of the neurotransmitter released from the presynaptic terminals. This release is determined by the presynaptic spiking, f_{in}, and spontaneous release, f_0, incorporated in the presynaptic current I_{pre} to unify the model formalism. In the mean field approach, we assume that uncorrelated synaptic events occur at different spatial sites and modeled them by the Poisson distribution of event timings and by mean field variables in space. The astrocyte is represented by spatially distributed astrocytic processes that function independently to locally modulate synaptic dynamics. The Ca^{2+} transients in the astrocyte determined the concentration of gliotransmitters in the synaptic sites, which were determined by mean field concentration variables, Y_1 (glutamate) and Y_2 (D-serine). Overall, the neuronal response was characterized by the average spike frequency (**Figure 2**). The feedback is characterized by linear correlation between input and output firing rates according to Equation (11).

Constants and parameters used in simulations of Equations (1–11) are listed in **Table 1**.

Table 1 | Model parameters.

Parameter	Value	Description
α_x	0.1 ms^{-1}	Neurotransmitter clearance constant
k_{pre}	2	The efficacy of neurotransmitter release
α_I	0.1 ms^{-1}	Rate constant of EPSCs
b_0	5–50	Scaling factor of gamma-distribution
θ_x	0.2	Midpoint of activation function $S(x)$ (Equation 5)
k_x	0.05	Slope of activation function $S(x)$ (Equation 5)
α_1	0.01 ms^{-1}	Clearance constant of glutamate released from astrocyte
α_2	0.01 ms^{-1}	Clearance constant of D-serine released from astrocyte
$\Theta_{1,2}$	0.3	Midpoint of gliatransmitter activation function $H_{1,2}(x)$ (Equation 7)
$k_{1,2}$	0.1	Slope of activation function $H_{1,2}(x)$ (Equation 7)
γ_1	−0.8	Presynaptic feedback gain describing the influence of astrocytic glutamate on the average amount of released neurotransmitter
γ_2	0.4	Postsynaptic feedforward gain describing the influence of astrocytic D-serine on EPSCs amplitudes
f_0	0.02–0.03 kHz	Frequency of spontaneous activation of the synaptic transmission
k_N	3	Correlation coefficient determining the gain of the network feedback (Equation 10)
τ_N	0.1 ms^{-1}	Rate of network feedback

RESULTS

SIGNAL TRANSMISSION IN THE TRIPARTITE SYNAPSE

We considered the dynamics of the signal transmission in the tripartite synapse for different input frequencies. We analysed a condition where the glutamate released from the astrocyte depresses neurotransmitter release (Semyanov and Kullmann, 2000). By setting $\gamma_1 < 0$ in the model, we found that such a depression decreases the spiking response (**Figure 5A**). Importantly, the astrocytic feedback did not give any significant impact at the low and high input frequencies. For the low input frequencies, the probability of astrocytic activation was low (Pasti et al., 1997; Marchaland et al., 2008) and, thus, there was no gliotransmitter modulation of the presynaptic release. At the high input frequencies, the mean field concentration of the neurotransmitter reached its saturation level (all possible postsynaptic receptors were occupied) and the neuronal response was similar to that observed in the control condition without the astrocytic feedback.

Another effect of astrocytic activation was the D-serine-mediated potentiation of postsynaptic responses that we modeled by changes in the EPSCs amplitudes as a function of the gliotransmitter concentration Y_2. **Figure 5B** illustrates the response curve in a model of the synapse, with $\gamma_2 > 0$. The scaling of the EPSCs probability distribution caused a corresponding scaling of the spiking response curve. The mechanism of such scaling can be explained by D-serine mediated activation of postsynaptic NMDA receptors, and amplification of EPSCs amplitudes with the same level of occupancy of postsynaptic receptors with glutamate.

Interestingly, astrocytic activation can have both potentiating and depressing effects that contribute differently depending on the input frequency, if an astrocyte has both a reduction in neurotransmitter release and a postsynaptic upscaling of the EPSCs amplitudes (**Figure 5C**). Increasing the gain of presynaptic depression ($-\gamma_1$) led to quite different absolute values of frequency change, $\Delta f_{\text{out}} = |f_{\text{out}} - f_{\text{out}}(\gamma_{1,2} = 0)|$ for different intensities of the input (**Figure 5D**). For lower input frequency the impact of the γ_1, i.e. Δf_{out}, was higher. The opposite situation was for the postsynaptic upscaling gain, γ_2 (**Figure 5E**). The impact of γ_2 is more significant for higher values of the input frequency. Note that for large values of γ_2 the upscaling reached the saturation level (magenta dependence in **Figure 5E**).

NETWORK IMPACT

Because we are interested in the time averaged dynamics, it is important to estimate the steady-state functions of the network

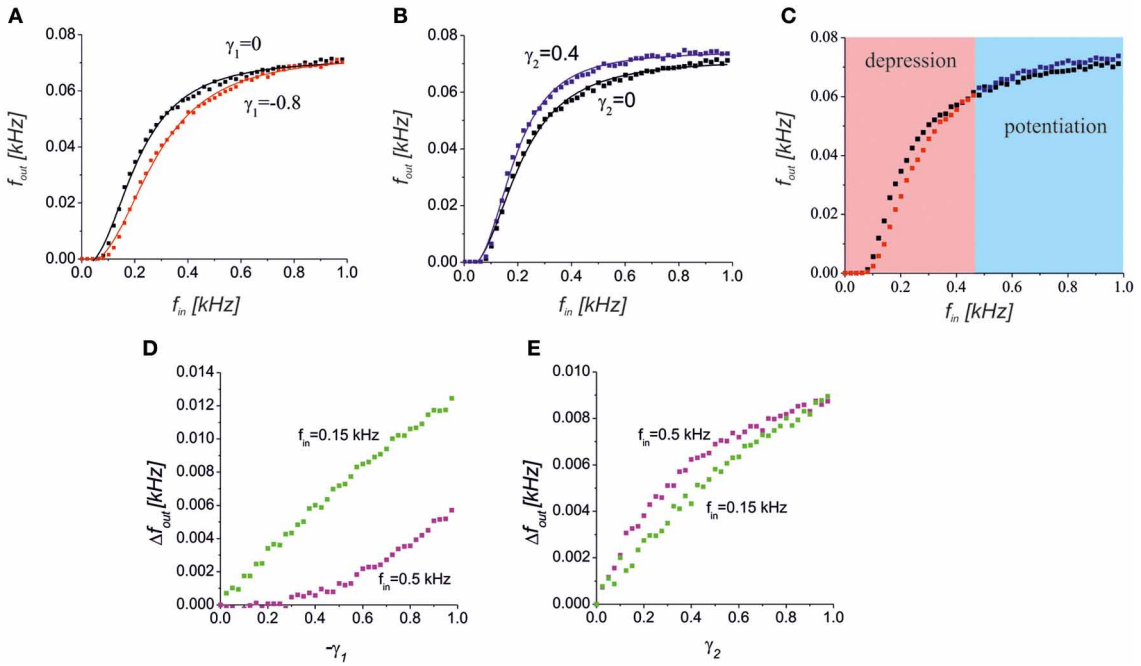

FIGURE 5 | (A) A reduction of the presynaptic release caused a decrease in the spiking response in the middle frequency band. The black and red points with the corresponding logistic curve fits show a response without ($\gamma_1 = 0$) and with ($\gamma_1 = -0.8$) astrocytic activation feedback, respectively. **(B)** Increase of the response spiking rate due to the D-serine-mediated postsynaptic feedforward effect for $\gamma_2 = 0.4$ (blue dots) relative to the control conditions (black dots). **(C)** The bidirectional effect of the astrocytic activation for both the presynaptic feedback and postsynaptic feedforward modulations for $\gamma_1 = -0.8$, $\gamma_2 = 0.4$. The red and blue areas show the frequency ranges when the response is depressed or potentiated, respectively. **(D,E)** The dependence of absolute output frequency changes $\Delta f_{\text{out}} = f_{\text{out}} - f_{\text{out}}(\gamma_{1,2} = 0)$ on the gain of presynaptic depression, γ_1, for $\gamma_2 = 0$ **(D)** and on the gain of postsynaptic upscaling, γ_2, for $\gamma_1 = 0$ **(E)**. Green and magenta points correspond to input frequencies $f_{\text{in}} = 0.5$ kHz and 0.15 kHz, respectively.

feedback. As we have illustrated in **Figure 2**, the neuronal response is converged to the input–output frequency curve, depending on the input frequency:

$$f_{\text{out}} = Q\left(f_{\text{in}}\right), \tag{12}$$

where the function $Q(f)$ can be approximated by a logistic curve (**Figure 2**). It is easy to determine that the network steady-state conditions will be given by the intersection points of the curves defined by Equations (10) and (12). There are three principle mutual arrangements of the steady-state curves (**Figure 6**). The level of network correlation is defined by the gain, k_N, which determines the slope of the line (10), $1/k_N$, in the phase plane (f_{in}, f_{out}). If the network gain, k_N, is small enough for low enough spontaneous activity, f_0, then the curves have a single intersection point with low activity (**Figure 6A**). In the linear relaxation limit, e.g., assuming that the dynamics of relaxation to the curve (12) and to the line (10) are independent, we find that the steady-state will be locally stable and for any initial conditions, the neuron-to-network dynamics will converge upon the state of low activity mainly defined by the spontaneous synaptic activation component, f_0. **Figure 6D** shows the evolution of the neuronal membrane potential in such conditions. The dynamics converges upon the state of low frequency and the network impact is negligible in this case. **Figure 6B** illustrates the opposite situation where there is a single intersection point in the upper branch of the frequency curve. Despite starting from low spontaneous activity, the network feedback brings the system to a relatively high spiking level (**Figure 6E**). The third alternative is a bistability when two stable states of low and high activity co-exist (**Figure 6C**). Depending on the initial conditions, the neuron may generate either high-frequency spiking, which is described as the "network-evoked" response or spontaneous firing. Interestingly, the application of a strong enough stimulus may come, for example, from another network group that may induce the switching of the neuron between the spontaneous and evoked modes, as illustrated in **Figure 6F**.

We further analysed the astrocytic impact on the synaptic transmission. In the steady-state approximation, we analysed the mutual arrangements of the curves under the influence of astrocytic feedback (**Figure 5**). A reduction of the presynaptic release may completely inhibit the activity by the network feedback in the middle frequency range (**Figure 7A**). A steady firing rate in control conditions (black dot in **Figure 7A**) shifts to a low firing level (red dot in **Figure 7A**) due to astrocytic activation. Increasing the gain of presynaptic depression above a critical value led to the transition to spontaneous firing defined by spontaneous release frequency f_0 (**Figure 7B**). In other words, the neuron may be temporally excluded from a coordinated network firing, which may be protective mechanism from hyperexcitation. Next, activation of astrocytes may modulate network firing dynamics by the emergence of bistability of the high- and the low-frequency firing

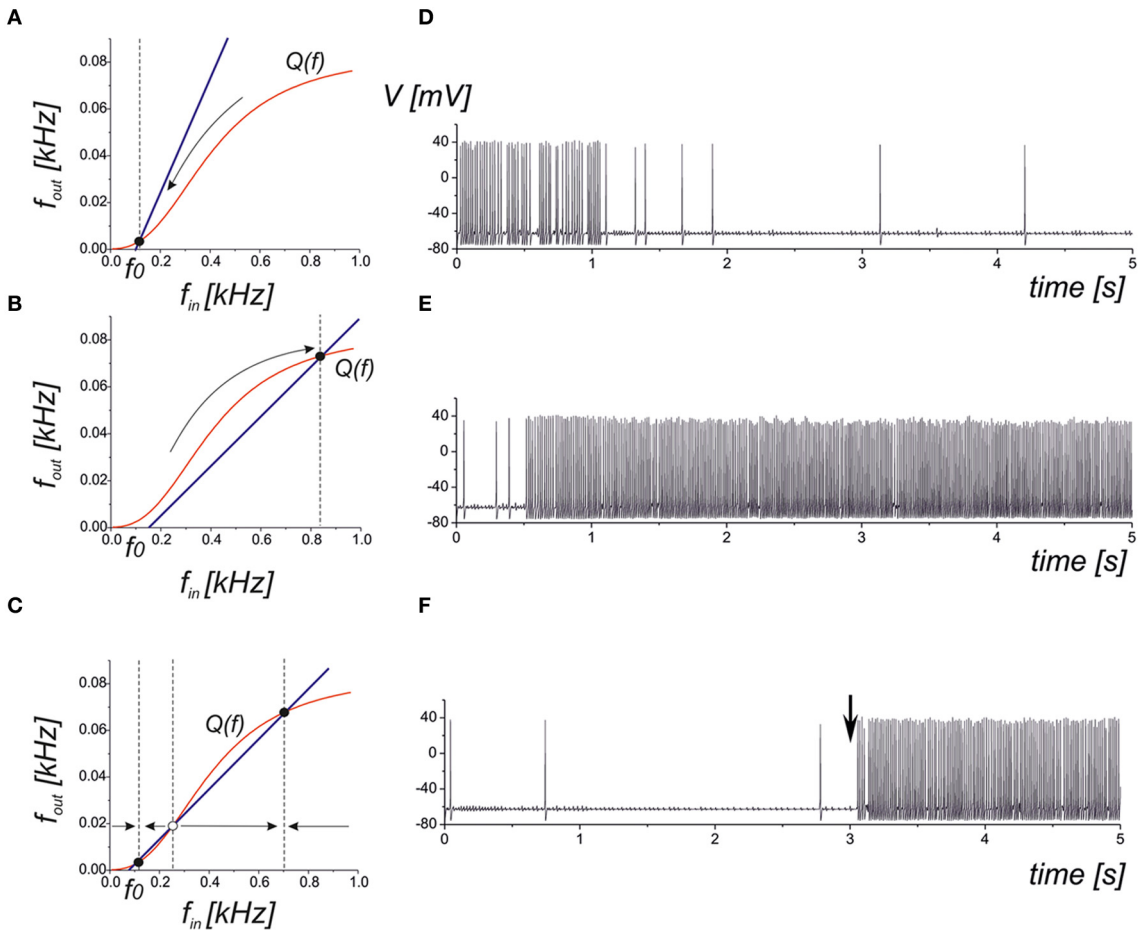

FIGURE 6 | Qualitative illustration of the network feedback dynamics in phase plane (f_{in}, f_{out}) and corresponding spiking sequences calculated from Equations (1–11). The curve $Q(f)$ represents the input–output characteristics of the tripartite synapse (red curve). The blue line shows the network feedback correlating with the output frequency and the input spike train in Equation (10). The f_0 is the frequency of spontaneous activation of the synaptic transmission. **(A)** Low activity mode. The neuronal dynamics are defined mainly by spontaneous firings. **(B)** High activity mode. The neuron fires at a high (close to saturation level) firing rate. **(C)** Bistable model. Low and high levels co-exist. Either level is realized depending on the initial conditions and/or due to the appropriate external stimulation. **(D)** Transition into spontaneous firing (panel **A**) for $k_N = 0.1$. **(E)** Transition into a higher activity state (panel B) for $k_N = 5$. **(F)** Bistability corresponding to phase plane in panel **(C)**. A stimulus in the form of a short high-frequency spike train injected into the input at $t = 3$ s induces the transition to a high activity level.

modes. **Figure 7C** illustrates the dependence of output firing rate on the strength of the correlation feedback. We assumed that without astrocytes the output rate was monotonic (black curve in **Figure 7C**). Bi-directional effect of astrocyte modulation leads to the appearance of two rate-encoded stable states of persistent neuronal firing for a certain range of feedback gains, k_N (red curves in **Figure 7C**). Importantly, the activation of astrocytes leads to two major modulation effects, as qualitatively demonstrated by the steady-state analysis. In particular, the threshold of the correlated firing leading to the high-activity state is changed due to the reduction of the neurotransmitter release probability. Thus, although models with a direct recurrent excitatory feedback can generate bistable neuronal firing (Koulakov et al., 2002; Goldman et al., 2003) in the absence of glial impact, the interval of bistability is broadening if the bi-directional effect of gliotransmitters on synaptic transmission is considered. In other words, in the presence of astrocytes the neuron can sustain its firing state (of high- or low-activity) for a wider range of network feedback, k_N.

