

Perisynaptic astroglial processes: dynamic processors of neuronal information

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Abstract Neuroglial interactions are now recognized as essential to brain functions. Extensive research has sought to understand the modalities of such dialog by focusing on astrocytes, the most abundant glial cell type of the central nervous system. Neuron–astrocyte exchanges occur at multiple levels, at different cellular locations. With regard to information processing, regulations occurring around synapses are of particular interest as synaptic networks are thought to underlie higher brain functions. Astrocytes morphology is tremendously complex in that their processes exceedingly branch out to eventually form multitudinous fine leaflets. The latter extremities have been shown to surround many synapses, forming perisynaptic astrocytic processes, which although recognized as essential to synaptic functioning, are poorly defined elements due to their tiny size. The current review sums up the current knowledge on their molecular and structural properties as well as the functional characteristics making them good candidates for information processing units.

Keywords Astrocytes · Neurons · Neuroglial interactions · Synapses · Processes · Neurotransmission

Introduction

Cellular composition of the brain is of two main kinds: neurons and glia. Neurons have for long been regarded as the exclusive basic structural element undertaking noble functions of the brain, allowing for subtle sensory inputs processing as well as higher abilities such as memory making and reflection. In contrast glial cells, which outnumber neurons, have been considered as a mere “glue” filling the space between neurons and supporting their activity by providing a metabolic bridge with blood capillaries. Although glia does play such roles, in the last decades the picture has become incredibly more complex. Astroglia, the most abundant cell type of the central nervous system, has indeed been found to regulate ionic homeostasis and neurotransmitter levels in the extracellular space and substantial evidence now shows that they are capable of releasing neuroactive molecules known as gliotransmitters, e.g., glutamate, D-serine, ATP (Parpura et al. 1994; Mothet et al. 2005; Pangrsic et al. 2007). Such finding provoked a fundamental conceptual change for neurosciences as synapses should now be considered as a party of three: the pre- and postsynaptic neurons as well as the astrocytes. Morphologically, the latter are characterized by a small soma from which expand manifold ramifications so that the portion of non-somatic membranes largely predominates. Classical astrocytic markers such as GFAP or SR101 actually enable to visualize only a very restricted portion of these ramifications (Fig. 1a, c–e). The radiance of astrocytes is in fact extremely complex and exceedingly branches out to eventually cover most confined intercellular spaces. The thinnest portions of these ramifications form sheet-like structures that are crucial for modeling fine brain organization during

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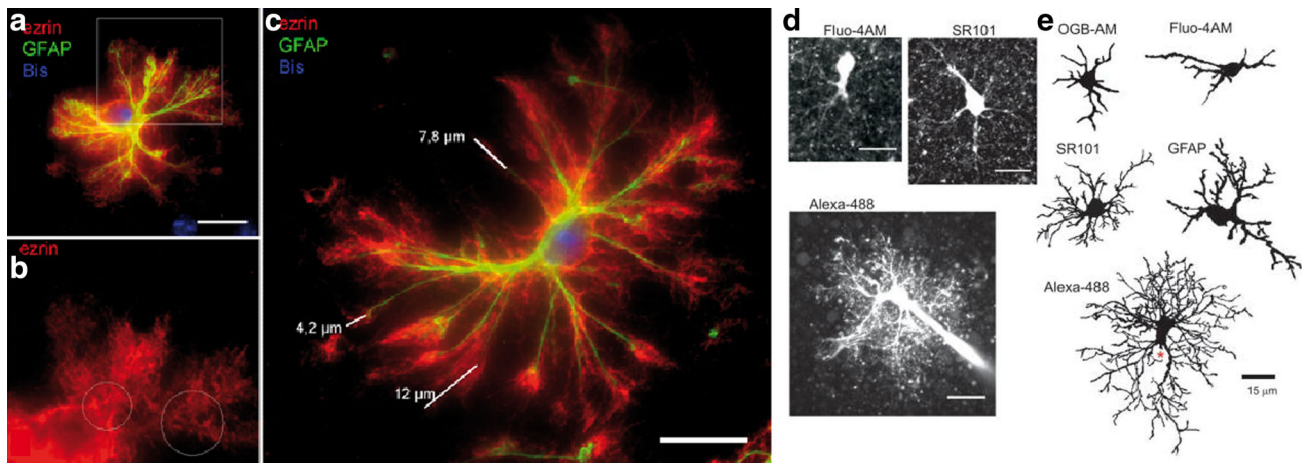


Fig. 1 The astrocyte arborescence viewed through different methodological approaches. **a–c** Immunolabeling of acutely dissociated and morphologically well-preserved mouse astrocytes showing that ezrin covers the entire astrocytic surface (**a**) and mainly localizes to the PAPs (**b**) whereas GFAP is restricted to the main processes. The ezrin IR-positive processes extend beyond the main processes by a mean distance of $6.64 \mu\text{m}$ (**c**). **d** Images of confocal Z-series projection for

mouse astrocytes loaded with Fluo-4AM, labeled with SR101 or dialyzed with Alexa-488. **e** Two-dimensional representation of an astrocyte arborization revealed by Alexa-488 dialysis, SR101 and GFAP labeling, and Ca^{2+} indicator (OGB-AM, Fluo-4AM) dye loading. Adapted from Reeves et al. (2011) (**d–e**) and Haseleu et al. (2013) (**a–c**)

development as well as in adulthood. One hallmark of such function is the insulation provided by oligodendrocyte myelin sheaths allowing for accelerated action potential propagation. Similarly, astroglial processes contacting nodes of Ranvier also influence axonal spreading of electrical activity (Butt et al. 1994). The so-called perisynaptic astrocytic processes (PAPs) play a particularly prominent role at synapses where they regulate their formation, by providing an astroglial “cradle” and positive molecular factors (Nedergaard and Verkhratsky 2012; Allen 2013), as well as elimination by direct engulfing and release of negative regulators (Allen 2013; Chung et al. 2013). The size and shape of PAPs enable them to come in direct apposition with the synaptic cleft (Fig. 2), where they are ideally placed to sense neuronal activity, notably through transport of potassium and glutamate (Dallérac et al. 2013). Although it is generally well-accepted that they respond by regulating neurotransmission, whether this is mediated by gliotransmission (i.e., release of neuroactive substances from astrocytes to modulate neuronal activity) or indirectly as a result of homeostatic regulations has been and remains subject of intense debates. Such dissensions are difficult to solve in that PAP properties are not well defined given the nanometric scale at which direct optical or physiological measures would have to be performed. Here, we review the current knowledge on the molecular and functional aspects of PAPs with an integrated point of view regarding the tripartite synapse during development as well as adulthood in health and diseases.

PAP as the third synaptic compartment

In the adult brain, protoplasmic astrocytes of the cortical gray matter exhibit a complex “spongiform” shape made of tridimensional ramifications of processes, generally organized such that on one side an end-foot process contacts a blood vessel and on the other side numerous fine processes nestle close to active synapses. As such cells do not display the clear polarity usually provided by a basolateral organization, it has recently been proposed to define astrocytic edges according to the cellular compartment their membrane domains contact as well as their unique protein expression patterns (Derouiche et al. 2012). In this respect, the most distal and finest leaflets of the astrocytic tree have been shown in brain slices to preferentially localize at the vicinity of glutamatergic synapses (Spacek 1985). These PAPs recruit a specific repertoire of proteins, display remarkable dynamic characteristics and form specialized signaling compartments. The current section describes these properties and further discusses how information arriving at the PAP may be conveyed to the astrocytic soma.

Specific molecular composition of PAPs

PAPs express a unique set of proteins that are particularly associated with glutamate metabolism and signaling, such as the catabolizing enzyme glutamine synthetase (GS, Derouiche and Frotscher 1991), the high-affinity glutamate transporters (GluT) GLT-1 and GLAST (Rothstein et al.

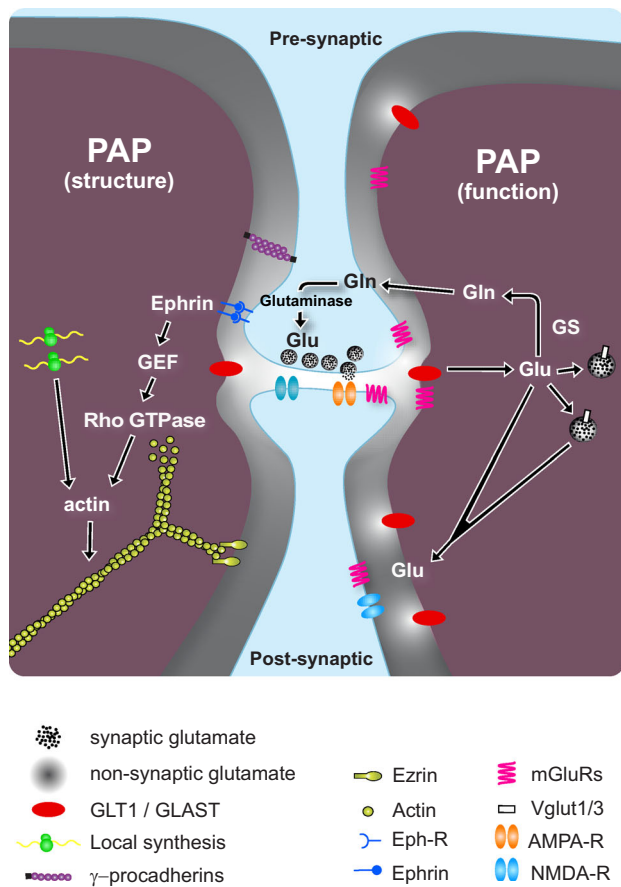


Fig. 2 The unique molecular composition of the PAP supports morphology dynamics and glutamate signaling. Structure (*left*): current knowledge on the molecular content of PAPs indicate that they express specific structural proteins such as dense actin fibers (unlike astrocytic branches that instead typically express intermediate filaments) as well as the ezrin protein, linking actin fibers to the plasma membrane. Spatial segregation of actin and possibly ezrin appears to arise from the compartmentalization of messenger RNAs followed by local synthesis. Regulation of the PAP morphology is thought to involve Ephrin signaling, implying the ephrin-receptor (Eph-R) and guanine exchange factors (GEF), which regulate extension and retraction of actin fibers through modulation of the Rho-GTPase activity. Glutamatergic function (*right*): in addition to the glutamate transporters (GLT1/GLAST), PAPs express a set of glutamate receptors mainly represented by the metabotropic types (mGluRs). The well-known astrocytic transport of glutamate is crucial for maintaining the tonic levels of glutamate at extremely low concentrations at the synaptic cleft (see Moussawi et al. 2011). Once in the PAP, glutamate is either recycled via transformation into glutamine and vice versa, or incorporated into astrocytic vesicle through the vesicular transporters Vglut1 and 3. Direct release of glutamate mediated by opening of hemichannels is also a possible fate of PAP glutamate