Several experimental studies have reported that astrocytic activation changes the frequency of spontaneous EPSCs (see, for example, Jourdain et al., 2007; Perea and Araque, 2007). An interesting prediction is derived from our model in terms of their influence on network dynamics. Assuming that the neuron output and input are correlated, as stated by Equation (10), we still have a network-independent parameter f_0 describing the frequency of spontaneous presynaptic activation. For example, let the neuron state be tuned into its bistable mode as shown in **Figure 7D** and set to its spontaneous firing mode with low activity. Then, even a small transient increase in spontaneous frequency, $f_0 + \delta f_0$, may occur due to astrocytic activation, which leads to a transition to the high-activity state. Generally, the backward transition with decreasing spontaneous frequency,

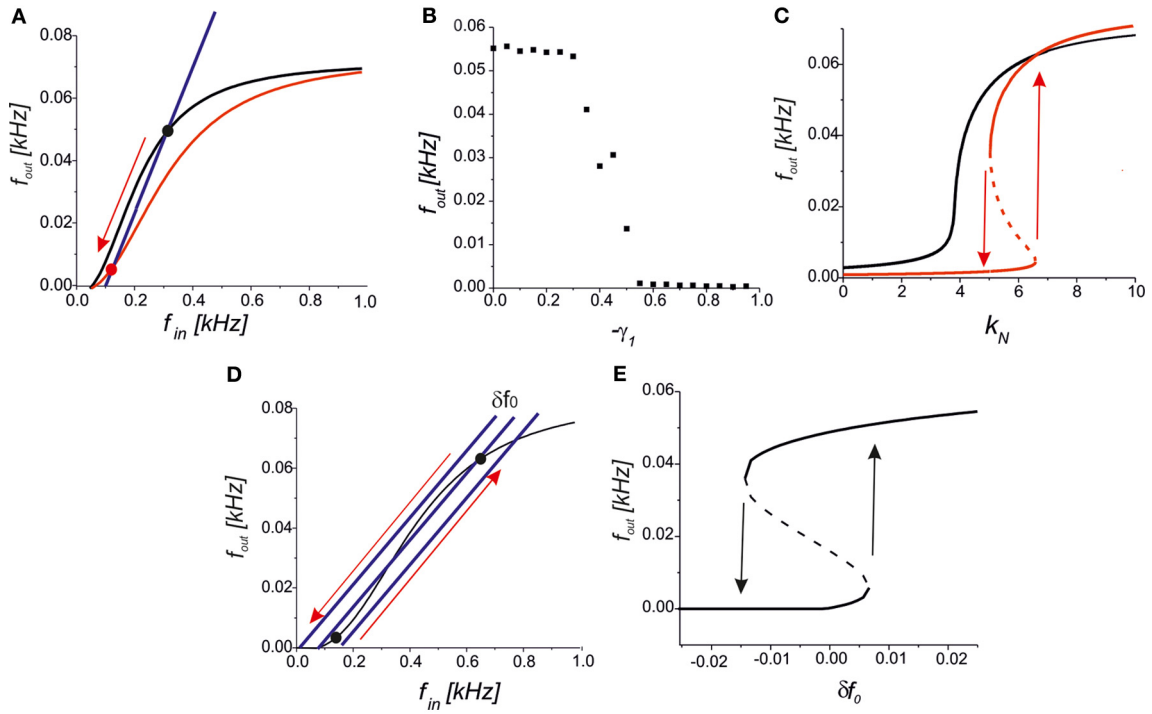

FIGURE 7 | (A) A qualitative view on the presynaptic feedback in the tripartite synapse leading to the suppression of the spiking activity of the network neuron. **(B)** The dependence of the firing rate on the gain of presynaptic feedback ($-\gamma_1$) for $f_0 = 0.09$ kHz, $k_N = 2.5$, $\gamma_2 = 0$. **(C)** Bifurcation diagram showing the output firing rate depending on the strength of the network feedback. For $f_0 = 0.07$ kHz the dependence is monotonic in control conditions (black curve). Interval of bistability appears for the bi-directional astrocyte feedback with $\gamma_1 = -0.8$, $\gamma_2 = 0.4$ (red curves). **(D)** A qualitative view of the firing rate changes due to small fluctuations in the spontaneous EPSCs frequency, δf_0. **(E)** Bifurcation diagram of output firing states depending on δf_0 for $k_N = 5$, $f_0 = 0.05$ kHz, $\gamma_{1,2} = 0$. Transitions between the low- and the high-activity states marked by arrows occur at the points of saddle-node bifurcations.

$f_0 - \delta f_0$, inhibits the neuron, excluding it from coordinated network firing (**Figure 7D**). **Figure 7E** illustrates the transitions between the low- and the high-activity states on a bifurcation diagram depending on the fluctuations of spontaneous frequency, δf_0.

To verify the predictions of the steady-state approximation, we simulated the model with a complete equation set to show how the output activity depends on the network impact for the synaptic transmission. **Figure 8** shows a bifurcation diagram of the average spiking rate depending on the network feedback gain, k_N. Increasing the gain led to a bistable dynamics marked by rectangle areas. Astrocyte activation shifted the boundary of bistability due to the depression of presynaptic release and enlarged the bistability interval due to the bi-directional regulation effects (red points in **Figure 8**).

DISCUSSION

We developed a computational model of astrocytic regulation of synaptic transmission predicting that the astrocytes can effectively modulate neuronal network firing. We considered a mean field network neuron. Network impact was modeled by a certain level of correlation between its output and input. Thus, the presynaptic dynamics consisted of spontaneous activity and the activity induced by the network feedback. The postsynaptic dynamics was modeled by a number of PSCs integrating at soma and leading to a spike generation. The astrocytic compartment was characterized by local release of glutamate and D-serine modulating the presynaptic release probability and the postsynaptic amplitudes of EPSCs, respectively.

Here we analysed only two potential effects of astrocyte feedback on the synapse: presynaptic depression of glutamate release and postsynaptic enhancement of these responses. These phenomena correspond to reports showing that astrocyte-released glutamate can reduce the release probability of neurotransmitter (Araque et al., 1998) while astrocytic release of D-serine enhances the response of postsynaptic NMDA receptors (Henneberger et al., 2010). Because the number of synapses to the target cell is finite, this limits the maximal amount of synaptic inputs, which can be simultaneously activated. Thus, the cell input still can be saturated even with decreased release probability by the increase in f_{in}. However, when f_{in} is low and does not saturate cell input, reduced release probability decreases f_{out}. The postsynaptic effect of gliotransmitter D-serine is principally different. D-serine is co-agonist of NMDA receptors and still required even if these receptors are bound to glutamate. Thus, D-serine increases the response of the postsynaptic cell to the same amount of glutamate (even saturating) because of additional recruitment of postsynaptic NMDA receptors. It allows a larger f_{out} at saturating conditions

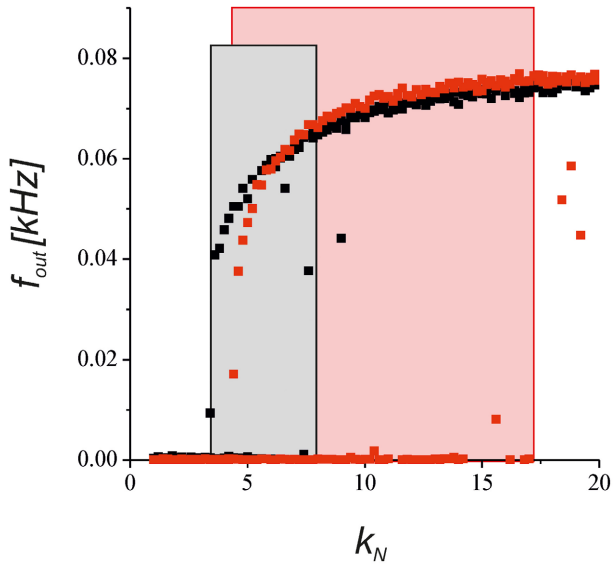

FIGURE 8 | The dependence of the neuronal firing rate on the network feedback gain, k_N, in Equations (1–11). The black points show the results for the synapse without the astrocytic feedback. The red dots illustrates the modulation by the astrocyte in the tripartite synaptic transmission for $\gamma_1 = -0.8$ and $\gamma_2 = 0.4$. The rectangular areas show the intervals of bistable dynamics where the two stable steady states for low and high activities co-exist. The model was simulated for the different initial conditions [low and high values of f_{in} $(t = 0)$] for each value of k_N.

at large f_{in}. When these presynaptic and postsynaptic effects coincide, f_{out} is reduced at low f_{in} because of a reduced release probability, but increases at a high f_{in} because of the increase in the level of saturation. Thus, simultaneous recruitment of two counteracting types of astrocytic modulation actually works as a high-pass filter. A similar phenomenon has been reported in hippocampal slices for glutamate acting presynaptically on both mGluRs and kainate receptors (Kullmann and Semyanov, 2002). mGluRs reduce the release probability, while kainate receptors increase presynaptic excitability. Co-activation of both types of receptors contrasted action potentials dependent synaptic GABA release versus spontaneous action potential independent release in CA1 interneurons. The dependence of the astrocytic effects on the density of receptors targeted by a gliotransmitter and on the efficiency of gliotransmitter clearance opens up the possibility that a relative weight of each influence is not a constant. These weights can be represented as a vector of parameters of gliotransmitter influence specific to the particular synapse. These vectors can be different for different synapses and determine differential modulation at different synapses by an equivalent amount of released gliotransmitter. Similar bi-directional changes in the efficacy of signal transmission by astrocytic modulation were recently found by De Pittà et al. (2011). They showed that gliotransmitter acting on presynaptic site can regulate the release differentially depending on frequency of input signal.

An interesting consequence of the proposed model for network computations is that such differential modulation can be effective in controlling network firing states. Indeed, we found that even small changes in the input–output function induced by transient astrocyte activations when superimposed with network feedback may lead to dramatic changes in neuron firing. In particular, the astrocyte may activate or deactivate specific neurons to be involved in network firing. Another interesting effect is the enforcement of the bistability by the astrocyte. Coexistence of two stable firing modes at the level of single neuron implies coexistence of multiple rate-encoded states in spiking networks. In such a treatment, the astrocytes may serve not only as local gate-keepers in synaptic transmission (Volman et al., 2006) but also as activity guiders coordinating information processing at the network level (Semyanov, 2008).

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Computational quest for understanding the role of astrocyte signaling in synaptic transmission and plasticity

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The complexity of the signaling network that underlies astrocyte-synapse interactions may seem discouraging when tackled from a theoretical perspective. Computational modeling is challenged by the fact that many details remain hitherto unknown and conventional approaches to describe synaptic function are unsuitable to explain experimental observations when astrocytic signaling is taken into account. Supported by experimental evidence is the possibility that astrocytes perform genuine information processing by means of their calcium signaling and are players in the physiological setting of the basal tone of synaptic transmission. Here we consider the plausibility of this scenario from a theoretical perspective, focusing on the modulation of synaptic release probability by the astrocyte and its implications on synaptic plasticity. The analysis of the signaling pathways underlying such modulation refines our notion of tripartite synapse and has profound implications on our understanding of brain function.

Keywords: astrocyte-synapse interactions, astrocyte modeling, calcium signaling, calcium encoding, gliotransmission, synaptic plasticity, metaplasticity, cortical maps

INTRODUCTION

The simultaneous recognition that astrocytes sense neighboring neuronal activity and release neuroactive agents (or "gliotransmitters") has been instrumental in the uncovering of the many roles played by these cells in the control of genesis, function and plasticity of synapses (Haydon, 2001; Ullian et al., 2004; Volterra and Meldolesi, 2005; Bains and Oliet, 2007; Santello and Volterra, 2009; Zorec et al., 2012). These findings initiated a conceptual revolution that leads to rethinking how brain communication works since they imply that information travels and is processed not just in the neuronal circuitry but in an expanded neuron-glial network (Haydon, 2001; Volterra and Meldolesi, 2005; Giaume et al., 2010). On the other hand the physiological need for astrocyte signaling in brain information processing and the modes of action of these cells in computational tasks remain largely undefined. This is due, to a large extent, both to the lack of conclusive experimental evidence, and to a substantial lack of a theoretical framework to address modeling and characterization of the many possible astrocyte functions. This review aims at introducing such a perspective providing a framework for future modeling efforts in the field based on preliminary theoretical studies on both astrocytic calcium signaling and gliotransmitter-mediated modulations of synaptic release probability.

Abbreviations: Adn, adenosine; AFM, amplitude and frequency modulation; AM, amplitude modulation; AMPA, 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl) propanoic acid; AMPAR, AMPA glutamate receptor; AP, action potential; ATP, adenosine triphosphate; Ca^{2+}, calcium; CICR, Ca^{2+}-induced Ca^{2+} release; D-ser, D-serine; EAAT, excitatory aminoacid transporter; ER, endoplasmic reticulum; FM, frequency modulation; GABA, γ-aminobutyric acid; GJC, gap-junction channel; Glu, glutamate; GluR, glutamate receptor; mGluRs, metabotropic glutamate receptor; GPCR, G_q protein-coupled receptor; IP-5P, inositol polyphosphate 5-phosphatase; IP_3, inositol 1,4,5-trisphosphate; IP_3-3K, IP_3 3-kinase; IP_3R, IP_3 receptor; LTD (LTP), long-term depression (potentiation); NMDA, *N*-methyl-d-aspartic acid; NMDAR, NMDA glutamate receptor; PLCδ (PLCβ), phospholipase Cδ (Cβ); PPR, paired-pulse ratio; PR, purinergic receptor; PSC, postsynaptic current; SIC, slow inward current; SNARE, soluble N-ethylmaleimide-sensitive factor attachment protein receptor; STDP, spike-timing-dependent plasticity; TACE, TNFα-converting enzyme; TNFα, tumor necrosis factor-α.

A THEORETICAL FRAMEWORK FOR ASTROCYTE-SYNAPSE INTERACTIONS

Control of synaptic transmission and plasticity by astrocytes subtends a complex signaling network, which involves different biochemical pathways (Volterra and Meldolesi, 2005; Zorec et al., 2012). In general, synaptically-released neurotransmitter can spill out of the synaptic cleft and bind to metabotropic receptors found on the neighboring astrocytic processes triggering there inositol 1,4,5-trisphosphate (IP_3)-mediated Ca^{2+} signaling. This was observed at both glutamatergic, cholinergic, noradrenergic, and GABAergic synapses in the hippocampus,

in the thalamus and in the cortex (Volterra and Meldolesi, 2005; Haydon and Carmignoto, 2006; Santello and Volterra, 2009; Halassa and Haydon, 2010; Navarrete et al., 2012a,b). **Figure 1A** summarizes a number of observations made at the level of hippocampal glutamatergic synapses (Bains and Oliet, 2007; Santello and Volterra, 2009). There, synaptic glutamate can trigger Ca^{2+} signaling in the surrounding astrocytic processes via metabotropic glutamate receptors (mGluRs) (Pasti et al., 1997; Fiacco and McCarthy, 2004; Panatier et al., 2011). There is also evidence in the dentate gyrus that ATP, possibly synaptically-released, triggers astrocytic Ca^{2+} signaling through the activation of metabotropic purinergic P_2Y_1 receptors (Jourdain et al., 2007; Di Castro et al., 2011; Larsson et al., 2011; Santello et al., 2011). Following elevation of intracellular Ca^{2+}, astrocytes can release glutamate as well as other chemical transmitters such as D-serine (D-ser) and ATP which can be converted into adenosine (Adn) in the extracellular milieu (Bezzi et al., 2004; Pascual et al., 2005; Montana et al., 2006; Henneberger et al., 2010; Parpura and Zorec, 2010). Astrocyte-released glutamate diffuses in the extrasynaptic space and may bind to glutamate receptors (GluRs), including mGluRs and NMDARs on neighboring presynaptic terminals, modulating the release of neurotransmitter (Fiacco and McCarthy, 2004; Jourdain et al., 2007; Perea and Araque, 2007; Bonansco et al., 2011; Di Castro et al., 2011). An analogous action on synaptic release could also be due to astrocyte-derived ATP and its derivative adenosine through presynaptic purinergic receptors (PRs), including both A_1 and A_2 receptors (Pascual et al., 2005; Halassa and Haydon, 2010; Panatier et al., 2011). On the postsynaptic side, astrocytic glutamate and D-serine may bind to extrasynaptic NR_2B-containing and postsynaptic NMDARs respectively, modulating neuronal firing and participating in the induction of long-term potentiation (Fellin et al., 2004; Bains and Oliet, 2007; Henneberger et al., 2010). Astrocyte could also release tumor necrosis factor-α (TNFα) by Ca^{2+}-dependent activation of TNFα-converting enzyme (TACE) (Bezzi et al., 2001; Santello and Volterra, 2012), which could strengthen excitatory synaptic transmission by promoting surface insertion of AMPA receptors (AMPARs) (Beattie et al., 2002; Stellwagen and Malenka, 2006; Bains and Oliet, 2007). This signaling route could also play a role in pathological states such as post-traumatic epilepsy (Balosso et al., 2009; Volman et al., 2011) or spinal cord injury (Stellwagen et al., 2005; Ferguson et al., 2008). On the other hand, extracellular levels of TNFα control glutamate release from astrocytes, ultimately modulating the astrocytic action on presynaptic function (Domercq et al., 2006; Santello et al., 2011). This intricate signaling network is further complicated by the possibility that astrocyte Ca^{2+} events are triggered by additional mechanisms, including the action of ATP released extracellularly by astrocytes themselves or of IP_3 that diffuses intracellularly, from one astrocyte to another, through gap junction (GJCs) (Kang et al., 2005; Scemes and Giaume, 2006). At Schaffer collateral synapses, astrocytic Ca^{2+} increases could also be promoted by retrograde endocannabinoid signaling from postsynaptic terminals via activation of endocannabinoid CB_1 receptors (Navarrete and Araque, 2008, 2010) (omitted from **Figure 1A** for clarity). Moreover, the relation between astrocytic Ca^{2+} and gliotransmitter release is not simple: some of the Ca^{2+} signals that can be generated in astrocytes are apparently not able to induce gliotransmitter release or its synaptic consequences (Fiacco et al., 2007; Agulhon et al., 2008, 2010; Petravicz et al., 2008; Lovatt et al., 2012), while gliotransmitters can also be released by mechanisms that are independent of Ca^{2+} signaling (Parpura and Zorec, 2010) (not included in **Figure 1A** for simplicity).

Despite its apparent complexity, the ensemble of astrocyte-synapse signaling interactions discussed above can be well captured by the modeling scheme of **Figure 1B**. This scheme shows the three essential components of astrocyte-regulated synapses, also referred to as "tripartite synapses" (Araque et al., 1999; Haydon, 2001): these are the pre- (PRE) and postsynaptic (POST) terminals, and the astrocyte, i.e., an astrocytic process surrounding the synaptic elements (ASTRO) (Araque et al., 1999; Haydon, 2001). Moreover, in addition to the classical neuronal path that leads from input presynaptic action potentials, commonly referred to as input spikes (IN), to the output postsynaptic current (OUT), further input and/or output pathways (I/O) could coexist due to the above-mentioned routes based on astrocytic Ca^{2+} signaling (Giaume et al., 2010).

Focusing on synapse-astrocyte coupling, three fundamental pathways are identified: one (**A**) from the synapse to the astrocyte whereby synaptically-released glutamate (or other synaptic agents) promotes Ca^{2+} signaling in the astrocyte and the other two (**B** and **C**) from the astrocyte to synaptic terminals, whereby glutamate or ATP released from the astrocyte affects synaptic function (Volterra and Meldolesi, 2005; Santello and Volterra, 2009). Additional pathways supported by other neuroactive agents such as D-serine or TNFα can also be evoked in parallel to those shown in **Figure 1B** but they would not alter the essence of the scheme. Moreover, although based on experimental results at excitatory synapses in the hippocampus, (Araque et al., 1998a,b; Fiacco and McCarthy, 2004; Jourdain et al., 2007; Perea and Araque, 2007; Andersson and Hanse, 2010; Santello et al., 2011), the modeling scheme in **Figure 1B** could also hold for other reported pathways such as GABA-evoked gliotransmission at interneuron-to-pyramidal cell synapses in the hippocampus (Kang et al., 1998; Serrano et al., 2006), or glia-mediated ATP release at hippocampal synapses (Pascual et al., 2005), in the hypothalamus (Gordon et al., 2009), and in the retina (Newman, 2003, 2005), or glial modulation of neuromuscular transmission (Robitaille, 1998; Rousse et al., 2010; Todd et al., 2010) (see **Table 1** for a summary of the possible signaling pathways).

Analysis of the scheme in **Figure 1B** reveals that astrocytes mediate two loops in the signal flow from presynaptic to postsynaptic terminal: a *feedforward* and a *feedback* one. The feedforward loop ends on the postsynaptic terminal and is activated when synaptic glutamate and/or ATP induces glutamate and/or D-serine release from the astrocyte to the postsynaptic element, i.e. the **A**–**C** path in **Figure 1B** (Bains and Oliet, 2007; Barres, 2008; Santello and Volterra, 2009). The feedback loop ends on the presynaptic terminal (the **A**–**B** path in **Figure 1B**) and is activated when synaptic glutamate or ATP trigger Ca^{2+}-dependent release of glutamate and/or ATP from the astrocyte to the presynaptic terminal, leading to modulation of synaptic release through specific

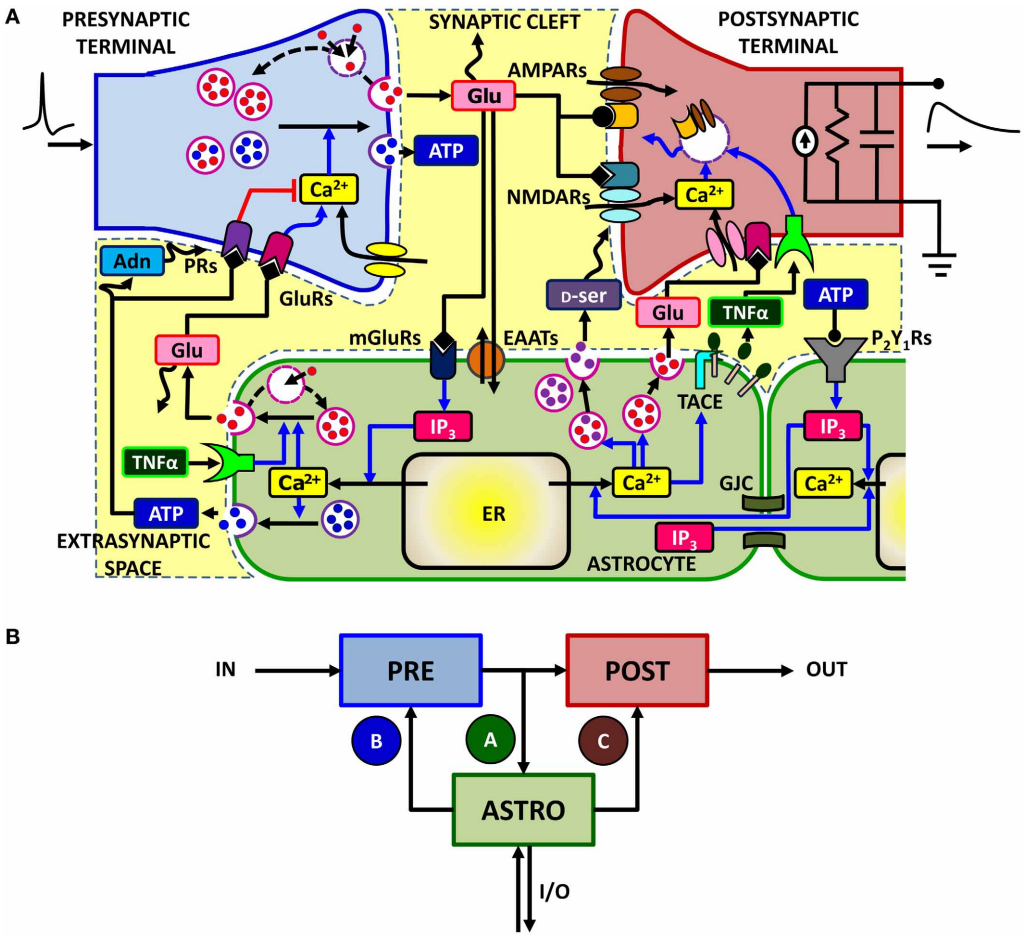

FIGURE 1 | The signaling network of astrocyte-synapse interactions. **(A)** A simplified scheme of the different signaling pathways between synaptic terminals and astrocytes for the case of excitatory synapses in the hippocampus (see text for a detailed description). Action potentials arriving at the presynaptic terminal trigger release of glutamate, which can spill over from the synaptic cleft. Perisynaptic astrocytes take up glutamate using their plasma membrane transporters (EAATs) while glutamate, by acting on astrocytic metabotropic receptors (mGluRs), triggers Ca^{2+} signaling in the astrocyte. This signaling pathway includes production of IP_3 and causes an increase of cytosolic Ca^{2+} due to efflux of this ion from the endoplasmic reticulum (ER). At some synapses, such as in the dentate gyrus, the same Ca^{2+} signaling pathway could also be mediated by astrocytic purinergic P_2Y_1 receptors, likely activated by synaptically-released ATP (see text for details). Astrocytic Ca^{2+} excitability can in turn lead to exocytotic release of several neuroactive substances (or "gliotransmitters") such as glutamate (Glu), D-serine (D-ser) or ATP which can target specific receptors on pre- and post-synaptic terminals and differentially modulate synaptic transmission. Glutamate acting on presynaptic GluRs could enhance synaptic release, whereas ATP and its derivate adenosine (Adn) could depress it (*red path*) through presynaptic purinergic receptors (PRs). On the postsynaptic spines [depicted here by a standard RC circuit (Ermentrout and Terman, 2010)], the ensuing effect of gliotransmitters could substantially modify postsynaptic currents by enhancing activation of NMDA receptors (D-serine) or by altering expressions of AMPA receptors therein. Astrocytes could also release TNFα by Ca^{2+}-dependent activation of the matrix metalloprotease TNFα-converting enzyme (TACE), while extracellular TNFα could in turn regulate glutamate release from the astrocyte as well as postsynaptic AMPAR expression. Moreover astrocytic Ca^{2+} could also propagate across different regions of the same cell or to other neighboring astrocytes by intracellular IP_3 diffusion through gap junction channels (GJCs) or via extracellular ATP-dependent pathways, extending gliotransmission to some distal sites away from the considered synapse. For clarity both endocannabinoid-mediated Ca^{2+} signaling (Navarrete and Araque, 2008), retrograde activation of presynaptic glutamate receptors (Navarrete and Araque, 2010), regulation of postsynaptic NMDARs by presynaptic adenosine receptors (Deng et al., 2011), and the possibility for astrocyte-derived adenosine to enhance synaptic release (Panatier et al., 2011) are not included in this scheme. **(B)** Equivalent modeling scheme for astrocyte-synapse interactions. The astrocyte (ASTRO) constitutes a third active element of the tripartite synapse in addition to the presynaptic (PRE) and postsynaptic (POST) terminals. In its presence, the flow of input (IN) signals to the output (OUT) is no more unidirectional but presynaptically released neurotransmitter can affect astrocyte function through the interaction pathway *A*. In turn, the astrocyte can regulate both synaptic terminals via pathways *B* and *C*. In addition, the astrocyte could receive additional inputs from or send output to remote synapses in a heterosynaptic fashion (I/O).

presynaptic receptors (Santello and Volterra, 2009; Halassa and Haydon, 2010).

In principle the two pathways could coexist at the same synapse where they are expected to display different dynamics and respond to different preferred input stimuli. Therefore their coexistence at the same synapse could give rise to complex effects that are hard to quantify when considered altogether. Accordingly, a common approach in experiments is to characterize their effects

Table 1 | Transmitters, targeted receptors, and major effects on synaptic transmission by the signaling pathways A, B, C in Figure 1B (*in situ* and *in vivo* studies only).

References	Sp.[1]	Prep.[2]	Area[3]	Syn. neurotr.[4]	Targeted receptor[5]	Gliotr.[6]	Targeted receptors[5]		Cell[7]	Effects[8]
					Pathway A		Pathway B	Pathway C		
Wang et al., 2006	M	VV	BC	Glu	mGluR-I					
Porter and McCarthy, 1996	R	ST	CA1	Glu	mGluR, iGluR					
Perea and Araque, 2007	R	ST	CA1			Glu	mGluR-I		PY	↑synaptic release probability
Liu et al., 2004a,b	R	ST	CA1			Glu	KAR		IN	↓mIPSCs (frequency)
Liu et al., 2004a,b	R	ST	HIP			Glu	mGluR-II/III		IN	↑synaptic release probability
Bardoni et al., 2010	R	ST	DH			Glu		NMDAR	SGN	SICs; synchronous Ca^{2+} elevations in neighboring neurons
Kang et al., 2005	R	ST	CA1			Glu		iGluR	PY	SICs
Parri et al., 2001	R	ST	VBT			Glu		NMDAR	TCN	SICs
Bezzi et al., 1998	R	ST, VT	CA1, VC, COR			Glu		GluR	PY	Ca^{2+} elevations in neighboring neurons
Newman and Zahs, 1997	R	ST	Ret^m			Glu*		iGluR	AC*	↑neuronal inhibition
Bonansco et al., 2011	R	ST	CA1			Glu	mGluR-I	NMDAR	PY	↑mEPSCs (frequency); SICs; control of t-LTP induction
Fiacco and McCarthy, 2004	M	ST	CA1	n.r.	n.r.	Glu	mGluR-I		PY	↑synaptic release probability
Pasti et al., 1997	R	ST	CA1, VC	Glu	mGluR	Glu*			PY	Ca^{2+} elevations in neighboring neurons
Pirttimaki et al., 2011	R	ST	VBT	n.r.	mGluR-I	Glu		NR_2B-NMDAR	TCN	Long-term enhancement of SIC frequency
D'Ascenzo et al., 2007	M	ST	NAcc	Glu	$mGluR_5$	Glu		NR_2B-NMDAR	MSN	SICs, ↑neuronal firing
Fellin et al., 2004	R	ST	CA1	Glu	n.r.	Glu		NR_2B-NMDAR	PY	SICs; synchronous Ca^{2+} elevations in neighboring neurons
Newman, 2005	R	ST	Ret^m	ATP	n.r.					
Piet and Jahr, 2007	R	ST	CER^b	Glu, ATP	AMPAR, P_2YR					
Rieger et al., 2007	M	ST	OB	Glu, ATP	$mGluR_1$, P_2Y_1R					
Beierlein and Regehr, 2006	R	ST	CER^b	Glu, ATP	$mGluR_1$, P_2YR					
Newman, 2003	R	ST	Ret^m			ATP		A_1R	RN	↑K^+ conductance; ↑neuronal inhibition

(Continued)

Table 1 | Continued

References	Sp.[1]	Prep.[2]	Area[3]	Syn. neurotr.[4]	Targeted receptor[5]	Gliotr.[6]	Targeted receptors[5]		Cell[7]	Effects[8]
					Pathway A		Pathway B	Pathway C		
Torres et al., 2012	M	ST	CA1			ATP		P_2Y_1R	IN	↑neuronal firing
Schmitt et al., 2012	M	ST, VV	HIP	n.r.	n.r.	ATP	A_1R		PY	↓fEPSP (slope)
Di Castro et al., 2011	R	ST	MLDG	ATP	P_2Y_1R	n.r.	n.r.		GC	↑synaptic release probability
Halassa et al., 2009	M	VV	COR	n.r.	n.r.	ATP	A_1R		CN	↓fEPSP (slope)
Jourdain et al., 2007	R	ST	MLDG	ATP*	P_2Y_1R	Glu	NR_2B-NMDARs		GC	↑synaptic release probability
Panatier et al., 2011	R	ST	CA1	Glu	$mGluR_5$	ATP	$A_{2A}R$		PY	↑synaptic release probability
Schipke et al., 2008	M	ST	BC	Glu	mGluR-I	ATP	A_1R, P_2YR		PY	↑neuronal inhibition; confinement of astrocytic Ca^{2+} signals
Pascual et al., 2005	M	VT, ST	CA1	Glu	n.r.	ATP	A_1R		PY	↓fEPSP (slope); heterosynaptic depression; control of long-term plasticity (metaplasticity)
Zhang et al., 2003, 2004a	R	ST, VT	CA1	Glu	n.r.	ATP	A_1R, P_2YR		PY	↓EPSP (amplitude); heterosynaptic depression
Todd et al., 2010	F	VT	NMJ	n.r.	n.r.	ATP	A_1R, $A_{2A}R$		MF	PTD (A_1R); PTP ($A_{2A}R$)
Robitaille, 1998	F	VT	NMJ			n.r.	n.r.		MF	PTP; PTD
Bowser and Khakh, 2004	M	ST	CA1	ATP, Glu	P_2Y_1R, mGluR-I	ATP		P_2Y_1R	IN	SICs, ↑neuronal firing
Gordon et al., 2009	R	ST	PVN	Glu	mGluR-I	ATP		P_2XR	MNC	↑mEPSCs (amplitude)
Araque et al., 2002	R	ST	CA1	ACh	mAChR					
Bélair et al., 2010	F	VV	NMJ	ACh, ATP	mAChR, P_2YR, P_2XR					
Navarrete et al., 2012a,b	R	ST, VV	CA1	ACh	mAChR	Glu	mGluR		PY	↑synaptic release probability; LTP
Chen et al., 2012	M	VV, ST	V1	ACh	mAChR	n.r.		NMDAR	V1N	SICs, ↑neuronal firing
Perea and Araque, 2005	R	ST	CA1	ACh, Glu	mAChR, mGluR	Glu		NMDAR	PY	SICs
Bekar et al., 2008	M	VV	COR	NE	αAR					
Kulik et al., 1999	M	ST	CER[b]	NE	α_1AR					