1994), the vesicular glutamate transporters VGLUT1 and 3 (Ormel et al. 2012a, b), the metabotropic glutamate receptors (mGluRs) 3 and 5 (Lavielle et al. 2011) (Fig. 2). Astroglial processes tend to make closer contacts with the postsynaptic rather than presynaptic element (Lehre and Rusakov 2002) and, in this regard, are further enriched

with a repertoire of transmembrane and membrane-anchored proteins supporting bidirectional neuroglial signaling. For instance, dendritic spines express Eph receptor tyrosine kinases, while PAPs contain ephrin cell-surface ligands (Murai and Pasquale 2011), together with γ -protocadherin (Garrett and Weiner 2009) and most likely integrin receptors (Hama et al. 2004; Herrera-Molina et al. 2012) (Fig. 2). Based on their small size (hundreds of nanometers thick), PAPs are generally assumed to not contain organelles, although they may enclose some mitochondria (Lovatt et al. 2007), which have been found in fine astroglial processes near synapses (Genoud et al. 2006; Lavielle et al. 2011). Yet, these domains do contain cytoskeletal elements that differ from their larger parent process; namely, PAPs are filled with dense cortical actin fibers (Fig. 2) and occasionally microtubules at their base, and appear devoid of intermediate filament, including the widely used molecular marker glial fibrillary acidic protein (GFAP, Fig. 1a, c) (Derouiche et al. 2002; Haseleu et al. 2013). Most interestingly, spatial segregation of such proteins in the astrocytic tree appears to arise from compartmentalization of messenger RNAs. Indeed, whereas mRNAs encoding for GFAP have been found in situ in retinal glial cells (Müller cells) to be confined in the cytoplasm, actin mRNAs are mainly localized in the cell processes (Sarthy et al. 1989). Further, it has recently been shown that astrocyte protrusions are enriched with additional mRNAs, such as Ras-related proteins, Plakophilin-4, Ankyrin Repeat Domain 25 and inositol polyphosphate-1-phosphatase (Thomsen and Lade Nielsen 2011). To recruit molecules at PAPs, astrocytes may require scaffolding proteins, as seen in perivascular endfeet, where dystrophin-associated complex proteins undertake the anchoring of Kir4.1 potassium channels and aquaporin-4 water channels (Enger et al. 2012). Accordingly, actin-associated proteins of the ezrin radixin moesin (ERM) family have been described to be specifically enriched in PAPs (Derouiche and Frotscher 2001), where they dynamically regulate membrane cytoskeleton linkage. However, most scaffolding proteins in PAPs are still unknown and, therefore, represent an exciting target for future studies. Moreover, compartmentalization of the various cytoskeletal elements along the astrocytic tree supposes serial mRNA and protein transport systems. For instance, the APC tumor-suppressor protein, known to be implicated in mRNA anchoring at the leading edge of migrating fibroblasts (Mili et al. 2008), has been shown to localize at the tip of microtubules within protrusions of migrating astrocytes (Etienne-Manneville and Hall 2003). It may, thus, be hypothesized that APC anchors PAPs mRNAs in migrating astrocytes during development and/or adulthood. Such compartmentalized distribution and translation of mRNAs in PAPs would enable local and adjustable accumulation of a specific

repertoire of proteins. However, given that mechanisms of cargo transports in astrocyte processes are still poorly understood, these hypotheses have yet to be thoroughly investigated *in situ*. This might be achieved using a recently described variant of the Boyden chambers assay, in which a membrane with small pores separates cultured cells from the bottom compartment, allows for the isolation of cell protrusions and thus enables genome-wide screenings of their components (Thomsen and Lade Nielsen 2011). Another freshly described method consists in an acute cell dissociation procedure based on cytospin centrifugation preserving the astrocytes fine morphology, which enables the precise *in vitro* localization of antigen in intact PAPs (Haseleu et al. 2013). In all, despite intense investigations of the third synaptic partner in the last decades, molecular functioning of PAPs remains poorly described; the aforementioned methodological advances may, therefore, become instrumental in unraveling their unique molecular features.

PAPs display particular dynamic morphological properties

Pioneering work performed by the group of Steven Smith in the early 90s demonstrated that cultured astrocytes respond to glutamate exposure by rapidly inducing sprouting of filopodial processes. Remarkably, similar reorganizations of the membrane surface can be induced by settling neurons on top of an astrocytic culture, suggesting that chemotropism toward neuronal diffusing signals might be the mechanism driving astrocytic plasma membrane motility (Cornell-Bell et al. 1990). Further studies in brain slices have demonstrated that whereas PAPs surrounding synapses display spontaneous remodeling within the time range of a minute, this motility is not observed at the level of astrocytic somata and endfeet (Hirrlinger et al. 2004; Haber et al. 2006). However, given the tremendous complexity of differentiated astrocyte morphology, elucidating *in situ* the general principle of this structural plasticity remains challenging. Thus, several *in vitro* assays have been developed to characterize the compartmentalized signaling pathways underpinning the asymmetric rearrangement of cellular elements in response to stimuli (Etienne-Manneville 2008). In particular, establishment of stellation in primary cultured astrocytes has been used to model the dynamic behavior of PAPs, as it fully relies on subcellular redistribution of cytoskeletal proteins (Safavi-Abbasi et al. 2001), and induces the rapid protrusion of filopodia at the cell surface, which are devoid of GFAP whilst expressing ezrin (Fig. 1a, b) and α -actin (Racchetti et al. 2012). Using stellation model, filopodia extension and retraction have been shown to be controlled by the small GTPases Rac1 and Rho, respectively. Thus, activated Rac1

would relocalize under the plasma membrane at the extremity of astrocyte protrusions (Racchetti et al. 2012), where it has been suggested to promote the polymerization of actin filaments as well as the recruitment of ezrin, which links actin to the plasma membrane when phosphorylated at T567 (Auvinen et al. 2007). In brain slices, T567-activated ezrin has been shown to be restricted to perisynaptic glial sheaths, providing a hypothetical mechanism for the stabilization of PAP remodeling after mGluRs activation (Lavialle et al. 2011). On the other hand, L-glutamate exposure *in vitro* has also been found to activate the small GTPase Rho, leading to suppression of astrocyte stellation via an as yet unknown mechanism involving GluTs (Chen et al. 2006). Therefore, the opposite effect of Rho and Rac1 activation on astrocyte morphology is consistent with the role they play in axonal growth cone and dendritic spine extension and retraction, respectively (Tashiro et al. 2000; Woo and Gomez 2006). Together, these GTPases-mediated antagonistic functions of glutamate suggest that the link between synaptic activity and morphological plasticity of PAPs may not be as trivial as a chemotropism toward glutamate and likely depends on the repertoire of membrane proteins expressed at a given process. An alternative mechanism for glutamate-induced PAP structural plasticity could rely on intracellular calcium (Ca^{2+}) rise upon mGluR-induced Gq GPCR activation setting off the canonical phospholipase C/inositol1,4,5-triphosphate (IP3) pathway. Recent data have indeed shown a reduced astrocytic coverage of hippocampal synapses in mice in which IP3R-induced Ca^{2+} release was downregulated by expressing a recombinant glutathione-S-transferase-IP3 buffer (“IP3 sponge”) specifically in astrocytes and in a temporally controlled manner (Tanaka et al. 2013). Moreover, neuroglial contact-mediated ephrin signaling may regulate the morphology of the tripartite synapse via the recruitment of guanine exchange factors (GEF) such as Ephexin (Shamah et al. 2001), Tiam1 (Tanaka et al. 2004) and the Rap1/R-Ras exchange factor SHEP1 (Dodelet et al. 1999), thereby controlling small G protein activity.

Strikingly, using more integrated approaches, it has recently been shown in hippocampal acute slices that inducing long-term synaptic plasticity in CA1 pyramidal neurons, through Schaffer collaterals high-frequency stimulation, leads to a fast G protein-mediated motility of astrocytes processes, which actually move away from the dendritic shaft (Perez-Alvarez et al. 2014). In this regard, we have recently shown that an unexpected key determinant of synaptic astroglial coverage in the hippocampus is the gap-junction protein connexin 30 (Cx30). Indeed, in the absence of the protein, astroglial processes literally penetrate synaptic clefts and get abnormally close to postsynaptic densities (Fig. 4), thereby reducing synaptic transmission as synaptic glutamate is removed directly

from the synaptic cleft by GluTs. Such finding indicates that Cx30 regulates the distance of the PAP from the synapse (Pannasch et al. 2014). Further, using a mutated form (Cx30T5M) that only impairs the channel function of the protein, we showed that such regulation involves channel-independent mechanisms. Insofar as Cx30 has recently been shown to interact with the cytoskeleton (Qu et al. 2009), it appears that such interaction is essential to confine PAPs to perisynaptic spaces, thereby limiting the sink effect of GluT accumulation away from releasing sites. Given that Cx30 only begins to be expressed around P10 in mice, it most certainly plays a prominent role in shaping synaptic networks during postnatal development. Recent studies reminiscent of our work assessed the impact of increasing GluT expression on synaptic and neuronal function, and found impairments in presynaptic short-term plasticity, long-term plasticity as well as information processing and memory formation (Carmona et al. 2009; Filosa et al. 2009). Further research is, however, needed to unravel the molecular pathway(s) enabling Cx30 to regulate the distance of the PAP to the synaptic cleft.