(Continued)

Table 1 | Continued

References	Sp.[1]	Prep.[2]	Area[3]	Syn. neurotr.[4]	Targeted receptor[5]	Gliotr.[6]	Targeted receptors[5]		Cell[7]	Effects[8]
					Pathway A		Pathway B	Pathway C		
Gordon et al., 2005	R	VT, ST	PVN	NE	α_1AR	ATP		P_2X_7R	MNC	↑mEPSCs (amplitude)
Min and Nevian, 2012	R	ST	BC	ECB^r	CB_1R	Glu	NMDAR		PY	↓EPSP (slope), t-LTD
Navarrete and Araque, 2010	M	ST	CA1	ECB^r	CB_1R	Glu	mGluR		PY	↑synaptic release probability
Navarrete et al., 2012a,b	H	ST	HIP, COR	n.r.	GluR, PR, CBR	Glu		NMDAR	PY	SICs
Navarrete and Araque, 2008	M	ST	CA1	ECB^r	CB_1R	Glu		NMDAR	PY	SICs
Panatier et al., 2006	R	ST	SON			D-ser		NMDAR	MNC	Control of long-term plasticity (metaplasticity)
Takata et al., 2011	M	VV	BC	ACh	mAChR	D-ser		NMDAR	PY	Control of LTP induction
Henneberger et al., 2010	R	ST	CA1	n.r.	n.r.	D-ser		NMDAR	PY	Control of LTP induction
Fellin et al., 2009	M	VV, ST	COR, HIP	n.r.	n.r.	D-ser		NMDAR	CN, PY	↑NMDAR-mediated currents
Yang et al., 2003	R	ST, VT	CA1	Glu	n.r.	D-ser		NMDAR	PY	Control of LTP induction
Lee et al., 2011	H	VT	COR			GABA				
Le Meur et al., 2012	R	ST	HIP			GABA, Glu		$GABA_AR$, NMDAR	PY	SOCs ($GABA_AR$); SICs (NMDAR)
Lee et al., 2010	M	ST	CER^b			GABA		$GABA_AR^s$	GC, PF^s	↑neuronal inhibition[s]
Kozlov et al., 2006	R	ST	OB			GABA, Glu		$GABA_AR$, NMDAR	MC	SOCs ($GABA_AR$); SICs (NMDAR)
Serrano et al., 2006	R	ST	CA1	GABA	$GABA_BR$	ATP	A_1R		PY	↓fEPSP (amplitude); heterosynaptic depression
Kang et al., 1998	R	ST	CA1	GABA	$GABA_BR$	n.r.	iGluR	iGluR	PY	↑mIPSCs (frequency and amplitude)
Stellwagen and Malenka, 2006	M	VT	HIP			TNFα		n.r.	PY	↑AMPAR; LTP
Beattie et al., 2002	R	ST, VT	HIP			TNFα		n.r.	PY	↑AMPAR
Santello et al., 2011	M	ST, VT	MLDG			TNFα, Glu				Control of Glu exocytosis from astrocytes
Bezzi et al., 2001	R, H	ST, VT	HIP			TNFα, Glu				Control of Glu exocytosis from astrocytes

(Continued)

Table 1 | Continued

Reference to in vitro studies is included whenever the latter are missing.

[1] Specimen. F, frog; H, human; M, mouse; R, rat.

[2] Preparation. VI, in vitro/cultures; ST, in situ/slices; VV, in vivo.

[3] Brain area or body area. BC, barrel cortex; CA1, Cornu Ammonis area 1; CER, cerebellum; COR, cortex, DH, dorsal horn; HIP, hippocampus; MLDG, molecular layer dentate gyrus; NAcc, nucleus accumbens; NMJ, neuromuscular junction; PVN, paraventricular nucleus of the hypothalamus; Ret, retina; VBT, ventrobasal thalamus; V1, primary visual cortex; VC, visual cortex.

[4] Synaptically-released neurotransmitter. Ach. acetylcholine; ECB, endocannabinoids; NE, norepinephrine.

[5] Astrocytic receptors targeted by synaptically-released neurotransmitters (pathway A) and neuronal receptors targeted by gliotransmitters (pathways B and C). αAR (α_1AR), α- (α_1-) adrenergic receptors; CBR, cannabinoid receptors; iGluR, ionotropic glutamate receptors; KAR, kainate receptor; mAChR, muscarinic receptors; mGluR-I, mGluR-II/III, group I (II/III) metabotropic glutamate receptors.

[6] Gliotransmitter released from astrocyte.

[7] Cell targeted by gliotransmitters, where targeted receptors and effects were reported. AC, amacrine cell; CN, cortical neuron; GC, granule cell; IN, interneuron; MF, muscle fiber; PF, parallel fiber axon; PY, pyramidal neuron; MNC, magnocellular neurosecretory cell; MSN, medium spiny neurons; SGN, substantia gelatinosa neuron; RN, retinal neuron; V1N, V1 excitatory neuron

*[8] Effects triggered by gliotransmission on pathways B or C in **Figure 1B**. (f)EPSP, (field) excitatory postsynaptic potential; mEPSC (m)PSC), miniature excitatory (inhibitory) postsynaptic current; t-LTP (t-LTD), spike-timing-dependent long-term potentiation (depression); LTP, long-term potentiation; PTD, post-tetanic depression; PTP, post-tetanic potentiation; SICs, slow inward (depolarizing) currents; SOCs, slow outward (hyperpolarizing) currents. Arrows denote increase (↑) or decrease (↓) and are followed by the associated signal, e.g. ↑(↓)EPSP (frequency)(amplitude)(slope): increase (decrease) in (frequency)(amplitude) (slope) of EPSPs. fEPSP, field EPSP.*

*Other: b, study on Bergmann glia cells; r, retrograde signaling; s, suggested by investigators; m, study on Müller glia cells; n.r., when existence of a specific signaling pathway is observed but details are not reported; * indirect evidence.*

on synaptic function by separate manipulations of pathways **A–C** (**Figure 1B**) using different techniques (Montana et al., 2004; Jourdain et al., 2007; Marchaland et al., 2008; Di Castro et al., 2011; Panatier et al., 2011; Santello et al., 2011). For example, characterization of the feedback pathway on the presynaptic terminal (**A–B**) can be carried out by opening the feedback loop (for example by inhibiting elements of **A** or **B**) and analyzing the signaling components separately. From a theoretical point of view, this approach is put forth by at least three steps of analysis that are: (1) characterization of Ca^{2+} dynamics in the astrocyte as a function of different synaptic inputs (that is, pathway **A**); (2) characterization of how gliotransmitter release from the astrocyte depends on different astrocytic intracellular Ca^{2+} dynamics; and (3) characterization of the effect on synaptic release of Ca^{2+}-dependent gliotransmitter release from the astrocyte (i.e. pathway **B**). These three aspects are discussed below from a modeling perspective, focusing on their possible roles in synaptic information processing.

CHARACTERISTICS OF ASTROCYTE Ca^{2+} EXCITABILITY AND ITS RELATIONSHIP WITH SYNAPTIC ACTIVITY

Intracellular Ca^{2+} elevations in the astrocyte are not simple on–off signals (Carmignoto, 2000; Zonta and Carmignoto, 2002; Di Castro et al., 2011). There are multiple and varied spatiotemporal patterns of Ca^{2+} elevation, which probably underlie different types of function, including generation of diverse output signals (Carmignoto, 2000; Volterra and Meldolesi, 2005; Zorec et al., 2012). Two main types of neuronal activity-dependent Ca^{2+} responses are observed in astrocytes (Grosche et al., 1999; Codazzi et al., 2001; Matyash et al., 2001; Zonta and Carmignoto, 2002; Scemes and Giaume, 2006): (1) transient Ca^{2+} increases that are confined to their distal processes (Pasti et al., 1997; Nett et al., 2002; Di Castro et al., 2011) and (2) Ca^{2+} elevations propagating along these processes as regenerative Ca^{2+} waves, often eventually reaching the cell soma (Pasti et al., 1997; Sul et al., 2004). This latter kind of response can even propagate to neighboring astrocytes, giving rise to intercellular Ca^{2+} waves (Tian et al., 2005; Kuga et al., 2011). On the other hand, intercellular Ca^{2+} propagation does not necessarily need propagation through the cell soma and has been observed across astrocytic processes or from an end foot to an end foot (Mulligan and MacVicar, 2004; Giaume et al., 2010).

The precise signaling cascades underlying the various forms of Ca^{2+} elevation are not completely understood. In general, Ca^{2+} signals in astrocytes are determined by an intricate interplay of amplification, buffering, and extrusion pathways linked to cytosolic Ca^{2+} elevations mediated by influx from the extracellular space (Malarkey et al., 2008; Shigetomi et al., 2011) and/or release from intracellular endoplasmic reticulum (ER) stores (Verkhratsky et al., 2012). IP_3-triggered Ca^{2+}-induced Ca^{2+} release (CICR) from the ER is considered the primary mechanism responsible for intracellular Ca^{2+} dynamics in astrocytes (Volterra and Meldolesi, 2005; Nimmerjahn, 2009). This mechanism, schematized in **Figure 2A**, is essentially controlled by the interplay of three fluxes: (1) a Ca^{2+} transfer from the cytosol to the ER (J_P) mediated by endoplasmic-reticulum Ca^{2+}-ATPase (SERCA) pumps which contributes to the maintenance of higher

Ca^{2+} concentrations in the ER stores than in the cytosol; (2) a passive Ca^{2+} leak (J_L) from the ER to the cytosol that is driven by the Ca^{2+} gradient between the ER and the cytosol; and (3) an efflux (J_{NL}) from the ER to the cytosol through IP_3 receptor (IP_3R) channels, which depends both on IP_3 and Ca^{2+} concentrations in the cytosol in a nonlinear fashion (Bezprozvanny et al., 1991; Ramos-Franco et al., 2000; Shinohara et al., 2011).

Cytosolic Ca^{2+} regulates IP_3Rs in a biphasic manner: Ca^{2+} release from the ER is potentiated at low cytosolic Ca^{2+} concentrations but is inhibited at higher Ca^{2+} concentrations (Iino, 1990; Bezprozvanny et al., 1991). On the other hand, IP_3 monotonically activates IP_3R channels at constant Ca^{2+} concentrations (Watras et al., 1991), but dynamically changes the Ca^{2+} sensitivity of the channel (Kaftan et al., 1997; Ramos-Franco et al., 2000; Mak et al., 2003). At low, subsaturating IP_3 concentrations, the optimal Ca^{2+} concentration for IP_3R modulation becomes lower, whereas at very high IP_3 concentrations, channel activity persists at supramicromolar Ca^{2+} concentrations (Kaftan et al., 1997; Mak et al., 2003). Thus, the level of IP_3 determines the dynamics of intracellular Ca^{2+}.

Both production and degradation of IP_3 depend on enzymes that are regulated by cytosolic Ca^{2+} (Berridge et al., 2003; De Pittà et al., 2009a,b). These include Ca^{2+}-dependent PLCδ-mediated IP_3 synthesis and Ca^{2+}-dependent IP_3 degradation by IP_3 3-kinase (IP_3-3K) and by inositol polyphosphate 5-phosphatase (IP-5P) (**Figure 2A**) (Zhang et al., 1993; Sims and Allbritton, 1998; Rebecchi and Pentyala, 2000). However, while the activity of IP_3-3K is stimulated by cytosolic Ca^{2+} (Communi et al., 1997), IP-5P is inhibited instead (Communi et al., 2001). This results in different mechanisms of IP_3 degradation depending on the Ca^{2+} concentration in the cytoplasm (Sims and Allbritton, 1998; Irvine et al., 2006). Thus, for example, for equally-expressed enzymes, at low Ca^{2+} concentrations, namely lower than 500 nM (Sims and Allbritton, 1998; De Pittà et al., 2009a,b), IP_3 degradation is promoted by both IP-5P and IP_3-3K, whereas for intermediate-to-high cytosolic Ca^{2+} concentrations, degradation by IP_3-3K becomes predominant (Sims and Allbritton, 1998). Theoretical investigation showed that the interplay of these two regimes is both necessary and sufficient to reproduce Ca^{2+} oscillations and pulsations observed experimentally (De Pittà et al., 2009a,b).

Intracellular levels of IP_3 can also be controlled by gap junction mediated diffusion of IP_3 from other regions of the same astrocyte or from neighboring cells (Giaume et al., 2010) (i.e., the I/O pathway in **Figure 1B**). Moreover, synaptic glutamate (or other synaptic agents) can bind to astrocytic G protein-coupled receptors (GPCRs) like mGluRs that are directly linked to intracellular IP_3 production by PLCβ (Zur Nieden and Deitmer, 2006) (pathway **A** in **Figure 1B**). In this fashion, astrocytic Ca^{2+} dynamics triggered by synaptically-controlled IP_3 production can be regarded as a form of encoding information about activity in neighboring synapses.

Encoding of synaptic activity by astrocytic Ca^{2+} is likely multimodal, depending on many possible intracellular properties (De Pittà et al., 2008, 2009a,b; Dupont et al., 2011). A widely adopted classification considers the amplitude and the frequency of Ca^{2+} increases from resting levels (Berridge, 1997; Falcke, 2004; De Pittà et al., 2008, 2009a,b). In this view, as summarized in **Figure 2B**, synaptic activity reflected by different intracellular IP_3 concentrations (STIMULUS), is encoded by the modulation of Ca^{2+} oscillations and pulsations either in their amplitude (AM), their frequency (FM), or both (AFM). While available experimental data suggest a preferential FM mode of encoding (Pasti et al., 1997), AM and AFM encoding of synaptic activity are also plausible mechanisms given that the amplitude of Ca^{2+} response can strongly depend on the stimulation intensity (Wang et al., 2006; Di Castro et al., 2011; Panatier et al., 2011; Torres et al., 2012). This is the case for example, of synaptic inputs that occur rapidly one after the other whereby the ensuing intracellular Ca^{2+} concentration builds up as a cumulation of such inputs (Perea and Araque, 2005; Torres et al., 2012). In further support of the AM/AFM encoding is the experimental observation that glutamate exocytosis from the astrocyte occurs only when Ca^{2+} increases beyond a threshold concentration (Newman and Zahs, 1997; Parpura and Haydon, 2000; Pasti et al., 2001; Auld and Robitaille, 2003; Montana et al., 2006). Hence, astrocytic Ca^{2+} increases in response to synaptic activity would not systematically trigger the release of glutamate or other gliotransmitters from the astrocyte. Acting on the amplitude of astrocytic Ca^{2+} signals, AM/AFM encodings could constitute a way to regulate astrocytic gliotransmitter release by synaptic activity. Further experiments are needed to elucidate the nature of the Ca^{2+} threshold for astrocytic exocytosis since this latter might be gliotransmitter-specific (Montana et al., 2006). Accordingly, AM/AFM encoding of Ca^{2+} dynamics could vary from one gliotransmitter to another.

Experimental evidence suggests that Ca^{2+} dynamics does not simply mirror synaptic activity but is more complex, to a point that astrocytes have been proposed to perform genuine processing of synaptic information (Perea and Araque, 2005; Perea et al., 2009). This possibility follows from the complex network of IP_3 and Ca^{2+} signaling and subtends a scenario where Ca^{2+} could be only one of the players in the encoding and processing of synaptic activity by astrocytes (Mishra and Bhalla, 2002). Yet many, if not all, of the other signals underlying the complex cascade of biochemical reactions that link synaptically-released glutamate to CICR, could also carry out encoding and processing (Barlow, 1996; Berridge et al., 2003). Theoretical investigations suggested that IP_3 could also encode for the glutamate stimulation levels via a systematic AFM encoding (De Pittà et al., 2009a,b) as shown in **Figure 2C**. When cytosolic Ca^{2+} levels are low, close to resting values, IP_3 generally increases with ongoing synaptic activity (Glu). With low Ca^{2+}, the activity of IP_3-3K is reduced and the resulting IP_3 degradation slows down. The contribution to IP_3 production by Ca^{2+}-dependent PLCδ is reduced as well, so that intracellular IP_3 mostly depends on the frequency of synaptic release. Rapid successions of synaptic release events produce crisp increases of IP_3 (essentially proportional to the number of successive synaptic release events) while, between two remote release events, IP_3 tends to relax to resting levels. As a result, IP_3 dynamics overall evolves as the integral of synaptic activity. If IP_3 reaches the CICR-triggering threshold, intracellular Ca^{2+} increases fast and so does IP_3-3K activity. Then, IP_3 is rapidly degraded and resting IP_3 levels are restored, thus resetting the integral of synaptic activity to initial values.

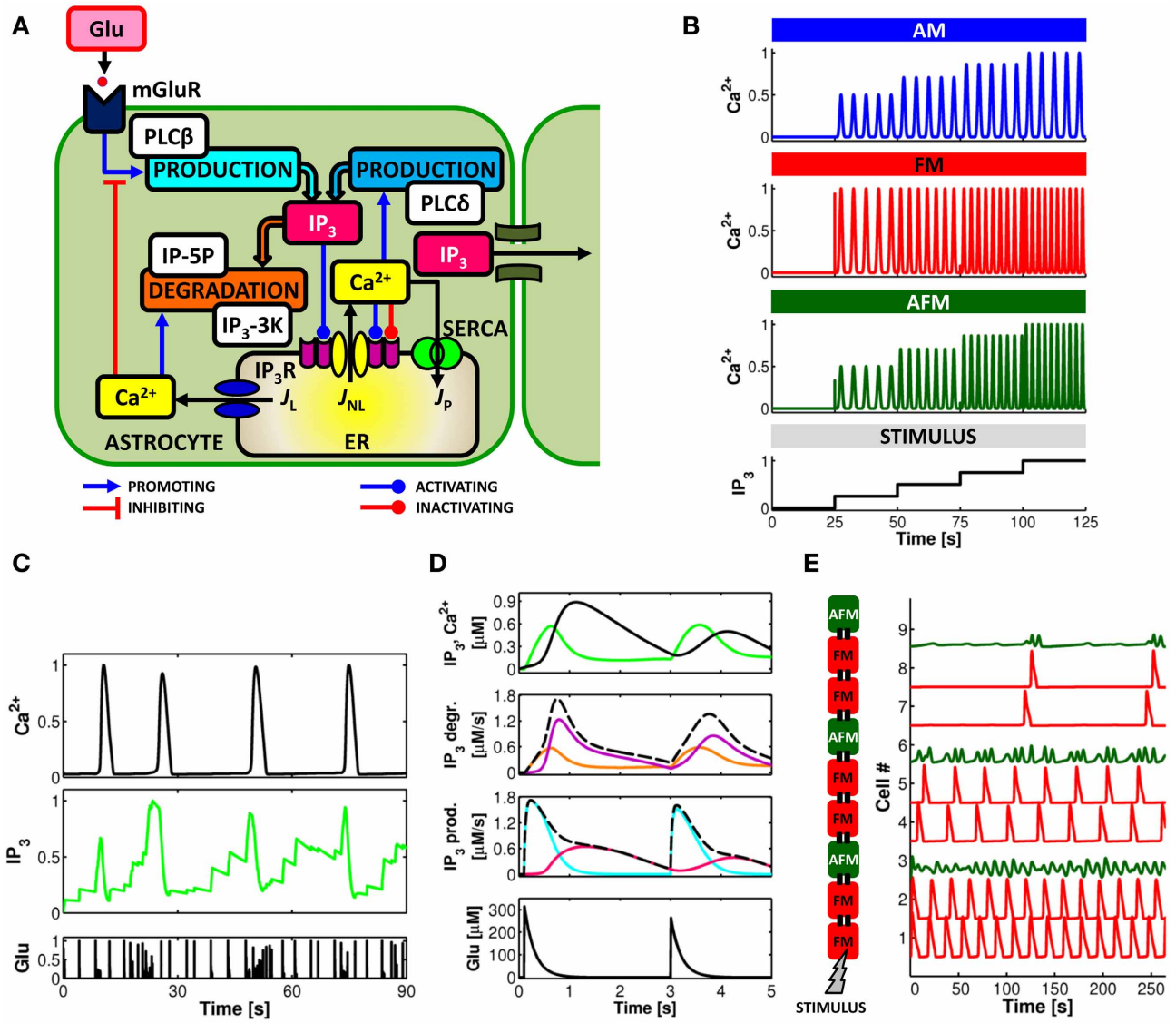

FIGURE 2 | Computational aspects of astrocytic Ca^{2+} signaling. **(A)** Scheme of IP_3-mediated Ca^{2+}-induced Ca^{2+} release in the astrocyte. Calcium and IP_3 signals are controlled by synaptic glutamate through metabotropic glutamate receptor- (mGluR-) PLCβ-mediated IP_3 production (see text for details). **(B)** IP_3 and Ca^{2+} signals can be envisioned to encode incoming synaptic activity through frequency and amplitude of their oscillations. Astrocytes could thus encode synaptic information either by modulations of the amplitude (AM), the frequency (FM), or both (AFM) of their Ca^{2+} oscillations. **(C)** Simulated Ca^{2+} and IP_3 patterns in response to sample synaptic glutamate release (Glu) in a model astrocyte (De Pittà et al., 2009a,b) reveals that IP_3 signals could be locked in the AFM-encoding independently of the encoding mode of the associated Ca^{2+} signals. This feature could allow the astrocyte to optimally integrate synaptic stimuli. (**D**, *top*) Simulated Ca^{2+} (*black trace*) and IP_3 (*green trace*) signals in the same astrocyte model as in **(C)**, and associated rates of IP_3 production (prod.) and degradation (degr.) (*middle panels*, *dashed black lines*) in response to two consecutive synaptic glutamate release events (*bottom panel*, Glu). The analysis of the contributions of different enzymes to IP_3 signaling (*solid colored traces*; *cyan*: PLCβ; *pink*: PLCδ; *orange*: IP-5P; and *purple*: IP_3-3K) reveals dynamical regulation by Ca^{2+} of different mechanisms of IP_3 production/degradation which could ultimately underlie dynamical regulation of astrocyte processing of synaptic stimuli. **(E)** Simulated propagation of Ca^{2+} waves in a heterogeneous linear chain composed of both FM (*red traces*) and AFM (*green traces*) astrocytes reveals that encoding of synaptic activity (STIMULUS) could change according to cell location along the chain. Adapted from Goldberg et al. (2010).

It is precisely the alternation between these two different phases of IP_3 degradation (a high Ca^{2+}—high IP_3-3K-activity phase and a low Ca^{2+}—low IP_3-3K-activity phase), that endows IP_3 signal with high amplitude variability. On the other hand, such AM features still allow fast variations, thus rich spectral content (i.e., FM features), in response to changes in frequency of synaptic release. This enticing possibility could endow the IP_3 signal with the necessary properties to function as optimal interface between synaptic stimuli and intracellular Ca^{2+} signals. Since neural information is carried by the timing of spikes rather than by their amplitude (Sejnowski and Paulsen, 2006), the capability of fast highly-variable amplitude changes corresponding to

rich spectral content of IP_3 signals, would fulfill this requirement, embedding the essential spectral features of the synaptic signal into the spectrum of the IP_3 transduction. On the other hand, because Ca^{2+} signals are triggered primarily by suprathreshold IP_3 elevations (Li et al., 1994; Keizer et al., 1995), the coexistence of AM features within the AFM IP_3 signal seems to be a necessary prerequisite in order to trigger CICR.

This could also help elucidate the origin of the integrative properties of Ca^{2+} signaling in astrocytes (Perea et al., 2009). These properties could result from at least two steps of integration: one step is the transduction of the agonist signal into the IP_3 signal; the other step is the cross-talk between IP_3 and Ca^{2+} signals. Hence, AFM-encoding IP_3 dynamics could operate a first preliminary integration by smoothing the highly indented synaptic stimulus. The inherent features of CICR would then bring forth a further integration step, yielding Ca^{2+} patterns that are even smoother than IP_3 signals (De Pittà et al., 2009a,b).

The tight dynamical coupling between IP_3 and Ca^{2+} signals also suggests that the way astrocytes process synaptic signals, i.e. their frequency response to synaptic stimuli, is not fixed but rather dynamical and dependent on the history of activation of the astrocyte. This is because different IP_3 signaling mechanisms that are dynamically regulated by Ca^{2+} likely correspond to different frequency responses of the astrocyte with respect to synaptic signals. **Figure 2D** shows the time course of IP_3 production (IP_3 prod.) and degradation (IP_3 degr.) (*dashed black lines*) underlying simulated IP_3 and Ca^{2+} signals (*top panel*, *green* and *black traces,* respectively) in response to two events of synaptic glutamate release (*bottom panel*). While Ca^{2+}-dependent IP_3 production by PLCβ (*cyan trace*) and PLCδ (*pink trace*) could modulate the threshold frequency of synaptic stimuli that triggers Ca^{2+} signaling in the astrocyte, existence of different regimes of IP_3 degradation within a single Ca^{2+} oscillation cycle could be responsible for different cutoff frequencies of synaptic release beyond which Ca^{2+}-mediated astrocyte processing of synaptic stimuli ceases. In particular, the cutoff frequency during low IP_3-3K activity could be mainly set by the rate of IP_3 degradation by IP-5P (*orange trace*). When Ca^{2+} is high instead, IP_3 degradation by IP_3-3K (*purple trace*) could also become very strong, thus sensibly reducing the cutoff frequency. That is, the cutoff frequency is dependent on the context of underlying Ca^{2+} signaling which, in turn, depends on the history of activation of the astrocyte by synaptic stimuli.

The subcellular arrangement of the enzymes underlying IP_3 signaling could also be responsible for spatial heterogeneity of the frequency response of the astrocyte. Although the subcellular localization of IP_3 production and degradation enzymes in astrocytes remains to be elucidated, studies in brain tissue suggest that PLCβ and IP-5P could localize mainly in proximity to the plasma membrane, whereas PLCδ and IP_3-3K are preferentially in the cytoplasm (Rebecchi and Pentyala, 2000; Irvine et al., 2006). Given that the ER distribution changes from astrocytic processes to soma (Pivneva et al., 2008), different subcellular regions of the astrocyte could correspond to different cytoplasmic volumes and thus to distinctly different expressions of enzymes mediating IP_3 signaling. The ensuing different subcellular arrangement of these enzymes could ultimately provide anatomical specificity to the astrocytic phosphoinositide signaling which underlies CICR-based astrocyte processing of synaptic activity (Fukaya et al., 2008). In this fashion, processing of synaptic stimuli by Ca^{2+} signaling at astrocytic processes could differ from that carried out in the soma by means of differently expressed IP_3 signaling-related enzymes.

COMPUTATIONAL ASPECTS OF PROPAGATING Ca^{2+} SIGNALS

Intracellular and intercellular propagation of Ca^{2+} could contribute new encoding and processing modes, in addition to those depicted in **Figure 2B**. However, despite the numerous modeling studies developed to account for the rich dynamics of astrocyte Ca^{2+} signaling (Bennett et al., 2008; Goldberg et al., 2010; Dupont et al., 2011); [for a recent review on calcium modeling see Falcke (2004)], we still lack a comprehensive theoretical framework to link the local Ca^{2+} signals that are restricted to small regions of the astrocytic processes, to their spatial dynamics and their possible propagation at larger spatial scales: intracellular propagation, global whole-cell signals or cell-to-cell Ca^{2+} waves.

Propagations at these various scales probably differ by their underlying mechanisms (Falcke, 2004; Scemes and Giaume, 2006). Fast-rising and short-lived local Ca^{2+} events, observed in response to even a single quantal release from synaptic terminals (Di Castro et al., 2011; Panatier et al., 2011) closely resemble spatially confined Ca^{2+} puffs or blips in other cell types (Thomas et al., 2000; Bootman et al., 2001) and could depend on spatial clustering of IP_3Rs along the ER structures or of mGluRs along the plasma membrane, or on both (Marchaland et al., 2008; Panatier et al., 2011; Arizono et al., 2012). The cumulative recruitment of these Ca^{2+} puffs could lead to spatially more extended Ca^{2+} events which could either be still confined within astrocytic processes (Di Castro et al., 2011) or propagate to other cellular regions or to other cells as regenerative Ca^{2+} waves (Pasti et al., 1997; Kuga et al., 2011).

Calcium could propagate by at least two routes [for a recent review see Scemes and Giaume (2006)]. One is intracellular, through GJCs, involving diffusion of IP_3 directly from cytoplasm to cytoplasm. The other route is extracellular, involving release of ATP from the astrocyte which binds to GPCRs of the same cell or neighboring astrocytes, increasing their IP_3 levels (Guthrie et al., 1999). The relative contribution of each of these pathways likely depends on developmental, regional and physiological states and could subtend different ranges of propagations as well as different temporal features (Haas et al., 2006; Scemes and Giaume, 2006; Giaume et al., 2010).

The restriction and clustering of mGluRs expression along astrocytic processes to subregions that colocalize with synaptic terminals (van den Pol et al., 1995; Arizono et al., 2012) hints the possibility of a subcellular compartmentalization of Ca^{2+} signals (Marchaland et al., 2008; Di Castro et al., 2011; Panatier et al., 2011). Local Ca^{2+} events would be spatially restricted to narrow regions around each mGluRs cluster thus defining independent signals within the same process. In this fashion, astrocytes could carry out parallel integration and processing of synaptic information on different temporal and spatial scales at different processes (Goldberg et al., 2010; Bernardinelli et al., 2011) or even in

different subregions of the same process which could constitute separate functional microdomains (Panatier et al., 2011). Furthermore, the resulting neuromodulatory action exerted by astrocytic gliotransmitters on synaptic terminals and their impact on neuronal network activity could vary from one process or microdomain to the other (Navarrete and Araque, 2011).

The spatial scale and the time window of Ca^{2+} events likely discriminate between different mechanisms of IP_3 and Ca^{2+} signaling. At the level of astrocytic processes and subcellular compartments, Ca^{2+} propagation could be mediated by fast intracellular IP_3 linear (i.e. Fickian) diffusion (Sneyd et al., 1994; Falcke, 2004). On the other hand, when considering intercellular propagation mediated by gap junctions, IP_3 transport from one cell to the other could be essentially nonlinear. This scenario was shown to be consistent with the observed variability of Ca^{2+} wave propagation distance (Goldberg et al., 2010) and could explain the long-range regenerative propagation of Ca^{2+} waves observed in cortical astrocytes (Scemes and Giaume, 2006; Tian et al., 2006).

Cell heterogeneity likely constitutes an additional critical aspect that substantially affects propagation patterns and extent of propagation of Ca^{2+} signals (Iacobas et al., 2006; De Pittà et al., 2008; Goldberg et al., 2010). This possibility is illustrated in **Figure 2E** by a toy example consisting of a heterogeneous linear chain of FM-encoding (*red traces*) and AFM-encoding (*green traces*) astrocytes. The synaptic stimulus is restricted to the first cell of the chain (i.e. cell number 1). Only FM-encoding cells guarantee regenerative propagation of Ca^{2+} signals, whereas AFM cells do not, acting like propagation barriers along the chain. Unlike in AFM cells, Ca^{2+}-dependent IP_3 production in FM cells guarantees IP_3 diffusion to the next cell in the chain to levels that are beyond the threshold of CICR thus promoting regenerative propagation (Goldberg et al., 2010). Moreover, the shape of the local Ca^{2+} events in each cell changes along the propagation path: after each AFM cell, the frequency of FM-encoding Ca^{2+} pulses is reduced, suggesting that different propagation patterns could carry out different processing of synaptic information (Goldberg et al., 2010).

The above scenario hints that the spatial distribution of astrocytes in different brain areas could be made to fulfill specific processing tasks. Indeed neighboring astrocytes in the brain are believed to be distributed in space in a non-random orderly fashion called "contact spacing" (Chan-Ling and Stone, 1991; Volterra and Meldolesi, 2005) or "tiling," where each astrocyte creates its micro-anatomical domain with its processes overlapping with adjacent astrocytes only at their periphery (Bushong et al., 2002). Such spatial arrangement, combined with the heterogeneity of astrocytic responses, could be important in intercellular Ca^{2+} wave propagations and the related computational tasks carried out by astrocyte networks. The latter could be relevant in particular for the emergence of astrocytic functional maps observed in several brain areas (Giaume et al., 2010).

Increasing evidence argues in fact for a functional organization of astrocytes, reminiscent of that of cortical neurons (Bernardinelli et al., 2011). In the ferret visual cortex astrocytes, like neurons, respond to visual stimuli, with distinct spatial receptive fields and sharp tuning to visual stimulus features, including orientation and spatial frequency (Schummers et al., 2008). The stimulus-feature preferences of astrocytes there can be mapped across the cortical surface, in close register with neuronal maps (Schummers et al., 2008). Similar observations were also reported for astrocytes in the motor cortex (Haas et al., 2006) and in the somatosensory cortex (Schipke et al., 2008) as well as in the olfactory bulb (De Saint Jan and Westbrook, 2005).