PAPs as specialized signaling compartments

PAPs account for ~80 % of the astrocytic plasma membrane area (Chao et al. 2002) and carry specific pools of ions channels, neurotransmitter receptors and transporters (Wolff and Chao 2004). Moreover, proteins in PAPs are expected to be concentrated in domains facing the synapse, where they would be adequately positioned to integrate synaptic events. In fact, this has recently been demonstrated for mGluRs (Pantatier et al. 2011) (Fig. 3b) and AMPA as well as NMDA receptors that are not directly aligned to neurotransmitter releasing sites have been shown to display a low opening probability due to their low affinity for glutamate (Raghavachari and Lisman 2004; Santucci and Raghavachari 2008). Thus, given that PAPs contact the majority of synapses in the neuropil (Bushong et al. 2002), they appear perfectly suited for locally sensing synaptic spillover. In this respect, astrocytes have been shown to respond to synaptic activation by increasing Ca^{2+} concentration in specific peripheral process subdomains (Grosche et al. 1999). Strikingly, the spatial extent of these Ca^{2+} rises seems directly correlated to the strength of neuronal activity, such that signaling events associated to basal synaptic functioning are restricted to the tip of the astrocytic tree (Pantatier et al. 2011) and do not involve somatic activation, which would rather be recruited during intense firing activity. Such compartmentalized Ca^{2+} transients most likely rely on the activation of metabotropic receptors and pathways downstream of Gq GPCR (Volterra and Meldolesi 2005; Agulhon et al. 2008). A report from Di Castro and collaborators has recently suggested that

astrocytic processes may receive spatially confined synaptic inputs activating separate GPCR clusters, as pharmacological blocking of downstream GPCRs abolishes Ca^{2+} signals in all compartments spanning astrocytic processes, whilst independently inhibiting different types of GPCR only suppresses Ca^{2+} signal at specific locations. Hence, the idea that PAPs integrate input-specific signals is now emerging and may for instance explain why CA1 hippocampal astrocytes do respond to activation of Schaffer collaterals (CA3–CA1), but remain silent in response to recurrent pathway (CA1–CA1) stimulation (Perea and Araque 2005). Mechanistically, the functional compartmentalization of PAPs is likely to be mediated by the localized trafficking and positioning of their receptors, as is known to occur in postsynaptic elements (Henley et al. 2011). In particular, activation of PAP mGluRs, which requires a minimal time of 50 ms glutamate exposure (Attwell and Gibb 2005), is predicted to be modulated by their distance from GluTs with which they compete for glutamate binding (Tzingounis and Wadiche 2007). Therefore, akin to neurons, glutamate-induced Ca^{2+} signaling in PAPs may not only depend on the repertoire of proteins they express, but would also be modulated by the spatial arrangement of these proteins. Noteworthy, protein trafficking and PAP morphogenesis are most likely synergistically regulated, as they both rely on activity-dependent cytoskeletal remodeling (Zhou and Sutherland 2004) and share the same purpose of placing the receptor at the optimal location with regard to glutamate releasing sites. Thence, understanding structure/function relationship in PAPs will most certainly solve conflicting results showing on the one hand that astrocyte-specific activation of Ca^{2+} -dependent release of glutamate does not influence excitatory synaptic activity (Fiacco et al. 2007; Agulhon et al. 2010), whereas on the other hand other reports show that glutamate gliotransmission directly modulates neurotransmission (Halassa et al. 2009; Fellin et al. 2009). Yet, insofar as PAPs are one order of magnitude smaller than light microscopy resolution, physiologically relevant Ca^{2+} “hot spot” in PAPs has never been observed and will require state-of-the art super resolution techniques (Henneberger and Rusakov 2010).

How is information integrated from PAPs to soma?

Since the early hypothesis of Smith in 1992, placing astrocytes as important contributors to information processing, a wealth of investigations have confirmed such role, in particular through the description of Ca^{2+} signaling occurring in astrocytes as a result of neuronal activation. In acute brain slices, bulk loading of membrane permeable Ca^{2+} dyes has allowed demonstrating that intercellular Ca^{2+} oscillations can occur independently of neuronal

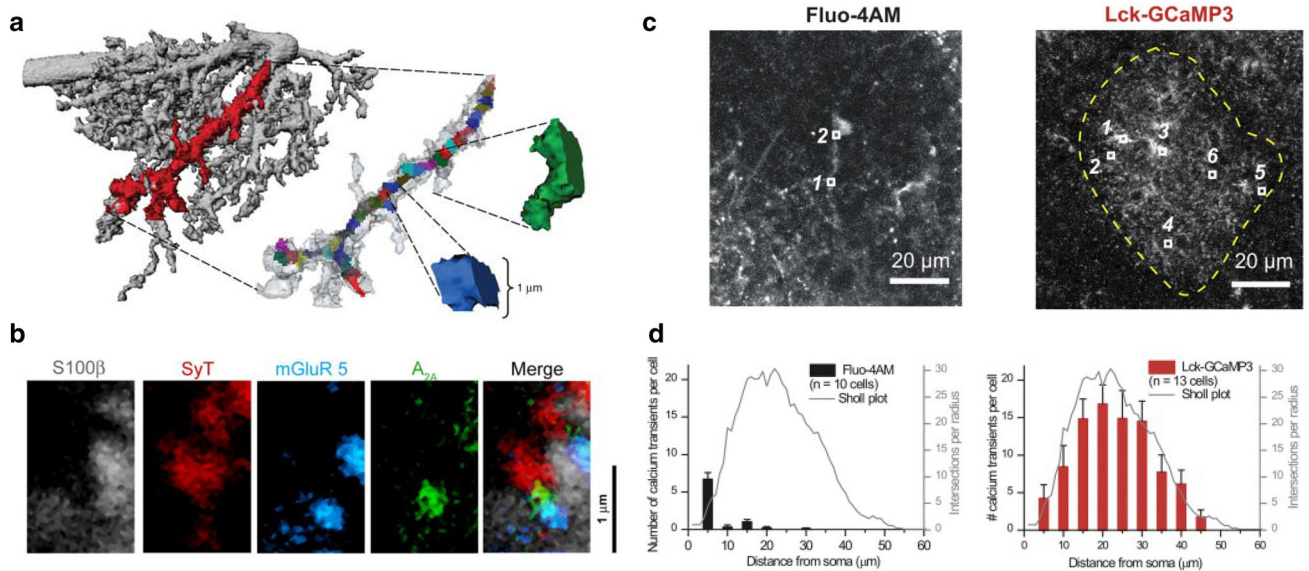


Fig. 3 Perisynaptic astrocyte processes have unique and compartmentalized activities. **a** Three-dimensional representation of a hippocampal astrocyte arborization derived from a two photon Z-series projection of Texas Red dextran signal. The astrocyte process (*red*) display locally confined Ca^{2+} activities within subdomains (*multicolor*). **b** High-magnification confocal images of hippocampal immunolabeling showing a portion of an S100 β -IR-positive astrocyte process (*gray*), synaptotagmin-IR-positive presynaptic

elements (SyT, *red*) bearing $\text{A}_{2\text{A}}$ receptors (*green*), and mGluR5 (*cyan*) decorating fine perisynaptic astrocytic leaflets. **c** Representative images of hippocampal astrocyte loaded with Fluo-4AM or expressing Lck-GCaMP3. **d** Quantification of the number of Ca^{2+} signals measured using Fluo-4AM bulk-loading or Lck-GCaMP3 infected astrocytes as a function of distance from the soma. *Gray line* shows an average astrocyte Sholl plot. Adapted from Di Castro et al. (2011) (a), Panatier et al. (2011) (b) and Shigetomi et al. (2013) (c–d)

stimulation (Nett et al. 2002). The latter has nevertheless been shown to regulate synchronization of spontaneous Ca^{2+} transients (Aguado et al. 2002). At the single cell level, dialysis of membrane-impermeable Ca^{2+} dyes via a patch pipette indicates that astrocytes can detect both basal and evoked synaptic activity in microdomains running along their processes by means of, respectively, “focal” ($\pm 4.2 \mu\text{m}$) and “expanded” ($\pm 12.9 \mu\text{m}$) Ca^{2+} elevations (Panatier et al. 2011; Di Castro et al. 2011). This body of work therefore puts forward the idea that spontaneous Ca^{2+} events may be spatially compartmentalized as long as they are not synchronized by activity (Fig. 3a). The occurrence of action potentials in the vicinity of PAPs may thus coordinate signaling events occurring in neighboring microcompartments and eventually lead to spreading of Ca^{2+} rises from PAPs to soma. This idea is further supported by in vivo data demonstrating that, after sensorimotor stimulation, Ca^{2+} signals in processes occur earlier than those recorded in the soma (Wang et al. 2006; Dombeck et al. 2007; Bekar et al. 2008). Further, activating different Gq GPCRs in astrocytic processes has been shown both in vitro and ex vivo (Yagodin et al. 1994; Fiacco et al. 2007) to induce comparable spatiotemporal patterns of Ca^{2+} spread, moving centripetally along the astrocytic tree. Such intracellular stream of Ca^{2+} may rely on local release from endoplasmic reticulum (ER) through the IP3 pathway, as ER membranes are known to express clusters of the type

2 IP3 receptors (Sheppard et al. 1997). However, limitations of the currently available Ca^{2+} imaging techniques, including the poor intracellular volume sampled by Ca^{2+} dyes after bulk loading (Reeves et al. 2011) and the wash out of cytoplasmic elements induced by dialysis inherent to whole cell patch clamp, have fostered much skepticism, particularly in regard of the sites where signals are monitored, as they are not the actual loci of Ca^{2+} signal induction (presumably PAPs). Such information will most probably be instrumental in our understanding of astroglial integration of synaptic inputs, as it has recently been shown that local Ca^{2+} events within PAPs regulate both synaptic coverage and spine stability (Bernardinelli et al. 2014). A recent study has demonstrated that genetically encoded Ca^{2+} indicators anchored to the membrane would be a more precise alternative, and was thus able, for the first time, to precisely monitor signaling activities occurring throughout the entire astrocytic volume (Shigetomi et al. 2013) (Fig. 3c, d). Remarkably, these experiments revealed that spontaneous Ca^{2+} events in acute brain slices are independently localized in space and equivalently sized, thus delimiting functional signaling “patches” distributed within the astrocytic territory (Shigetomi et al. 2013) (Fig. 3c, d). Although such investigation opens up new perspectives in astrocyte physiology, the challenge of ascertaining Ca^{2+} activation in PAPs still represents a major caveat that will need to be filled.

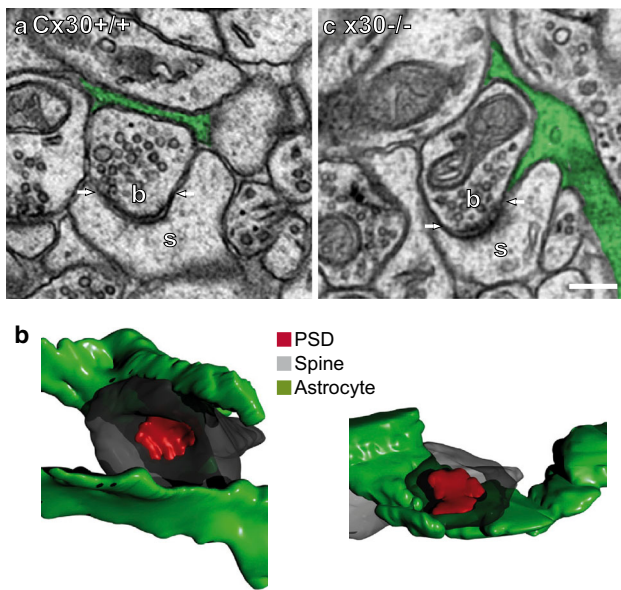


Fig. 4 Astroglial processes can invade synaptic clefts. **a** Serial electron microscopy images illustrating a hippocampal asymmetric synapse [PSD, marked by *arrow*, axonal bouton (**b**), dendritic spine (**S**)] contacted by astroglial processes (*green*) in *Cx30*^{+/+} and *Cx30*^{-/-} mice. Deficiency for astroglial *Cx30* leads to penetration of synaptic clefts by glial processes. *Scale bar*, 0.2 μm . **b** Reconstructions of the entire dendritic spine (*gray*) with PSD (*red*) and the surrounding astrocytic elements (*green*) are illustrated for *Cx30*^{+/+} (**c**) and *Cx30*^{-/-} mice (**d**), showing a closer association of *Cx30*^{-/-} astrocytic processes to dendritic spines and a shorter distance to PSD. Adapted from Pannasch et al. (2014)

PAP–spine as the processing unit of local neuroglial interactions

Astroglial coverage of spines is heterogenous. Electron microscopy investigations indeed show that spine coverage varies from no contact within olfactory bulb glomeruli (Chao et al. 2002) to ensheathment of almost all spines ($\sim 90\%$) in hippocampal mushroom/perforated synapses (Witcher et al. 2007). Such ultrastructural studies, however, provide “snapshot” overviews at a given timepoint, and do not appreciate the actually dynamic nature of glia–synapse interactions. Morphological relationships between the three synaptic partners have indeed been shown to change during development (Nixdorf-Bergweiler et al. 1994; Bushong et al. 2004), as well as in response to neuronal activity (Güldner and Wolff 1996; Oliet et al. 2001) and plasticity (Wenzel et al. 1991; Lushnikova et al. 2009). The following section presents the physiological properties of PAPs in these dynamic contexts and further explains how alterations of the function and three-dimensional distribution of astrocytic processes may contribute to synaptic activity.

Establishment of PAP–spine contacts during development

Onset of astrocytic filopodial process sprouting is concomitant to the expression of astrocytic mGluRs and GluT5 at postnatal week one (Schools and Kimelberg 1999; Regan et al. 2007) and is hence thought to occur as a result of neuronal excitatory transmission. Over the two following weeks, astroglial processes fully infiltrate the neuropil and actively participate in the activity of synaptic circuits (Freeman 2010). Formation of differentiated yet postsynaptically silent synapses is then subtended by the astrocytic matricellular proteins thrombospondins, especially TSP1 and 2, of which the peak of secretion is reached during early postnatal synaptogenesis (Christopherson et al. 2005). TSPs interact with various neuronal receptors, such as integrins (DeFreitas et al. 1995), neuroligins (Xu et al. 2010) and voltage-gated Ca^{2+} channels (Eroglu et al. 2009), thereby promoting the recruitment of adhesive as well as scaffolding proteins to nascent synapses. Thereafter, the maintenance of such connections is undertaken by trans-synaptic clustering of adhesion molecules, namely neuroligins and neurexins (Clarke and Barres 2013), mediated by the matricellular hevin–SPARC couple (Kucukdereli et al. 2011), whose expression is kept high in astrocytes during adulthood. Finally, functional maturation of synapses is regulated by the insertion and clustering of AMPA receptors in the postsynaptic density, which is directly supervised by astrocyte-secreted glypican proteins (Allen et al. 2012). Remarkably, injection into adult cat visual cortex of immature astrocytes, which can regulate ECM composition, has been shown to be sufficient to reactivate the critical period of ocular dominance plasticity (Müller and Best 1989). During the third and fourth postnatal weeks, glial coverage of maturing synapses and ramification of the finest leaflet of the astrocytic tree establish proper astrocytic territories by limiting processes overlap (Bushong et al. 2004). Mechanisms behind these morphological changes involve release of glutamate from axon terminals, acting as a signal for both dendritic spines and PAPs (Hirrlinger et al. 2004; Haber et al. 2006). Interestingly, dendritic remodeling mediated by glutamate spillover occurring in the hippocampus (Verbich et al. 2012) is associated with an adjustment of astrocytic process morphology, weakening PAP association with spines, and hence dynamically reducing GluT activity. PAP–spine association is also regulated through activation of dendritic EphA4 receptors with the astrocytic ligand ephrinA3, which results in spine retraction and reduces the lifetime of newly generated dendrites (Murai et al. 2003; Nishida and Okabe 2007). Lastly, although the formation of dendritic spine does not require the presence of astrocytes, the ability

to receive synapses may not be an intrinsic neuronal property and has been shown in retinal ganglion cells to require contacts with astrocytes (Hama et al. 2004; Barker et al. 2008). In regard of such findings, the idea that astrocyte processes function as bona fide “synaptic cradle” nursing synaptic development has now emerged (Nedergaard and Verkhratsky 2012).

PAPs control synaptic transmission

Through shielding of chemical synapses by means of their fine distal processes, astrocytes ensure efficient spine-to-spine transmission via insulation of neurotransmitter releasing sites. In particular, astroglial synaptic enwrapping is thought to prevent neurotransmitters spillover and activation of extrasynaptic receptors, thereby preserving release probability (Oliet et al. 2001). In this respect, PAPs are thought to rule for synaptic versus volume mode of neurotransmission by shaping extracellular neurotransmitter diffusion and uptake (Piet et al. 2004; Syková and Nicholson 2008). Volume transmission implies a diffusion-mediated intersynaptic crosstalk that occurs when concomitant vesicular releases from distinct presynaptic elements lead to neurotransmitter spillover and activation of receptors from neighboring synapses. Interestingly, the role of volume transmission as a purely modulatory pathway has recently been revisited, as glutamate spillover (Semyanov and Kullmann 2001) and glutamate gliotransmission (Liu et al. 2004) can induce action potential firing, suggesting that PAPs allow to bypass synaptic connections. Finally, coverage of excitatory synapses has been proposed to bring astrocytic vesicles competent for glutamate release (Parpura and Zorec 2010) at sufficient proximity to the synaptic cleft to activate both pre- and postsynaptic GluRs. In the same way, astrocytic release of glutamate co-agonists has recently been shown to control synaptic transmission by unlocking the gate of NMDARs (Papouin et al. 2012). Crucially, these mechanisms of PAP-mediated tuning of neurotransmission are nevertheless inconsistent with the functional competition brought by astrocytic glutamate transporters at the extrasynaptic level (Huang et al. 2004). Indeed, GLT-1 and GLAST affinity for glutamate is comparable to NMDARs or mGluRs and their glutamate binding velocity is similar to AMPARs (Huang et al. 2004). Thus, given the high expression levels of GluTs at astrocyte membranes (Lehre and Danbolt 1998), glutamate released by PAPs would have to run away from thousands of glial GluTs to stand a chance of activating neuronal GluRs. Therefore, to solve this discrepancy, PAP glutamatergic gliotransmission must imply the spatiotemporal segregation of glutamate uptake and release events. Interestingly, such patterned membrane expression of transporters and transmitter releasing sites at PAPs has been

proposed to functionally result in compartmentalization of extracellular glutamate concentrations (Moussawi et al. 2011), an hypothesis supported by *in vitro* data suggesting that, conversely, extracellular fluctuations of glutamate control membrane distribution of glial transporters (Poitry-Yamate et al. 2002; Nakagawa et al. 2008). However, most of the arguments brought forward to explain how astrocytes set the mode of synaptic transmission remain inaccurate in that the role of PAPs is often omitted. Indeed, PAPs are difficult to study in living tissue, as they are not directly accessible to traditional electrophysiological recordings, cannot be readily isolated for biochemistry, and are smaller than the optical resolution of regular light microscopy (Reichenbach et al. 2010). Therefore, despite interesting emergent hypotheses, the current lack of understanding of the PAP functional architecture is a major issue that will need to be addressed to decipher their actual role in neurotransmission.