Anatomical compartmentalization of astrocyte networks could underlie such functional organization. Both in the somatosensory barrel cortex and in the olfactory bulb, astrocytes are preferentially connected by gap junctions within the same barrel or olfactory glomerulus rather than between adjacent barrels or glomeruli (Houades et al., 2008; Roux et al., 2011). However, spatial confinement of gap junctions within single barrels/glomeruli might not fully account for the selective activation of astrocyte by electrical stimulation. Indeed, similar Ca^{2+} signals in response to the same stimulus could be observed in the same barrel yet with pharmacological block of astrocyte gap junctions (Schipke et al., 2008). Thus, additional factors must contribute to the astrocyte tuned response, which likely emerges as a result of the dynamical interactions with surrounding neurons (Rouach et al., 2004) and could ultimately depend on location and nature of activated cells among the other astrocytes in the network (Matyash and Kettenmann, 2010; García-Marqués and Lópes-Mascaraque, 2012). In the case of **Figure 2E**, for example, stimulated FM-encoding cells could trigger Ca^{2+} signaling in neighboring cells in a regenerative fashion thus extending their tuned response in space. On the other hand, AFM cells, acting as propagation barriers, could shape the borders of this tuned response, eventually drawing the topographical features of the ensuing functional map (Lallouette and Berry, 2012).

RELATING Ca^{2+} SIGNALS TO GLIOTRANSMITTER EXOCYTOSIS FROM THE ASTROCYTE

There is a number of possible routes by which astrocytes could release gliotransmitters (Ni et al., 2007; Parpura and Zorec, 2010; Zorec et al., 2012), but Ca^{2+}-dependent exocytosis is likely the major one on a physiological basis (Barres, 2008; Parpura et al., 2011). However, the identity of incoming inputs, the underlying molecular mechanism and the physiological conditions that govern gliotransmitter exocytosis largely remain to be elucidated (Montana et al., 2006; Ni et al., 2007; Santello and Volterra, 2009; Parpura and Zorec, 2010). Calcium-dependent exocytosis of glutamate or ATP from astrocytes, for example, may strictly depend on the nature of the upstream Ca^{2+} signal (Perea and Araque, 2005; Li et al., 2008; Marchaland et al., 2008; Pryazhnikov and Khiroug, 2008; Malarkey and Parpura, 2011); including the type of neurotransmitter involved and the type of receptor engaged (Enkvist and McCarthy, 1992; Muyderman et al., 2001; Coco et al., 2003; Bezzi et al., 2004; Blomstrand and Giaume, 2006). The influence of astrocytic glutamate or ATP on synaptic activity also likely depends both on the type of incoming stimulus and on the specific localization of the engaged receptor in the astrocyte (Perea and Araque, 2005; Santello and Volterra, 2009).

A large amount of evidence suggests that gliotransmitter exocytosis from astrocytes bears several similarities with its synaptic homologous (Bergersen and Gundersen, 2009; Santello and Volterra, 2009; Bergersen et al., 2012) (**Figure 3A**). Astrocytes

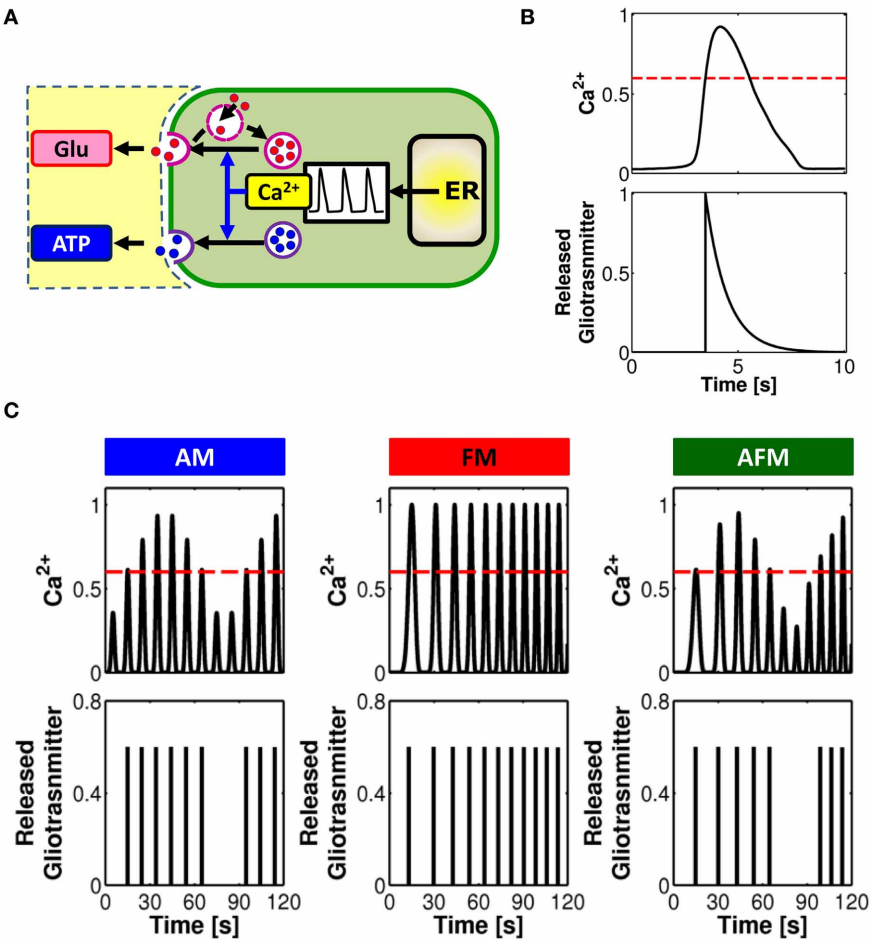

FIGURE 3 | Linking gliotransmitter exocytosis to various Ca^{2+} encoding modes. (A) Calcium-dependent glutamate and ATP exocytosis from astrocytes are both brought forth by a vesicular compartment in the astrocyte competent for regulated exocytosis. The frequency of exocytotic events is directly controlled by the shape and frequency of Ca^{2+} oscillations. **(B)** Modeling concept for an "exocytosis event" from the astrocyte. Calcium (*top trace*) triggers exocytosis of glutamate or ATP every time it increases beyond a certain threshold concentration value (*red dashed line*). The overall release can then be approximated, under proper assumptions, by an exponentially-decaying pulse of extracellular concentration of glutamate or ATP (*bottom trace*). **(C)** Distinct Ca^{2+} encoding patterns could translate into distinct rates of gliotransmitter exocytosis events. In this way, synaptic activity encoded by astrocytic Ca^{2+} signals is linked to the frequency of glutamate/ATP release from the astrocyte in a unique fashion. Adapted from De Pittà et al. (2011).

possess vesicular compartments that are competent for the regulated exocytosis of glutamate (Bezzi et al., 2004; Bergersen and Gundersen, 2009) and ATP (Coco et al., 2003; Jaiswal et al., 2007; Zhang et al., 2007). Similarly to synapses, astrocytes express soluble N-ethylmaleimide-sensitive factor attachment protein receptors (SNARE) necessary for exocytosis (Parpura et al., 1995; Schubert et al., 2011) as well as proteins responsible for concentrating glutamate or ATP into vesicles (Bezzi et al., 2004; Montana et al., 2004; Zhang et al., 2004b; Sawada et al., 2008). Fusion with the plasma membrane, trafficking and recycling of astrocytic glutamate and ATP secretory vesicles have been observed (Bezzi et al., 2004; Chen et al., 2005; Crippa et al., 2006; Jaiswal et al., 2007; Pangršic et al., 2007; Stenovec et al., 2007), which are indicative of quantal glutamate and ATP release (Del Castillo and Katz, 1954; Pasti et al., 2001; Domercq et al., 2006; Jaiswal et al., 2007; Pangršic et al., 2007; Marchaland et al., 2008; Santello et al., 2011).

Glutamate exocytosis from cultured astrocytes evoked by GPCRs is observed with short delay, i.e. 50–100 ms, after the rise in submembrane Ca^{2+}, and is mediated by a rapid succession of fusion events which peaks within ∼500 ms from the onset and decays to baseline much more slowly (>1 s), though generally before the recovery of basal Ca^{2+} levels (Domercq et al., 2006; Marchaland et al., 2008; Santello et al., 2011) (refer also to **Table 2**). In addition to this "exocytotic burst," larger delays with slower rate of sustained vesicular fusion events have also been reported (Kreft et al., 2004; Malarkey and Parpura, 2011). The delayed onset with respect to the Ca^{2+} rise is consistent with a threshold Ca^{2+} concentration for release (Pasti et al., 1997; Parpura and Haydon, 2000). On the other hand the overall release of glutamate is characterized by a rising phase much faster than its decay and can be approximated by an exponential function like synaptic exocytosis yet with a decay time much slower than the latter (Marchaland et al., 2008; De Pittà et al., 2011; Santello et al.,

Table 2 | Comparison of time scales of rise, decay and full-width half-maximum (FWHM) duration of changes of intracellular Ca^{2+}, extracellular glutamate (Glu) and ATP in astrocytes and neurons.

Signal	Origin	τ_{rise} [s]	τ_{decay} [s]	FWHM [s]	Essential references
Ca^{2+}	Astrocyte (soma)	2–20[f]	3–25[f]	5–160	Hirase et al., 2004; Nimmerjahn et al., 2004; Wang et al., 2006
	Astrocyte (soma)[a]	∼0.5	∼1.1	∼2–4	Winship et al., 2007
	Neuron (soma)	$2–5{\cdot}10^{-3}$	0.1–0.4	∼0.1–0.3	Svoboda et al., 1997; Waters et al., 2003; Nimmerjahn et al., 2004
	Astrocyte (process)	0.1–0.2	0.2–4[f]	∼0.5–4	Di Castro et al., 2011; Panatier et al., 2011
	Neuron (presynaptic bouton)	$0.5–5{\cdot}10^{-3}$	∼0.1–2	0.1–1*	Regehr et al., 1994; Emptage et al., 2001
Glu	Astrocyte	0.2–0.5	0.5–1.5	1–6	Domercq et al., 2006; Marchaland et al., 2008; Santello et al., 2011
	Neuron (synapse)	$1–5{\cdot}10^{-4}$	0.01–0.1	0.01–0.1*	Raghavachari and Lisman, 2004; Herman and Jahr, 2007; Okubo et al., 2010
ATP	Astrocyte	0.1–0.5	2–3	2–20	Pangršic et al., 2007; Li et al., 2008
	Neuron (synapse)	$1–5{\cdot}10^{-4}$*	0.2–1*	0.15–0.5	Dundwiddie and Masino, 2001; Pankratov et al., 2007

Glutamate and ATP values refer to transient increases of their extracellular concentrations following release in a Ca^{2+}-dependent fashion. Therefore they describe the time course of the overall glutamate and ATP released by an exocytotic burst rather than by a single exocytotic event which can be much faster and occur within the first ∼50 ms from Ca^{2+} rise (see text). Indicative rise and decay time constants as well as FWHM values are reported in terms of min–max ranges. Fast calcium signals imaging in astrocyte in the somatosensory cortex reported by Winship et al. (2007) are reported separately and dubbed by "a." The letter "f" stands for values that were obtained by fitting of experimental data by a biexponential function such as $f(t) = C{\cdot}(\exp(-t/\tau_{decay}) - \exp(-t/\tau_{rise}))$ with C being a proper scaling factor. Asterisk "" denotes values estimated by a model of astrocytic and synaptic release introduced in De Pittà et al. (2011).*

2011) (**Figure 3B**). Although much less characterized than its glutamate counterpart, astrocytic ATP exocytosis could occur in a qualitatively similar fashion in spite of different underlying kinetics (Pangršic et al., 2007; Pryazhnikov and Khiroug, 2008; Li et al., 2008). Based on these arguments, it was proposed that astrocytic gliotransmitter release could be modeled using the same mathematical description of synaptic release, although the two mechanisms are likely different in their molecular machinery, with the kinetics of astrocyte release much slower than synaptic release (De Pittà et al., 2011; Schubert et al., 2011).

How are different modes of Ca^{2+} encoding translated into glutamate or ATP release? Assuming proper conditions about the rate of clearance of these gliotransmitters with respect to the underlying intracellular Ca^{2+} dynamics that mediate their exocytosis (Abbracchio et al., 2009), an intriguing theoretical prediction is that various patterns of Ca^{2+} oscillations could mostly correspond to different rates of gliotransmitter release (De Pittà et al., 2011). This is presented in **Figure 3C** where, for three stereotypical patterns of Ca^{2+} oscillations, that is AM, FM, and AFM, the corresponding timing of gliotransmitter release from the astrocyte is shown. In this example, FM-encoding Ca^{2+} oscillations always cross the threshold for exocytosis (*dashed red line*), triggering gliotransmitter release every time. Conversely, AM or AFM oscillations may not be large enough to reach such threshold, resulting in some oscillations that fail to trigger gliotransmitter release. In this fashion, while FM Ca^{2+} oscillations trigger gliotransmitter exocytosis at their own frequency, the amplitude of AM and AFM oscillations could selectively discriminate which Ca^{2+} pulse triggers exocytosis, eventually dictating the frequency of "measured" glutamate or ATP release events (De Pittà et al., 2011). Further experimental investigations are required to elucidate whether such prediction could effectively mimic reality.

While astrocytic Ca^{2+} signals could be triggered both by spontaneous and evoked synaptic release, gliotransmitter release might be not (Di Castro et al., 2011; Panatier et al., 2011). Blockade of Ca^{2+}-dependent glutamate release in astrocytes in the dentate gyrus was indeed observed to be effective in reducing the frequency of synaptic release events only when these were evoked by action potentials, but not when they happened spontaneously. This suggests that release of glutamate from astrocytes in this region could effectively occur only in presence of evoked synaptic activity (Di Castro et al., 2011). Interestingly, the Ca^{2+} elevations in astrocyte processes triggered by action potentials were reported to be more complex, larger in amplitude and more extended in space, than those generated by spontaneous synaptic release events. Since glutamate could be released from several sites along the same astrocyte process and this latter could contact several synapses (Panatier et al., 2011), one of such Ca^{2+} elevations could then generate multiple spatially-distinct glutamate release events modulating synaptic release at several other synapses. The same Ca^{2+} elevation however, could bring forth at subcellular regions of the process, different local Ca^{2+} dynamics, in close analogy to the behavior of the heterogeneous AM/AFM oscillations in the astrocyte chain in **Figure 2E**. Such different local Ca^{2+} dynamics could in turn result in different rates of glutamate release and thus in different modulations of synaptic release in a non-random fashion. In this way, the same astrocytic process or a segment of it, could carry out multiple regional modulations of synaptic release, depending both on the temporal and spatial dynamics of synaptic release. It is however possible that the mechanisms underlying Ca^{2+}-dependent gliotransmission differ in different brain areas (Matyash and Kettenmann, 2010; Zhang and Barres, 2010). Indeed, in contrast with the above observations in the dentate gyrus, astrocytes in the stratum radiatum

of the hippocampus were reported to release glutamate even in the absence of synaptic activity evoked by action potentials (Bonansco et al., 2011).

Another issue is what makes a single astrocyte release one gliotransmitter rather than another. A possibility is that different gliotransmitters are released in response to different stimuli. *In situ* studies indeed suggest that purinergic GPCR-mediated astrocytic Ca^{2+} signals could preferentially trigger glutamate release (Jourdain et al., 2007; Perea and Araque, 2007; Di Castro et al., 2011) (see also **Table 1**). In contrast, Ca^{2+} elevations triggered by glutamate could bring forth ATP release (Pascual et al., 2005; Gordon et al., 2009; Panatier et al., 2011). Overall these observations hint that a tight association likely exists between the type of targeted astrocytic receptor and the secretory machinery of gliotransmitters (Zorec et al., 2012). Moreover, in the same astrocyte, different gliotransmitters could be contained in different organelles with different secretory properties in response to Ca^{2+} signals (Coco et al., 2003). Indeed, while glutamate seems to be preferentially released by synaptic-like microvesicles (Bezzi et al., 2004; Jourdain et al., 2007; Bergersen et al., 2012), ATP is likely released by dense-core granules (Coco et al., 2003), and/or lysosomes (Jaiswal et al., 2007; Zhang et al., 2007; Li et al., 2008). Although the underlying molecular machinery of exocytosis remains to be elucidated, each organelle population is likely secreted in a different fashion (Pryazhnikov and Khiroug, 2008). *In vitro* evidence showed in fact that Ca^{2+} signals that triggered release of glutamate-containing vesicles (Marchaland et al., 2008) did not release ATP-filled organelles and vice versa (Coco et al., 2003; Li et al., 2008), ultimately suggesting that glutamate and ATP could be released in response to different Ca^{2+} signals (Parpura and Zorec, 2010).