Activity-dependent plasticity of PAP–spine functional interactions

Subtle changes in the efficacy of synaptic transmission are believed to be at the core of memory formation and can be experimentally assessed through the induction of long-term potentiation (LTP) and long-term depression, respectively corresponding to activity-dependent increase and decrease in synaptic strength (Bliss and Lomo 1973; Bliss and Collingridge 1993; Cooke and Bliss 2006). The so-called Hebbian synapse increases its efficacy when pre- and postsynaptic activity concurs, and, conversely, decreases its strength as a result of disparate activity. NMDAR-dependent LTP and LTD require coincident presynaptic glutamate release and depolarization of the postsynaptic membrane, enabling synchronous Mg^{2+} block relief and NMDAR activation (Lüscher and Malenka 2012). Strikingly, astrocytes have recently been proposed to provide additional sources of activity underlying atypical form of Hebbian plasticity. NMDAR-independent LTP of presynaptic neurotransmitter release could indeed be induced by gliotransmission of glutamate coupled to postsynaptic depolarization (Perea and Araque 2007), thereby implying that astrocytes would contribute to the long-term storage of synaptic information. Although this hypothesis has recently been validated *in vivo* for both LTP and LTD (Min and Nevian 2012; Navarrete et al. 2012), we are still far from understanding the exact mechanisms underlying these astrocyte-dependent forms of plasticity. For instance, signaling dynamics at the PAPs may be several orders of magnitude slower than neurotransmission events, which would imply that the pairing of pre- and postsynaptic activities is likely achieved long before gliotransmission has occurred. In this respect, a recent study proposed that for

certain patterns of correlated activity, astrocytes may act as a “thresholding unit”, meaning that PAPs-to-neuron signaling would be triggered in response to local build-up of neurotransmitters accumulated as a result of repeated stimulations (Min and Nevian 2012). However, further studies are still required to fully decipher how PAPs manage to sense differences in extracellular neurotransmitters concentration. In a more integrated perspective, astrocytes may control metaplasticity, i.e., the ability of synapses to exhibit long-term modifications. Indeed both LTP (Henneberger et al. 2010) and LTD (Zhang et al. 2008) are in part gated by the astrocyte-secreted NMDAR coagonist D-serine, known to be enriched in glial processes (Schell et al. 1995). Thus, PAPs have been proposed to set the “sliding threshold” for long-term synaptic plasticity by controlling the number of NMDARs that may be activated during synaptic transmission (Panatier et al. 2006). This hypothesis has, however, recently been challenged by reports showing that serine racemase (SR), the enzyme undertaking the synthesis of D-serine, is predominantly expressed in glutamatergic neurons and that genetic suppression of neuronal, but not astroglial, SR leads to alteration in synaptic functions and plasticity (Rosenberg et al. 2010; Benneyworth et al. 2012). Moreover, given that PAPs enwrap ~60 % of hippocampal excitatory synapses and support activity-dependent spreading of Ca²⁺ signaling, such structures are ideally positioned to coordinate plasticity at neighboring inactivated synapses along dendritic shafts (known as heterosynaptic plasticity). It has, thus, been proposed that gliotransmitter-mediated homeostatic plasticity may serve as an efficient way to regulate the overall neuronal outputs, via coordinated scaling of synaptic weights (Beattie et al. 2002; Gordon et al. 2005). In addition to the functional strengthening or weakening of pre-existing synapses, activity-dependent long-term plasticity is known to induce the de novo formation and elimination of synapses.

Strikingly, it has also recently been described that LTP induces the structural remodeling of PAPs. Indeed, LTP was found to induce a transient increase in PAP motility (Bernardinelli et al. 2014; Perez-Alvarez et al. 2014), leading to a decreased astrocytic coverage of dendritic spines (Perez-Alvarez et al. 2014) although an increased coverage preferentially occurring on enlarged spines was also reported (Bernardinelli et al. 2014). Thus, LTP regulates, both in vitro and in vivo, synaptic coverage and, in doing so, influences synaptic fate. Remarkably, a decrease in astroglial synapse coverage in the amygdala was also recently evidenced during consolidation of contextual memory (Ostroff et al. 2014). These latter evidence suggest that astrocytic withdrawal may promote synaptic efficacy.

Finally, as astrocyte territories contact several individual neurons, PAPs most probably connect synaptic ensembles

belonging to separate neuronal circuits. It would, therefore, be interesting to ascertain in future investigations whether astrocytes may compute information stored from dispersed synaptic networks.

Implication of PAP–spine interactions in synaptopathies?

Homeostatic fluctuation of extracellular glutamate levels is a key modulator of synaptic function. The amount of neurotransmitters flowing in the extracellular space is set by the dynamic balance between neuroglial uptake and release events, as well as the coordinated synaptic recycling of neurotransmitter precursor and metabolites (Papura and Verkhratsky 2012). Alterations in the core elements making up this system results in a vast repertoire of neurodegenerative and neuropsychiatric disorders. For instance, excessive or prolonged activation of excitatory amino acid receptors results in the massive influx of ions, and eventually leads to activation of Ca²⁺-dependent inflammatory pathways and cell death cascades (Puyal et al. 2013). Such glutamatergic excitotoxic signaling is heavily involved in the etiology of neurodegenerative disorders such as Alzheimer’s or Huntington’s disease (Hynd et al. 2004; Mehta et al. 2013), multiple sclerosis (Kostic et al. 2013) and neuropathic pain (Osikowicz et al. 2013). Excessive glutamatergic gliotransmission (Ding et al. 2007), reversion of astrocytic glutamate transporters activity (Grewer et al. 2008), connexin (and likely pannexin) hemichannels (Bennett et al. 2012) and glutamate-permeable ion channels opening (Woo et al. 2012), may represent a significant source of glutamate release leading to excitotoxicity. Supporting this notion, Papouin et al. have recently demonstrated that PAP-secreted D-serine gates the glutamate-induced Ca²⁺ overload that mediates neuronal death in hippocampal slices, hence suggesting that perisynaptic neuroglial interactions play a crucial role in neurodegeneration (Papouin et al. 2012). Other mechanisms subtending neurological disorders rely on long-lasting adaptation of glutamate homeostasis regulators such as the glial glutamate transporter EAAT2/GLT-1 and the glutamate-catabolizing enzyme GS as a result of prolonged exposure to inadequate ECS glutamate concentrations (Javitt et al. 2011). These persistent changes have indeed been shown to mediate symptoms of mental disorders with known environmental causes, such as substance abuse (Kalivas 2009) and mood disorders (Mathews et al. 2012). Moreover, failure of the system to maintain the overall excitation/inhibition balance of synaptic inputs is thought to result in a pathophysiological pathway shared by most psychiatric conditions (Yizhar et al. 2011). In this respect, “glutamate hypotheses” accounting for neuropsychiatric disorders such as schizophrenia (Moghaddam and Javitt

2012), Huntington's chorea (Cummings et al. 2009) and Down's syndrome (Baroncelli et al. 2011) have emerged. Interestingly, this framework is currently being reconsidered in light of the tripartite nature of the synapse. For instance, the function of astrocytic cysteine/glutamate exchangers and glutamate transporters has been shown to be strongly altered after prolonged exposure to drugs of abuse, thereby mediating symptoms of drug-seeking patients (Reissner and Kalivas 2010). In the same way, given that astrocytes processes could control the synchronized activity of neuronal networks dispersed across their territory of influence (Halassa et al. 2007; Pannasch and Rouach 2013), astroglial processes have been proposed to participate in the spatiotemporal coordination of excitation/inhibition balance via the release of gliotransmitters (Fellin et al. 2006). Therefore, in all, implication of the third synaptic compartment in our understanding of synaptopathies has yet to be considered and is likely to bring forth new pharmacological targets and therapeutic strategies.

Conclusions and perspectives

The PAP has recently been recognized as the third active element of the chemical synapse (Araque et al. 1999), thereby challenging the previous neurocentric dogma of synaptic transmission. The unique molecular composition as well as structural plasticity and signaling properties of perisynaptic glial sheaths endow them with the ability to interfere dynamically with neuronal information processing. Although the PAP is now viewed as the initiation locus of astrocyte excitability, most studies have, however, yielded unreliable monitoring of these synaptically evoked glial events due to spatial resolution limitations of conventional approaches at the nanometric scale. Thus, force is to conclude that our understanding of the mechanisms used by PAP for sensing and responding to ongoing neurotransmission is still lagging behind in experimental evidences and many questions consequently linger. Do all PAPs cover synapses the same way? Does this depend on brain areas? On neuronal populations? On the type of synapses? What are the signaling cues regulating glia–synapse contacts? Are all PAPs expressing the same pool of proteins or mRNAs? Are all PAPs equipped for gliotransmission? Addressing these questions of fine brain organization will most probably require the synergistic combination of cutting-edge optical and electrophysiological methodologies.

New optical approaches for monitoring PAP structure and function

Three-dimensional reconstruction of electron microscopic serial section is a suitable technique for resolving the

ultrastructural architecture of the tortuous perisynaptic astroglial membranes (Ventura and Harris 1999; Witcher et al. 2010). Although electron microscopy provides the most accurate description and quantification of PAP morphology and synapse coverage, such approach can, however, only provide static observations on fixed tissue and is notoriously tedious.

Alternative approaches have recently been developed by tempting to overcome the diffraction resolution limit inherent to light microscopy (Maglione and Sigrist 2013), thus providing a way to study PAPs dynamics in physiological contexts. Stimulated emission depletion (STED) microscopy is the first modality of super-resolution techniques that successfully broke the diffraction limit (Hell and Wichmann 1994) by reducing the size of the blurry excitation spot called point-spread function (PSF). This is achieved by restricting the emission light to a central spot which is slightly smaller than the diffraction barrier. Such technique has been used to resolve changes in synapse shape (Tønnesen et al. 2014), neurotransmitter vesicles fusion (Willig et al. 2006) as well as single-molecule trafficking in dendritic spines (Blom et al. 2013; Westin et al. 2014). STED microscopy is thus a particularly promising approach to unravel the intimate structure of the tripartite synapse. However, one may keep in mind that STED microscopy induces relatively high phototoxicity, does not improve Z-axis spatial resolution and displays a lower temporal resolution than traditional scanning microscopy techniques. In addition, to work in tissues at a reasonable depth, STED microscopy should be combined to 2-photon excitation, otherwise limiting access to cultured cells or organotypic slices. More recent methodologies have further emerged, offering alternative possibilities to investigate PAPs dynamics. Photo-activated localisation microscopy (PALM) and stochastic optical reconstruction microscopy (STORM) combine the iterative and random switching on/off of fluorophores with the computational localization of its PSF center (Henriques et al. 2011). Such multicolor photo-switchable super-resolution imaging has for example recently enabled highly specific single molecules detection with less than 50 nm lateral resolution (Bates et al. 2007). However, PALM and STORM are thus far only applicable to *in vitro* cultured cells. Future development of these techniques may enable unraveling PAPs proteins/mRNAs compartmentalization and activation by fusing chromatically distinguishable photo-activatable probes (Heilemann 2009) to neurotransmitter receptors and transporter molecules. Furthermore, novel Ca^{2+} indicators have recently been generated. Of particular interest, the recent use of a membrane-targeted “GCaMP”, a well-known Ca^{2+} indicator expressed *in situ* or *in vivo* in astrocytes (Shigetomi et al. 2010, 2013), has revealed unprecedented intracellular near-membrane Ca^{2+} events

within astrocyte processes microdomains (Shigetomi et al. 2010, 2013) (Fig. 3c, d). Noteworthy, this non-invasive approach enables to assess Ca^{2+} signaling in fine astroglial processes. One should nevertheless not overlook the fact that high-affinity Ca^{2+} indicators can strongly buffer intracellular Ca^{2+} , thereby altering important signaling pathways, while low-affinity indicators possibly operate below detection level. Finally, the use of membrane voltage reporters has not yet been exploited to study PAP excitability but should, in the near future, help to unravel the mechanisms enabling PAPs to endorse synaptic neuroglial ionic regulation.

Adapting electrode-based electrophysiological techniques to record PAP signaling

Electrophysiology is the gold standard for assessing electrical activity. Techniques such as patch clamp recordings are usually performed at cell bodies, enabling the detection of currents originating mostly from somas and proximal processes. At distal processes, currents might be detectable at the somatic level only in the context of strong and coordinated activity generated in multiple loci. However, basal activity of channels and receptors from individual PAPs is currently hardly detectable. This is partly due to restricted spatial and temporal control of astrocyte membrane potentials and currents recorded in situ with the whole cell patch clamp technique. Thus, despite the fact that the surface of the numerous tiny distal astroglial processes exceeds by far the membrane area of soma and main proximal processes, up to now, we cannot directly and reliably record their electrophysiological activity. This is particularly unfortunate, since PAPs precisely contain the receptors and channels that are functionally relevant and are most likely the ones playing a crucial role in regulating activity of adjacent synapses. There is, therefore, an urge to develop refined patch clamp approaches designed to overcome current limitations and record from fine astroglial distal processes with dimensions typically below hundreds of nanometers, as performed nowadays for dendrites (Davie et al. 2006). One of the main challenges is to visualize PAPs as their dimensions, typically below hundreds of nanometers, cannot be resolved by regular light microscopy. In addition, they can only be identified topologically, based on their close association with synapses as there is, as yet, no specific known marker for PAPs. Thus, only super resolution techniques such as STED microscopy coupled to two photon excitations can provide the required resolution to visualize dynamically such structures in live tissues. To identify PAPs, one therefore needs to label both astrocytes, using for instance dye loading via a patch pipette (e.g., Alexa-488, Fig. 1e), and spines, taking for

example advantage of transgenic mice such as Thy1-YFPH mice where spines are sparsely labeled. Another important challenge is to improve the signal to noise ratio of the patch clamp technique, since the presumably small electrophysiological responses of tiny astrocytic processes are likely to be below the present current threshold detection, i.e., 3–5 pA. Finally one also needs to design electrodes with tip size below 1 μm . Although this can be presently achieved using filamented glass with micropipette puller, nanotechniques such as focused ion beam microscopy (Qiao et al. 2005) or carbon nanotubes (Krapf et al. 2006) are very promising approaches to produce finer electrodes with a better control. Remarkably, such electrodes will not only enable to patch tiny processes, but shall also contribute to a better signal to noise ratio due their high resistance, as well as minimize deformation of tissue (Scanziani and Häusser 2009). In fact, in depth study of the intimate dialog between PAPs and adjacent synapses will most likely rely in combinations of electrophysiology and Ca^{2+} imaging, as both approaches give access to complementary relevant information at the level of PAPs. Indeed, whilst Ca^{2+} imaging only provides information about one, yet crucial signaling molecule involved in numerous transduction pathways and thought to engage astroglial excitability, patch clamp recordings give access to quantitative information concerning all ionic currents induced by activation of membrane receptors and channels. Such interdisciplinary strategy should, therefore, be the key to unravel online the dynamics of neuroglial signaling between PAPs and adjacent synapses, as well as their role in processing brain information.

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References

- Aguado F, Espinosa-Parrilla JF, Carmona MA, Soriano E (2002) Neuronal activity regulates correlated network properties of spontaneous calcium transients in astrocytes in situ. *J Neurosci* 22:9430–9444
- Agulhon C, Petravic J, McMullen AB et al (2008) What is the role of astrocyte calcium in neurophysiology? *Neuron* 59:932–946. doi:[10.1016/j.neuron.2008.09.004](https://doi.org/10.1016/j.neuron.2008.09.004)
- Agulhon C, Fiacco TA, McCarthy KD (2010) Hippocampal short- and long-term plasticity are not modulated by astrocyte Ca^{2+} signaling. *Science* 327:1250–1254. doi:[10.1126/science.1184821](https://doi.org/10.1126/science.1184821)
- Allen NJ (2013) Role of glia in developmental synapse formation. *Curr Opin Neurobiol* 23:1027–1033. doi:[10.1016/j.conb.2013.06.004](https://doi.org/10.1016/j.conb.2013.06.004)