Intriguingly, stimulation of astrocyte GPCRs can evoke fast gliotransmitter exocytosis within few hundreds of milliseconds (Bezzi et al., 2004; Domercq et al., 2006; Marchaland et al., 2008; Santello et al., 2011), indicating that the Ca^{2+}-dependent process that couples stimulus with secretion must be fast. Indeed, the peak of GPCR-mediated Ca^{2+} release from the ER can be as fast as 50–250 ms (Marchaland et al., 2008; Di Castro et al., 2011; Panatier et al., 2011) and Ca^{2+}-dependent exocytosis of single glutamate vesicles can occur within less than 5–20 ms from Ca^{2+} elevation (Chen et al., 2005; Bowser and Khakh, 2007; Marchaland et al., 2008; Santello et al., 2011). Therefore, to assure fast stimulus-secretion coupling, IP_3 diffusion from the site of production by GPCRs at the plasma membrane to IP_3 receptors on the ER membrane must also be of the order of tens of milliseconds and so must Ca^{2+} diffusion from the mouth of IP_3Rs to the Ca^{2+} sensor of exocytosis (Zhang et al., 2003, 2004a; Ni et al., 2007). Given that the diffusion times of IP_3 or Ca^{2+} can be estimated as the half of the square of the diffusion distance from the site of their production in the cytoplasm divided by the respective diffusion constants (Syková and Nicholson, 2008), which can be as high as ∼200–300 $\mu m^2/s$ for IP_3 (Allbritton et al., 1992; Sneyd et al., 1994) and 30 $\mu m^2/s$ for Ca^{2+} (Kang and Othmer, 2009), then diffusion times of the order of milliseconds could be obtained only for diffusion lengths at most in the micron range. This prediction is fully supported by the functional evidence of local GPCR-evoked, ER-dependent Ca^{2+} microdomains in astrocytic processes (Di Castro et al., 2011; Panatier et al., 2011) and by the morphological evidence that at astrocytic processes the ER stores are found at ∼200–600 nm distance both from the plasma membrane and gliotransmitter-containing vesicles (Bezzi et al., 2004; Marchaland et al., 2008; Bergersen et al., 2012). Moreover, GPCR agonist-mediated Ca^{2+} transients were observed restricted areas beneath the plasma membrane, in close proximity to the sites of exocytosis (Marchaland et al., 2008), suggesting that both GPCRs and secretory vesicles must be spatially close to the IP_3Rs responsible for Ca^{2+} release from the ER. This indicates that the location of IP_3Rs along the ER membrane in astrocyte processes might be non-random (Blaustein and Golovina, 2001), in tight spatial association with astrocytic GPCRs on the plasma membrane and releasable gliotransmitter organelles (Marchaland et al., 2008; Panatier et al., 2011). Such spatial coupling could ultimately underlie the preferential occurrence of functional gliotransmission from astrocytic processes rather than from the soma (Gordon et al., 2009), entailing strict spatiotemporal requirements for Ca^{2+} signals to trigger release of gliotransmitters from the astrocyte and modulate synaptic transmission.

MODULATION OF SYNAPTIC RELEASE BY ASTROCYTIC GLUTAMATE AND ATP

Astrocyte-derived glutamate and ATP or adenosine can modulate synaptic transmission, either increasing neurotransmitter release (Araque et al., 1998a,b; Fiacco and McCarthy, 2004; Jourdain et al., 2007; Perea and Araque, 2007; Bonansco et al., 2011; Di Castro et al., 2011; Panatier et al., 2011) or decreasing it (Zhang et al., 2003, 2004a; Pascual et al., 2005; Andersson and Hanse, 2010, 2011) depending on the type of presynaptic receptor involved and the brain area (pathway **B** in **Figure 1B**; see also **Table 1**). At excitatory synapses in the hippocampal dentate gyrus, glutamate is released from surrounding astrocytic processes in close proximity to presynaptic NR_2B-containing NMDA receptors (**Figure 4A**). Activation of these receptors results in increased synaptic release and strengthening of synaptic transmission (Jourdain et al., 2007). At Schaffer collateral synapses in the CA1 hippocampal area a similar effect is mediated by presynaptic mGluRs (Fiacco and McCarthy, 2004; Perea and Araque, 2007). Besides directly targeting presynaptic receptors, astrocyte-released glutamate could also bind ionotropic receptors found along the axons of hippocampal CA3 pyramidal cells, broadening the profile of propagating action potentials (APs) (Sasaki et al., 2011). The broadened APs in turn, trigger larger Ca^{2+} elevations in presynaptic boutons, resulting in larger synaptic release probability.

Conversely, astrocyte-released ATP and its derivative adenosine, bind to presynaptic PRs, i.e. P_2Y_1Rs or A_1Rs, respectively, decreasing synaptic release (Zhang et al., 2003, 2004a; Pascual et al., 2005). However, astrocyte-derived adenosine, could also target A_{2A} receptors which can increase synaptic release (Panatier et al., 2011) (omitted from **Figures 1A** and **4A** for simplicity). Both effects of adenosine—inhibitory via A_1Rs and stimulatory via A_{2A}Rs—have been described at hippocampal CA3-CA1 synapses (Zhang et al., 2003, 2004a; Pascual et al., 2005; Serrano et al., 2006; Panatier et al., 2011), and the prevalence of one on

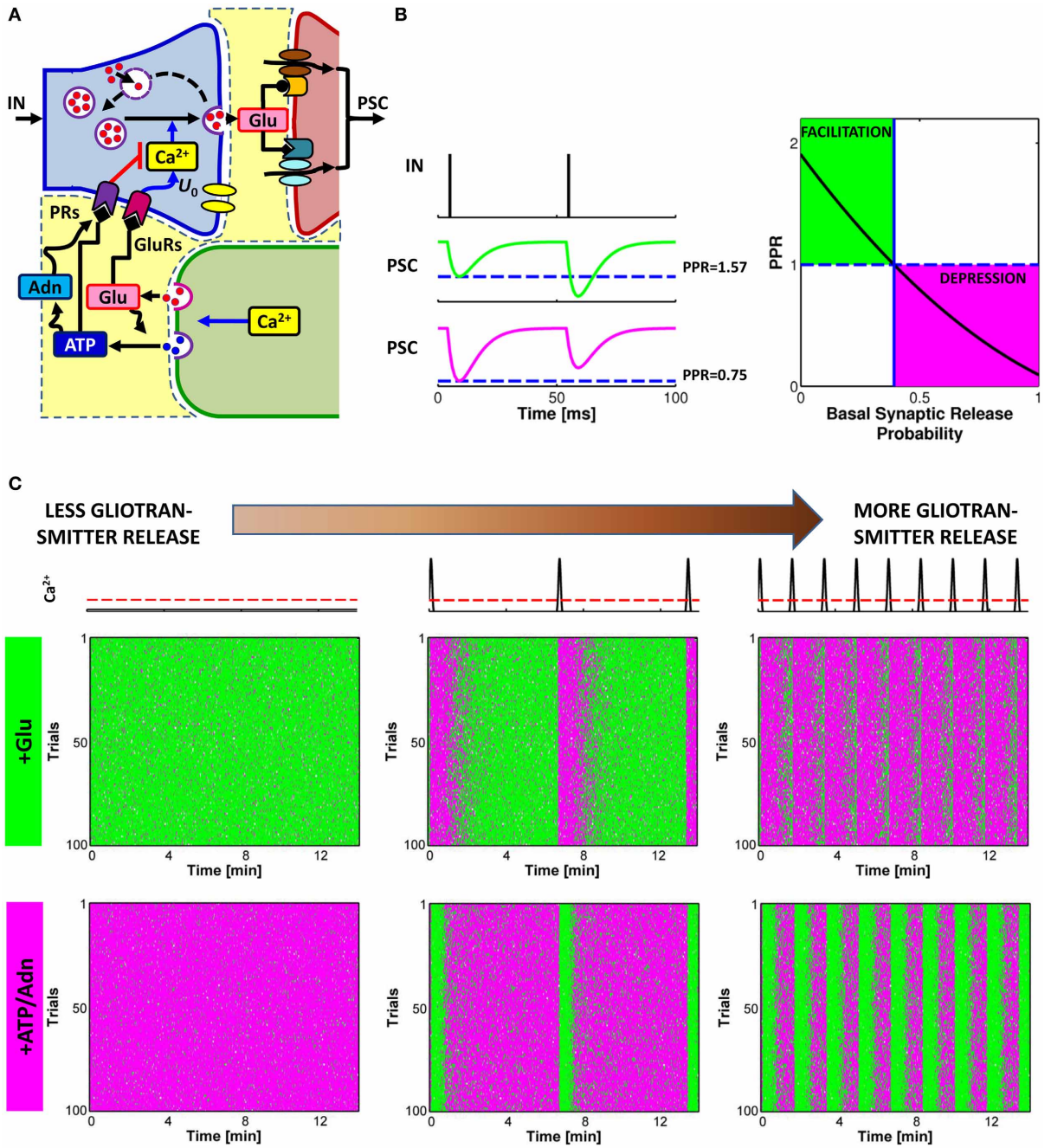

FIGURE 4 | Glutamate or ATP released from astrocytes regulates transitions between facilitation and depression of synaptic transmission. (A) Conceptual framework for the regulation of synaptic release probability at basal conditions by astrocytes. Astrocyte-released glutamate increases basal synaptic release probability (U_0), whereas astrocyte-released ATP/Adn generally decreases it. **(B)** Changes in synaptic release probability due to astrocytic gliotransmitters can be detected by variations of paired-pulse plasticity quantified by paired-pulse ratio (PPR). Paired-pulse facilitation (*left, green traces*) of postsynaptic currents (PSCs) corresponds to PPR values above 1 (*right, green-shaded area*) whereas paired-pulse depression (*left*, magenta traces) are associated with PPR values below 1 (*right, magenta-shaded area*). **(C)** Raster plots of simulated PSC pairs for 100 different input spike trains with same statistics colored according to the paired-pulse ratio: *green* for facilitation, PPR > 1; *magenta* for depression, PPR < 1. For increasing rates of exocytosis of gliotransmitter from the astrocyte, mimicked by increasing rates of Ca^{2+} crossing beyond the threshold for exocytosis (*top row, red dashed line*), synaptic plasticity could be progressively changed to its opposite depending on the type of gliotransmitter. Astrocytic glutamate could thus turn facilitating synapses into depressing (*middle row*) whereas astrocyte-derived ATP or adenosine could turn depressing synapses into facilitating (*bottom row*). Simulations are based on a model of astrocyte-regulation of synaptic release introduced in De Pittà et al. (2011). "Basal Synaptic Release Probability" in **(B)** refers to the probability of synaptic release at rest, that is when synaptic activity is assumed to be very low and the amount of neurotransmitter released upon arrival of an action potential to the presynaptic terminal is essentially independent of previous release events (Zucker and Regehr, 2002; De Pittà et al., 2011).

the other likely depends on the level of synaptic activity (Panatier et al., 2011). This would be in line with observations at the frog's neuromuscular junction, where different stimulations of the tibial nerve differentially activated A_1Rs or A_{2A}Rs in association with different Ca^{2+} dynamics in the peri-junctional glial cell (Todd et al., 2010). Intriguingly, at hippocampal synapses different synaptic stimuli could differentially change the morphology of astrocyte perisynaptic processes (Haber et al., 2006; Lavialle et al., 2011) thus reshaping, in an activity-dependent fashion, the extracellular space of interaction between astrocyte-derived adenosine and presynaptic receptors. This could dynamically regulate access of adenosine to one receptor with respect to the other (Haber et al., 2006; Syková and Nicholson, 2008) ultimately modulating synaptic release in a complex fashion. The functional consequences of astrocytic remodeling were indeed demonstrated in the supraoptic nucleus, where dynamic changes in the astrocytic wrapping of synapses during lactation could regulate the extent of synaptic glutamate spillover and thereby control heterosynaptic depression of GABAergic transmission by presynaptic mGluRs (Oliet et al., 2001; Piet et al., 2004).

The opposite effects due to astrocytic glutamate or ATP/adenosine could endow astrocytes with the capacity to exert non-stereotyped bimodal control of synaptic transmission (Volterra and Meldolesi, 2005). On the other hand, the temporal concurrence of both these effects due to co-expression of inhibitory and stimulatory receptors at the same synaptic terminals (Shigemoto et al., 1997; Rebola et al., 2005), could result in occlusion, i.e. no net effect on synaptic release by the astrocyte (De Pittà et al., 2011). Alternatively, balanced activation (and possibly occlusion) of A_1 and A_{2A} receptors by astrocyte-derived adenosine could set synaptic release in basal conditions (Panatier et al., 2011). Hence, the ensuing regulation of synaptic transmission triggered by gliotransmitters in response to stimuli, could result instead from an unbalance of activation of these receptors rather than by the distinct activation of one receptor type with respect to the other, ultimately providing a high degree of complexity in the control of synaptic transmission by astrocytes. For the sake of clarity, in the following we will consider only release-decreasing effects of astrocyte ATP or its derivative, adenosine.

The details of the biochemical mechanism underlying modulation of synaptic release by astrocytic glutamate or ATP (or adenosine) likely depend on the type of targeted presynaptic receptors and are not fully understood (Pinheiro and Mulle, 2008). The simplest explanation would be that astrocytic glutamate and ATP lead to a modulation of presynaptic intracellular Ca^{2+} levels which eventually results in a modulation of synaptic release probability (Zucker and Regehr, 2002; Pinheiro and Mulle, 2008) with significant repercussions on synaptic plasticity, including short-term depression and facilitation.

Short-term facilitation and depression can be assessed by measuring the paired-pulse ratio (PPR), i.e. the ratio between the amplitudes of successive postsynaptic currents (PSCs) recorded in response to a pair of action potentials in rapid succession as illustrated in **Figure 4B** (Zucker and Regehr, 2002). When the value of the peak postsynaptic current associated to the second incoming spike is larger than the peak current recorded in coincidence with the first spike (*green traces*), then synaptic release is increasing for incoming spikes, i.e. facilitation occurs, and the corresponding PPR is larger than unity. Conversely, when the second peak is less than the first peak (*magenta traces*), this marks a decrease of neurotransmitter release from the presynaptic terminal which reflects depression, and the corresponding value of PPR is less than one. It should be noted that, by varying the interval between two pulses, the same synapse can be either depressed or facilitated. For simplicity we omit the interpulse interval as a variable in this description.

In general, in basal conditions, i.e. in response to an individual action potential, the value of synaptic release probability of an individual synapse or of an ensemble of synapses defines the nature of synaptic transmission at that synapse/ensemble, namely whether it is facilitating or depressing, with low values of probability favoring facilitation and high values favoring depression (Abbott and Regehr, 2004) (**Figure 4B**, *right*). Thus, any modulation of synaptic release probability by gliotransmitters that changes the PPR from below unity to values above it or vice versa, could switch the mode of synaptic transmission from depressing to facilitating or vice versa. This scenario was theoretically addressed in De Pittà et al. (2011) and it was shown to substantially agree with experiments. Indeed, at hippocampal synapses, the increase of synaptic release probability due to astrocytic glutamate correlates with a *decrease* of the PPR (Jourdain et al., 2007; Perea and Araque, 2007; Bonansco et al., 2011). Conversely, a decrease of synaptic release due to the action of astrocyte-derived ATP (or adenosine) is accompanied by an *increase* of the PPR (Zhang et al., 2003, 2004a). The frequency of astrocytic glutamate (Glu) and/or ATP exocytosis are likely crucial in the regulation of the extent of the modulation of synaptic release by astrocytes (De Pittà et al., 2011), a principle illustrated in **Figure 4C**. This figure shows the simulated peak postsynaptic currents for the same synapse in response to 100 trials of presynaptic spike trains with identical statistics (*raster plots*). Each column in **Figure 4C** corresponds to a different frequency of astrocytic Ca^{2+} pulses (*top row*), yielding to different frequencies of gliotransmitter release when Ca^{2+} crosses the threshold for exocytosis (*dashed red line*) (see also **Figure 3B**). Colors in the raster plots refer to paired-pulse plasticity quantified by PPR. For two consecutive presynaptic spikes, if the second spike releases synaptic neurotransmitter more than the first spike, then it is PPR > 1, paired-pulse facilitation is observed, and the peak postsynaptic current associated to the second spike is colored in *green*. Vice versa, if the amount of neurotransmitter released by the second spike is less than that released by the first one, then PPR < 1, that is paired-pulse depression occurs, and the second peak postsynaptic current is colored in *magenta*.