- Allen NJ, Bennett ML, Foo LC et al (2012) Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature* 486:410–414. doi:[10.1038/nature11059](https://doi.org/10.1038/nature11059)
- Araque A, Parpura V, Sanzgiri RP, Haydon PG (1999) Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci* 22:208–215
- Attwell D, Gibb A (2005) Neuroenergetics and the kinetic design of excitatory synapses. *Nat Rev Neurosci* 6:841–849. doi:[10.1038/nrn1784](https://doi.org/10.1038/nrn1784)
- Auvinen E, Kivi N, Vaheri A (2007) Regulation of ezrin localization by Rac1 and PIPK in human epithelial cells. *Exp Cell Res* 313:824–833. doi:[10.1016/j.yexcr.2006.12.002](https://doi.org/10.1016/j.yexcr.2006.12.002)
- Barker AJ, Koch SM, Reed J et al (2008) Developmental control of synaptic receptivity. *J Neurosci* 28:8150–8160. doi:[10.1523/JNEUROSCI.1744-08.2008](https://doi.org/10.1523/JNEUROSCI.1744-08.2008)
- Baroncelli L, Braschi C, Spolidoro M et al (2011) Brain plasticity and disease: a matter of inhibition. *Neural Plast* 2011:286073. doi:[10.1155/2011/286073](https://doi.org/10.1155/2011/286073)
- Bates M, Huang B, Dempsey GT, Zhuang X (2007) Multicolor super-resolution imaging with photo-switchable fluorescent probes. *Science* 317:1749–1753. doi:[10.1126/science.1146598](https://doi.org/10.1126/science.1146598)
- Beattie EC, Stellwagen D, Morishita W et al (2002) Control of synaptic strength by glial TNF α . *Science* 295:2282–2285. doi:[10.1126/science.1067859](https://doi.org/10.1126/science.1067859)
- Bekar LK, He W, Nedergaard M (2008) Locus coeruleus alpha-adrenergic-mediated activation of cortical astrocytes in vivo. *Cereb Cortex* 18:2789–2795. doi:[10.1093/cercor/bhn040](https://doi.org/10.1093/cercor/bhn040)
- Bennett MVL, Garré JM, Orellana JA et al (2012) Connexin and pannexin hemichannels in inflammatory responses of glia and neurons. *Brain Res* 1487:3–15. doi:[10.1016/j.brainres.2012.08.042](https://doi.org/10.1016/j.brainres.2012.08.042)
- Benneyworth MA, Li Y, Basu AC et al (2012) Cell selective conditional null mutations of serine racemase demonstrate a predominate localization in cortical glutamatergic neurons. *Cell Mol Neurobiol* 32:613–624. doi:[10.1007/s10571-012-9808-4](https://doi.org/10.1007/s10571-012-9808-4)
- Bernardinelli Y, Randall J, Janett E et al (2014) Activity-dependent structural plasticity of perisynaptic astrocytic domains promotes excitatory synapse stability. *Curr Biol* 24:1679–1688. doi:[10.1016/j.cub.2014.06.025](https://doi.org/10.1016/j.cub.2014.06.025)
- Bliss TV, Collingridge GL (1993) A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361:31–39
- Bliss TV, Lomo T (1973) Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 232:331–356
- Blom H, Rönnlund D, Scott L et al (2013) Spatial distribution of DARPP-32 in dendritic spines. *PLoS One* 8:e75155. doi:[10.1371/journal.pone.0075155](https://doi.org/10.1371/journal.pone.0075155)
- Bushong EA, Martone ME, Jones YZ, Ellisman MH (2002) Protoplasmic astrocytes in CA1 stratum radiatum occupy separate anatomical domains. *J Neurosci* 22:183–192
- Bushong EA, Martone ME, Ellisman MH (2004) Maturation of astrocyte morphology and the establishment of astrocyte domains during postnatal hippocampal development. *Int J Dev Neurosci* 22:73–86. doi:[10.1016/j.ijdevneu.2003.12.008](https://doi.org/10.1016/j.ijdevneu.2003.12.008)
- Butt AM, Duncan A, Berry M (1994) Astrocyte associations with nodes of Ranvier: ultrastructural analysis of HRP-filled astrocytes in the mouse optic nerve. *J Neurocytol* 23:486–499
- Carmona MA, Murai KK, Wang L et al (2009) Glial ephrin-A3 regulates hippocampal dendritic spine morphology and glutamate transport. *Proc Natl Acad Sci USA* 106:12524–12529. doi:[10.1073/pnas.0903328106](https://doi.org/10.1073/pnas.0903328106)
- Chao TI, Rickmann M, Wolff JR (2002) The synapse-astrocyte boundary: an anatomical basis for an integrative role of glia in synaptic transmission. In: Volterra A, Haydon PG (eds) *The tripartite synapse: glia in synaptic transmission*, Oxford University Press, Oxford, pp 3–23
- Chen C-J, Ou Y-C, Lin S-Y et al (2006) L-glutamate activates RhoA GTPase leading to suppression of astrocyte stellation. *Eur J Neurosci* 23:1977–1987. doi:[10.1111/j.1460-9568.2006.04728.x](https://doi.org/10.1111/j.1460-9568.2006.04728.x)
- Christopherson KS, Ullian EM, Stokes CCA et al (2005) Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* 120:421–433. doi:[10.1016/j.cell.2004.12.020](https://doi.org/10.1016/j.cell.2004.12.020)
- Chung W-S, Clarke LE, Wang GX et al (2013) Astrocytes mediate synapse elimination through MEGF10 and MERTK pathways. *Nature* 504:394–400. doi:[10.1038/nature12776](https://doi.org/10.1038/nature12776)
- Clarke LE, Barres BA (2013) Emerging roles of astrocytes in neural circuit development. *Nat Rev Neurosci* 14:311–321. doi:[10.1038/nrn3484](https://doi.org/10.1038/nrn3484)
- Cooke SF, Bliss TVP (2006) Plasticity in the human central nervous system. *Brain* 129:1659–1673. doi:[10.1093/brain/awl082](https://doi.org/10.1093/brain/awl082)
- Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ (1990) Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* 247:470–473
- Cummings DM, André VM, Uzgil BO et al (2009) Alterations in cortical excitation and inhibition in genetic mouse models of Huntington's disease. *J Neurosci* 29:10371–10386. doi:[10.1523/JNEUROSCI.1592-09.2009](https://doi.org/10.1523/JNEUROSCI.1592-09.2009)
- Dalléac G, Chever O, Rouach N (2013) How do astrocytes shape synaptic transmission? Insights from electrophysiology. *Front Cell Neurosci* 7:159. doi:[10.3389/fncel.2013.00159](https://doi.org/10.3389/fncel.2013.00159)
- Davie JT, Kole MHP, Letzkus JJ et al (2006) Dendritic patch-clamp recording. *Nat Protoc* 1:1235–1247. doi:[10.1038/nprot.2006.164](https://doi.org/10.1038/nprot.2006.164)
- DeFreitas MF, Yoshida CK, Frazier WA et al (1995) Identification of integrin alpha 3 beta 1 as a neuronal thrombospondin receptor mediating neurite outgrowth. *Neuron* 15:333–343
- Derouiche A, Frotscher M (1991) Astroglial processes around identified glutamatergic synapses contain glutamine synthetase: evidence for transmitter degradation. *Brain Res* 552:346–350
- Derouiche A, Frotscher M (2001) Peripheral astrocyte processes: monitoring by selective immunostaining for the actin-binding ERM proteins. *Glia* 36:330–341
- Derouiche A, Anlauf E, Aumann G et al (2002) Anatomical aspects of glia-synapse interaction: the perisynaptic glial sheath consists of a specialized astrocyte compartment. *J Physiol Paris* 96:177–182
- Derouiche A, Pannicke T, Haseleu J et al (2012) Beyond polarity: functional membrane domains in astrocytes and Müller cells. *Neurochem Res* 37:2513–2523. doi:[10.1007/s11064-012-0824-z](https://doi.org/10.1007/s11064-012-0824-z)
- Di Castro MA, Chuquet J, Liaudet N et al (2011) Local Ca²⁺ detection and modulation of synaptic release by astrocytes. *Nat Neurosci* 14:1276–1284. doi:[10.1038/nn.2929](https://doi.org/10.1038/nn.2929)
- Ding S, Fellin T, Zhu Y et al (2007) Enhanced astrocytic Ca²⁺ signals contribute to neuronal excitotoxicity after status epilepticus. *J Neurosci* 27:10674–10684. doi:[10.1523/JNEUROSCI.2001-07.2007](https://doi.org/10.1523/JNEUROSCI.2001-07.2007)
- Dodelet VC, Pazzagli C, Zisch AH et al (1999) A novel signaling intermediate, SHEP1, directly couples Eph receptors to R-Ras and Rap1A. *J Biol Chem* 274:31941–31946
- Dombeck DA, Khabbaz AN, Collman F et al (2007) Imaging large-scale neural activity with cellular resolution in awake, mobile mice. *Neuron* 56:43–57. doi:[10.1016/j.neuron.2007.08.003](https://doi.org/10.1016/j.neuron.2007.08.003)
- Enger R, Gundersen GA, Haj-Yasein NN et al (2012) Molecular scaffolds underpinning macroglial polarization: an analysis of retinal Müller cells and brain astrocytes in mouse. *Glia* 60:2018–2026. doi:[10.1002/glia.22416](https://doi.org/10.1002/glia.22416)
- Eroglu C, Allen NJ, Susman MW et al (2009) Gabapentin receptor alpha2delta-1 is a neuronal thrombospondin receptor responsible for excitatory CNS synaptogenesis. *Cell* 139:380–392. doi:[10.1016/j.cell.2009.09.025](https://doi.org/10.1016/j.cell.2009.09.025)
- Etienne-Manneville S (2008) Polarity proteins in glial cell functions. *Curr Opin Neurobiol* 18:488–494. doi:[10.1016/j.conb.2008.09.014](https://doi.org/10.1016/j.conb.2008.09.014)