In absence of astrocytic gliotransmitter (*left column*), the raster plot of a facilitating synapse (*middle row*) shows predominant paired-pulse facilitation, i.e. mostly *green* dots. However, in presence of release of glutamate from the astrocyte (+Glu), as explained above, the stimulatory effect of this gliotransmitter on synaptic release changes paired-pulse plasticity which is marked by the appearance of *magenta* bands in the raster plots (*middle* and *right columns*). These bands locate time intervals where paired-pulse depression becomes prominent (i.e. predominance

of *magenta* dots) on the overall paired-pulse facilitation background (*green dots*). Notably these bands are almost in coincidence with glutamate release from the astrocyte (*top row*) and their number increases with the glutamate release frequency. Therefore, for the same time window, the same originally-facilitating synapse gets increasingly depressing as the rate of glutamate release from the astrocyte increases. The opposite is observed for an otherwise depressing synapse (i.e. predominance of paired-pulse depression, thus mostly *magenta* dots in the *bottom left* raster plot). In this case, in presence of release of ATP and its derivative adenosine (+ATP/Adn) from the astrocyte, due to the inhibitory effect of astrocyte-derived purines on synaptic release considered in this example, *green* bands appear in the raster plots which mark the onset of periods of predominant paired-pulse facilitation. The number of these bands grows for higher rates of ATP release from the astrocyte so that the same originally-depressing synapse behaves more akin of a facilitating one as the rate of ATP release from the astrocyte increases.

An intriguing prediction that follows from the above arguments is that the frequency of gliotransmitter release, by modulating synaptic release probability at basal conditions, could dynamically control the nature of synaptic transmission as elucidated in **Figure 5A**. In particular, under certain conditions, a threshold frequency for gliotransmitter release (*blue line*) could exist above which the astrocyte can switch the nature of synaptic transmission, turning depressing synapses into facilitating or vice versa, facilitating synapses into depressing (De Pittà et al., 2011). Hence, the plasticity mode at such synapses is not fixed but rather is set by the release rate of gliotransmitters from neighboring astrocytic processes.

Remarkably, the threshold frequency of gliotransmitter release that discriminates between facilitation and depression at one synapse can be as low as 0.05 Hz or less (as in the case of **Figure 5A**) thus falling within the range of Ca^{2+} oscillations observed in astrocytes in basal conditions (Parri et al., 2001; Aguado et al., 2002; Bonansco et al., 2011; Di Castro et al., 2011). In such conditions as shown in **Figure 5B** (*top*), intracellular Ca^{2+} levels in astrocytes spontaneously fluctuate in a highly stochastic fashion (Hirase et al., 2004; Di Castro et al., 2011) and can similarly cross the threshold for gliotransmitter release. The ensuing release of gliotransmitter, however, could be sufficient to tonically set the basal tone of synaptic transmission (**Figure 5B**). Inasmuch as the rate of gliotransmitter release could also correlate with the incoming synaptic stimulus through Ca^{2+} dynamics in the astrocytes (Aguado et al., 2002; Di Castro et al., 2011), this scenario discloses the possibility that astrocytes act as endogenous regulators of the efficacy of synaptic transmission (Haydon, 2001; Giaume et al., 2010; Halassa and Haydon, 2010; Di Castro et al., 2011; Panatier et al., 2011). That is, through integration of synaptic activity by means of their Ca^{2+} signals, astrocytes could adapt synaptic strength according to the history of the synapse.

IMPLICATIONS OF ASTROCYTE MODULATION OF SYNAPTIC TRANSMISSION AND PLASTICITY

The effect of modulation of synaptic release probability by astrocytic gliotransmitters may decay more slowly than the Ca^{2+} elevation triggering astrocyte exocytosis (Fiacco and McCarthy, 2004; Serrano et al., 2006), and still be present upon gliotransmitter release by following Ca^{2+} increases (Volman et al., 2007). Therefore astrocytic Ca^{2+} activity resulting in high rates of gliotransmitter release, could bring forth long-lasting modulations of synaptic release. Insofar as synaptically-released neurotransmitter could shape postsynaptic signaling at the basis of long-term synaptic plasticity (Bliss and Collingridge, 1993), persistent modulations of synaptic release probability by astrocyte could ultimately contribute to long-term changes of synaptic strength underlying neural processing, memory formation and storage of information. Support to this scenario comes from studies on hippocampal synapses for which the temporal coincidence of postsynaptic depolarization with the increase of Ca^{2+} elevations in neighboring astrocytes was shown to induce long-term potentiation (LTP) of synaptic transmission (Perea and Araque, 2007; Navarrete and Araque, 2010; Navarrete et al., 2012a,b). Notably, this form of LTP is independent of the activation of postsynaptic NMDARs but rather, it depends on glutamate released from astrocytes, which persistently potentiates synaptic transmitter release through activation of presynaptic mGluRs (Perea and Araque, 2007). On the other hand, at synapses between excitatory neurons in layers 4 and 2/3 in the rat barrel cortex, activity-dependent induction of long-term depression (LTD) also requires astrocyte Ca^{2+} signaling (Min and Nevian, 2012). At these synapses, postsynaptically-released endocannabinoids mediate Ca^{2+}-dependent release of glutamate from astrocytes which targets presynaptic NMDARs bringing forth LTD (Sjöström et al., 2003; Rodríguez-Moreno and Paulsen, 2008).

The differential induction of LTP and LTD in neighboring synapses has been suggested to determine the size and shape of cortical functional topographic units such as ocular dominance columns in the primary visual cortex and whisker barrels in the primary somatosensory cortex (Feldman and Brecht, 2005; Hensch, 2005; Li et al., 2009). Thus, the possible involvement of astrocytes in LTP and LTD suggests that these cells could contribute to the plasticity of cortical maps and the development of corresponding sensory representations (Rossi, 2012).

Modulation of synaptic release probability by astrocytic gliotransmitters could also alter the temporal order of correlated pre- and postsynaptic spiking that critically dictates spike-timing-dependent plasticity (STDP) (Pascual et al., 2005). Inhibition of spontaneous glutamate release from astrocytes in hippocampal CA1 synapses, consistent with a decrease of synaptic release probability, was indeed reported to modify the threshold for induction of spike-timing-dependent LTP (Bonansco et al., 2011). In this fashion, astrocytes by gliotransmission-mediated regulations of synaptic release probability could control not only different mechanisms of synaptic plasticity but also the threshold of synaptic activity required for their onset, thus playing a role in metaplasticity too, that is the plasticity of synaptic plasticity (Abraham, 2008).

Gliotransmitters do not control synaptic plasticity only via presynaptic actions, but also by actions on postsynaptic receptors. Indeed, the induction of LTP itself appears to be uniquely

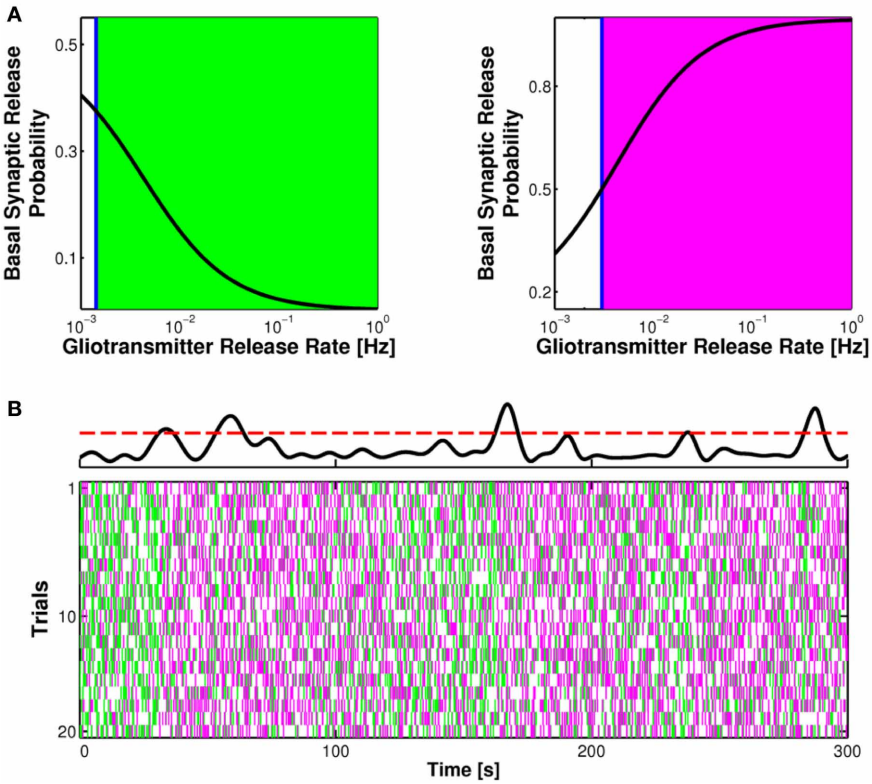

FIGURE 5 | Short-term synaptic plasticity is physiologically set by the rate of gliotransmitter release from the astrocyte. (A) The rate of glutamate or ATP release from the astrocyte could differently affect basal synaptic release probability. In particular, a threshold frequency for the release of these gliotransmitters could exist (*blue vertical line*) beyond which a depressing synapse could turn into facilitating (*right, green area*) or vice versa, a facilitating synapse could become depressing (*left, magenta area*). Adapted from De Pittà et al. (2011). **(B)** In basal conditions, synaptic release is due to sporadic neuronal network firing and the possible frequency of Ca^{2+} fluctuations beyond the threshold for exocytosis (*dashed red line*) in the astrocyte (*top trace*). In this fashion plastic changes in paired-pulse ratio could be inherently regulated by astrocytic gliotransmitters, as shown here for the case of an originally facilitating synapse under the effect of astrocytic glutamate exocytosis (20 trials with identical input statistics). Adapted from Berry et al. (2011). The color code for the raster plot is the same as in **Figure 4C**.

controlled by astrocytes through the release of D-serine (Santello and Volterra, 2010) (pathway **C** in **Figure 1B**). Both at hippocampal and cortical synapses astrocytic D-serine rather than glycine, is the endogenous co-agonist of synaptic NMDA receptors (Henneberger et al., 2010; Takata et al., 2011; Papouin et al., 2012). By controlling the level of co-agonist site occupancy of postsynaptic NMDARs, astrocytic D-serine affects the level of activation of these receptors and thus activity-dependent long-term synaptic changes (Bains and Oliet, 2007). In particular, the additional burst of activation of postsynaptic NMDARs (about 25% more) induced by astrocyte D-serine release seems necessary for LTP induction (Henneberger et al., 2010). Moreover, in conditions of reduced synaptic coverage by astrocytes, such as during lactation in the hypothalamus, higher presynaptic activity is required to obtain LTP while the same level of activity that normally induces LTP results instead in LTD (Panatier et al., 2006). In such conditions, astrocyte-released D-serine is diluted in the larger extracellular space resulting in a reduced number of postsynaptic NMDARs recruited by synaptic activity, which ultimately translates into smaller postsynaptic Ca^{2+} increases. Therefore, experimental protocols that would be expected to cause LTP, elicit LTD instead. This is in agreement with the Bienenstock-Cooper-Munro (BCM) model for variation of the threshold for LTP, which predicts that the relationship between synaptic activity and persistent changes in synaptic strength can vary according to the number of NMDARs available during synaptic activation (Bienenstock et al., 1982; Abraham and Bear, 1996). Effectively, by adjusting the D-serine occupancy of the NMDAR co-agonist-binding site, astrocytes can shift the relationship between activity and synaptic strength (Panatier et al., 2006).

Besides D-serine, experimental evidence hints that glutamate and ATP released from astrocytes could also directly bind postsynaptically-located receptors and accordingly, play a role in regulation of long-term synaptic plasticity. In the paraventricular nucleus of the hypothalamus for example, ATP released from astrocytes could directly target postsynaptic P_2X_7 receptors, promoting insertion of postsynaptic AMPARs which results in LTP of synaptic transmission (Gordon et al., 2005). Interestingly, ATP release is mediated by Ca^{2+} dynamics

triggered in astrocytes by noradrenergic afferents which, in the hypothalamus, generally lack direct postsynaptic contacts (Sawyer and Clifton, 1980), thus hinting that signaling in this vital homeostatic circuit may require dynamic neuron-glia interactions.

Glutamate released from astrocyte could also target extrasynaptically-located NR_2B-containing NMDA receptors at postsynaptic terminals, triggering slow inward currents (SICs) (Fellin et al., 2004; D'Ascenzo et al., 2007; Navarrete and Araque, 2008; Bardoni et al., 2010; Pirttimaki et al., 2011; Navarrete et al., 2012a,b) mainly mediated by Ca^{2+} ions (Cull-Candy et al., 2001), whose depolarizing action could affect postsynaptic neuronal firing (D'Ascenzo et al., 2007; Pirttimaki et al., 2011). In the primary visual cortex, nucleus basalis-mediated cholinergic activation of astrocytes mediates an increase of SICs frequency which correlates with a long-lasting increase of firing activity in visual responses of V1 excitatory neurons (Chen et al., 2012). The ensuing modulations of firing activity of these neurons by astrocyte-mediated SICs might ultimately affect STDP at individual synapses controlling orientation-specific responses of V1 neurons to visual stimuli (Jia et al., 2010).

Modulation of synaptic transmission by Ca^{2+}-dependent gliotransmission may not be limited to the very synapses that trigger Ca^{2+} activity in the astrocyte but it could also affect farther synaptic domains in a multimodal fashion (Kozlov et al., 2006), depending both on the morphology of the sites of astrocyte-synapse reciprocal communication (Ventura and Harris, 1999; Haber et al., 2006) and the functional connectivity of the astrocytic network (Pannasch et al., 2011). High-frequency activity of a Schaffer collateral fiber can trigger the potentiation of synaptic transmission at the same fiber but heterosynaptic suppression of another, adjacent fiber, by inducing ATP release from an astrocyte interposed between the two fibers (Zhang et al., 2003, 2004a; Pascual et al., 2005). In the somatosensory cortex in particular, astrocyte-mediated heterosynaptic suppression could modulate GABAergic inhibitory transmission (Benedetti et al., 2011) which plays a dominant role in the control of cortical neuronal excitability (Swadlow, 2002). Given that both experimental observations and theoretical arguments suggest that excitation and inhibition are globally balanced in cortical circuits (Shadlen and Newsome, 1994; Troyer and Miller, 1997; Shu et al., 2003; Haider et al., 2006), one may speculate that this mechanism could be involved in gating of signal transmission (Buzsáki, 2010). That is, by modulating inhibitory synaptic transmission, astrocytes could favor network excitation resulting in neuronal firing consistent with the transmission, i.e. gating "on," of specific stimuli rather than others (Vogels and Abbott, 2009).

The latter idea could also bring to a possible role of astrocyte signaling in coherent function of neural networks underlying potential behavioral states (Engel et al., 2001). In cortical slices, for example, stimulation of a single astrocyte was reported to activate large portions of the astrocytic network and to result in an increase of synchronized neuronal depolarizations (Poskanzer and Yuste, 2011). This phenomenon was suggested to modulate the induction of cortical UP and DOWN states, possibly involved in determining the oscillatory activity observed in slow-wave sleep (Fellin et al., 2004; Halassa and Haydon, 2010; Poskanzer and Yuste, 2011), and is consistent with reports of sleep perturbations in mice lacking astrocytic gliotransmitter exocytosis (Fellin et al., 2009; Halassa et al., 2009; Fellin et al., 2012).

CONCLUSIONS

A large body of evidence has accumulated over the last years, suggesting an active role of astrocytes in many brain functions. Collectively these data have fuelled the concept that synapses could be tripartite rather than bipartite, since in addition to the pre- and post-synaptic terminals, the astrocyte could be an active element in synaptic transmission (Araque et al., 1999; Haydon, 2001). While the tripartite synapse concept captures well the essence of astrocyte-regulated synapses, the inclusion of astrocytic signaling within our current knowledge of synaptic transmission could add more than just one level of complexity. Existing evidence suggests that astrocytes could produce not just tonic and diffuse modulatory influences on synapses but also engage in more focused, spatially precise and constrained communications with synaptic terminals (Anderson and Swanson, 2000; Jourdain et al., 2007; Santello and Volterra, 2009; Bergersen et al., 2012). This calls to rethink the definition of a functional synapse, to include the contribution from surrounding astrocytes. To conclude, the growing appreciation that astrocytes can regulate synaptic information at many levels, from activity of single synapses to network levels and behavioral states (Fellin et al., 2009; Halassa et al., 2009; Zorec et al., 2012) changes our understanding of brain communication and the role of glial cells in synaptic transmission. This resulting novel scenario offers an enticing platform for future theoretical investigations that we are just beginning to appreciate in its potential far-reaching implications.

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