- Etienne-Manneville S, Hall A (2003) Cdc42 regulates GSK-3 β and adenomatous polyposis coli to control cell polarity. *Nature* 421:753–756. doi:[10.1038/nature01423](https://doi.org/10.1038/nature01423)
- Fellin T, Gomez-Gonzalo M, Gobbo S et al (2006) Astrocytic glutamate is not necessary for the generation of epileptiform neuronal activity in hippocampal slices. *J Neurosci* 26:9312–9322. doi:[10.1523/JNEUROSCI.2836-06.2006](https://doi.org/10.1523/JNEUROSCI.2836-06.2006)
- Fellin T, Halassa MM, Terunuma M et al (2009) Endogenous nonneuronal modulators of synaptic transmission control cortical slow oscillations in vivo. *Proc Natl Acad Sci USA* 106:15037–15042. doi:[10.1073/pnas.0906419106](https://doi.org/10.1073/pnas.0906419106)
- Fiacco TA, Agulhon C, Taves SR et al (2007) Selective stimulation of astrocyte calcium in situ does not affect neuronal excitatory synaptic activity. *Neuron* 54:611–626. doi:[10.1016/j.neuron.2007.04.032](https://doi.org/10.1016/j.neuron.2007.04.032)
- Filosa A, Paixão S, Honsek SD et al (2009) Neuron-glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. *Nat Neurosci* 12:1285–1292. doi:[10.1038/nn.2394](https://doi.org/10.1038/nn.2394)
- Freeman MR (2010) Specification and morphogenesis of astrocytes. *Science* 330:774–778. doi:[10.1126/science.1190928](https://doi.org/10.1126/science.1190928)
- Garrett AM, Weiner JA (2009) Control of CNS synapse development by γ -protocadherin-mediated astrocyte-neuron contact. *J Neurosci* 29:11723–11731. doi:[10.1523/JNEUROSCI.2818-09.2009](https://doi.org/10.1523/JNEUROSCI.2818-09.2009)
- Genoud C, Quairiaux C, Steiner P et al (2006) Plasticity of astrocytic coverage and glutamate transporter expression in adult mouse cortex. *PLoS Biol* 4:e343. doi:[10.1371/journal.pbio.0040343](https://doi.org/10.1371/journal.pbio.0040343)
- Gordon GRJ, Baimoukhametova DV, Hewitt SA et al (2005) Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat Neurosci* 8:1078–1086. doi:[10.1038/nn1498](https://doi.org/10.1038/nn1498)
- Grewer C, Gameiro A, Zhang Z et al (2008) Glutamate forward and reverse transport: from molecular mechanism to transporter-mediated release after ischemia. *IUBMB Life* 60:609–619. doi:[10.1002/iub.98](https://doi.org/10.1002/iub.98)
- Grosche J, Matyash V, Möller T et al (1999) Microdomains for neuron-glia interaction: parallel fiber signaling to Bergmann glial cells. *Nat Neurosci* 2:139–143. doi:[10.1038/5692](https://doi.org/10.1038/5692)
- Güldner FH, Wolff JR (1996) Complex synaptic arrangements in the rat suprachiasmatic nucleus: a possible basis for the “Zeitgeber” and non-synaptic synchronization of neuronal activity. *Cell Tissue Res* 284:203–214
- Haber M, Zhou L, Murai KK (2006) Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. *J Neurosci* 26:8881–8891. doi:[10.1523/JNEUROSCI.1302-06.2006](https://doi.org/10.1523/JNEUROSCI.1302-06.2006)
- Halassa MM, Fellin T, Takano H et al (2007) Synaptic islands defined by the territory of a single astrocyte. *J Neurosci* 27:6473–6477. doi:[10.1523/JNEUROSCI.1419-07.2007](https://doi.org/10.1523/JNEUROSCI.1419-07.2007)
- Halassa MM, Florian C, Fellin T et al (2009) Astrocytic modulation of sleep homeostasis and cognitive consequences of sleep loss. *Neuron* 61:213–219. doi:[10.1016/j.neuron.2008.11.024](https://doi.org/10.1016/j.neuron.2008.11.024)
- Hama H, Hara C, Yamaguchi K, Miyawaki A (2004) PKC signaling mediates global enhancement of excitatory synaptogenesis in neurons triggered by local contact with astrocytes. *Neuron* 41:405–415
- Haseleu J, Anlauf E, Blaess S et al (2013) Studying subcellular detail in fixed astrocytes: dissociation of morphologically intact glial cells (DIMIGs). *Front Cell Neurosci* 7:54. doi:[10.3389/fncel.2013.00054](https://doi.org/10.3389/fncel.2013.00054)
- Heilemann M (2009) Light at the end of the tunnel. *Angew Chem Int Ed Engl* 48:3908–3910. doi:[10.1002/anie.200900696](https://doi.org/10.1002/anie.200900696)
- Hell SW, Wichmann J (1994) Breaking the diffraction resolution limit by stimulated emission: stimulated-emission-depletion fluorescence microscopy. *Opt Lett* 19:780–782
- Henley JM, Barker EA, Glebov OO (2011) Routes, destinations and delays: recent advances in AMPA receptor trafficking. *Trends Neurosci* 34:258–268. doi:[10.1016/j.tins.2011.02.004](https://doi.org/10.1016/j.tins.2011.02.004)
- Henneberger C, Rusakov DA (2010) Synaptic plasticity and Ca²⁺ signalling in astrocytes. *Neuron Glia Biol*. doi:[10.1017/S1740925X10000153](https://doi.org/10.1017/S1740925X10000153)
- Henneberger C, Papouin T, Oliet SHR et al (2010) Long-term potentiation depends on release of D-serine from astrocytes. *Nature* 463:232–236. doi:[10.1038/nature08673](https://doi.org/10.1038/nature08673)
- Henriques R, Griffiths C, Hesper Rego E, Mhlanga MM (2011) PALM and STORM: unlocking live-cell super-resolution. *Biopolymers* 95:322–331. doi:[10.1002/bip.21586](https://doi.org/10.1002/bip.21586)
- Herrera-Molina R, Frischknecht R, Maldonado H et al (2012) Astrocytic α V β 3 integrin inhibits neurite outgrowth and promotes retraction of neuronal processes by clustering Thy-1. *PLoS One* 7:e34295. doi:[10.1371/journal.pone.0034295](https://doi.org/10.1371/journal.pone.0034295)
- Hirrlinger J, Hülsmann S, Kirchhoff F (2004) Astroglial processes show spontaneous motility at active synaptic terminals in situ. *Eur J Neurosci* 20:2235–2239. doi:[10.1111/j.1460-9568.2004.03689.x](https://doi.org/10.1111/j.1460-9568.2004.03689.x)
- Huang YH, Sinha SR, Tanaka K et al (2004) Astrocyte glutamate transporters regulate metabotropic glutamate receptor-mediated excitation of hippocampal interneurons. *J Neurosci* 24:4551–4559. doi:[10.1523/JNEUROSCI.5217-03.2004](https://doi.org/10.1523/JNEUROSCI.5217-03.2004)
- Hynd MR, Scott HL, Dodd PR (2004) Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer’s disease. *Neurochem Int* 45:583–595. doi:[10.1016/j.neuint.2004.03.007](https://doi.org/10.1016/j.neuint.2004.03.007)
- Javitt DC, Schoepp D, Kalivas PW et al (2011) Translating glutamate: from pathophysiology to treatment. *Sci Transl Med*. doi:[10.1126/scitranslmed.3002804](https://doi.org/10.1126/scitranslmed.3002804)
- Kalivas PW (2009) The glutamate homeostasis hypothesis of addiction. *Nat Rev Neurosci* 10:561–572. doi:[10.1038/nrn2515](https://doi.org/10.1038/nrn2515)
- Kostic M, Zivkovic N, Stojanovic I (2013) Multiple sclerosis and glutamate excitotoxicity. *Rev Neurosci* 24:71–88. doi:[10.1515/revneuro-2012-0062](https://doi.org/10.1515/revneuro-2012-0062)
- Krapf D, Quinn BM, Wu M-Y et al (2006) Experimental observation of nonlinear ionic transport at the nanometer scale. *Nano Lett* 6:2531–2535. doi:[10.1021/nl0619453](https://doi.org/10.1021/nl0619453)
- Kucukdereli H, Allen NJ, Lee AT et al (2011) Control of excitatory CNS synaptogenesis by astrocyte-secreted proteins Hevin and SPARC. *Proc Natl Acad Sci USA* 108:E440–E449. doi:[10.1073/pnas.1104977108](https://doi.org/10.1073/pnas.1104977108)
- Lavialle M, Aumann G, Anlauf E et al (2011) Structural plasticity of perisynaptic astrocyte processes involves ezrin and metabotropic glutamate receptors. *Proc Natl Acad Sci USA* 108:12915–12919. doi:[10.1073/pnas.1100957108](https://doi.org/10.1073/pnas.1100957108)
- Lehre KP, Danbolt NC (1998) The number of glutamate transporter subtype molecules at glutamatergic synapses: chemical and stereological quantification in young adult rat brain. *J Neurosci* 18:8751–8757
- Lehre KP, Rusakov DA (2002) Asymmetry of glia near central synapses favors presynaptically directed glutamate escape. *Biophys J* 83:125–134. doi:[10.1016/S0006-3495\(02\)75154-0](https://doi.org/10.1016/S0006-3495(02)75154-0)
- Liu Q-S, Xu Q, Kang J, Nedergaard M (2004) Astrocyte activation of presynaptic metabotropic glutamate receptors modulates hippocampal inhibitory synaptic transmission. *Neuron Glia Biol* 1:307–316. doi:[10.1017/S1740925X05000190](https://doi.org/10.1017/S1740925X05000190)
- Lovatt D, Sonnewald U, Waagepetersen HS et al (2007) The transcriptome and metabolic gene signature of protoplasmic astrocytes in the adult murine cortex. *J Neurosci* 27:12255–12266. doi:[10.1523/JNEUROSCI.3404-07.2007](https://doi.org/10.1523/JNEUROSCI.3404-07.2007)
- Lüscher C, Malenka RC (2012) NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harb Perspect Biol*. doi:[10.1101/cshperspect.a005710](https://doi.org/10.1101/cshperspect.a005710)
- Lushnikova I, Skibo G, Muller D, Nikonenko I (2009) Synaptic potentiation induces increased glial coverage of excitatory

- synapses in CA1 hippocampus. *Hippocampus* 19:753–762. doi:10.1002/hipo.20551
- Maglione M, Sigris SJ (2013) Seeing the forest tree by tree: super-resolution light microscopy meets the neurosciences. *Nat Neurosci* 16:790–797. doi:10.1038/nn.3403
- Mathews DC, Henter ID, Zarate CA (2012) Targeting the glutamatergic system to treat major depressive disorder: rationale and progress to date. *Drugs* 72:1313–1333. doi:10.2165/11633130-000000000-00000
- Mehta A, Prabhakar M, Kumar P et al (2013) Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur J Pharmacol* 698:6–18. doi:10.1016/j.ejphar.2012.10.032
- Mili S, Moissoglou K, Macara IG (2008) Genome-wide screen reveals APC-associated RNAs enriched in cell protrusions. *Nature* 453:115–119. doi:10.1038/nature06888
- Min R, Nevian T (2012) Astrocyte signaling controls spike timing-dependent depression at neocortical synapses. *Nat Neurosci* 15:746–753. doi:10.1038/nn.3075
- Moghaddam B, Javitt D (2012) From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. *Neuropsychopharmacology* 37:4–15. doi:10.1038/npp.2011.181
- Mothet J-P, Pollegioni L, Ouanounou G et al (2005) Glutamate receptor activation triggers a calcium-dependent and SNARE protein-dependent release of the gliotransmitter D-serine. *Proc Natl Acad Sci USA* 102:5606–5611. doi:10.1073/pnas.0408483102
- Moussawi K, Riegel A, Nair S, Kalivas PW (2011) Extracellular glutamate: functional compartments operate in different concentration ranges. *Front Syst Neurosci* 5:94. doi:10.3389/fnsys.2011.00094
- Müller CM, Best J (1989) Ocular dominance plasticity in adult cat visual cortex after transplantation of cultured astrocytes. *Nature* 342:427–430. doi:10.1038/342427a0
- Murai KK, Pasquale EB (2011) Eph receptors and ephrins in neuron-astrocyte communication at synapses. *Glia* 59:1567–1578. doi:10.1002/glia.21226
- Murai KK, Nguyen LN, Irie F et al (2003) Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. *Nat Neurosci* 6:153–160. doi:10.1038/nn994
- Nakagawa T, Otsubo Y, Yatani Y et al (2008) Mechanisms of substrate transport-induced clustering of a glial glutamate transporter GLT-1 in astroglial-neuronal cultures. *Eur J Neurosci* 28:1719–1730. doi:10.1111/j.1460-9568.2008.06494.x
- Navarrete M, Perea G, Maglio L et al (2012) Astrocyte calcium signal and gliotransmission in human brain tissue. *Cereb Cortex* 23:1240–1246. doi:10.1093/cercor/bhs122
- Nedergaard M, Verkhratsky A (2012) Artifact versus reality—how astrocytes contribute to synaptic events. *Glia* 60:1013–1023. doi:10.1002/glia.22288
- Nett WJ, Oloff SH, McCarthy KD (2002) Hippocampal astrocytes in situ exhibit calcium oscillations that occur independent of neuronal activity. *J Neurophysiol* 87:528–537
- Nishida H, Okabe S (2007) Direct astrocytic contacts regulate local maturation of dendritic spines. *J Neurosci* 27:331–340. doi:10.1523/JNEUROSCI.4466-06.2007
- Nixdorf-Bergweiler BE, Albrecht D, Heinemann U (1994) Developmental changes in the number, size, and orientation of GFAP-positive cells in the CA1 region of rat hippocampus. *Glia* 12:180–195. doi:10.1002/glia.440120304
- Oliet SH, Piet R, Poulain DA (2001) Control of glutamate clearance and synaptic efficacy by glial coverage of neurons. *Science* 292:923–926. doi:10.1126/science.1059162
- Ormel L, Stensrud MJ, Bergersen LH, Gundersen V (2012a) VGLUT1 is localized in astrocytic processes in several brain regions. *Glia* 60:229–238. doi:10.1002/glia.21258
- Ormel L, Stensrud MJ, Chaudhry FA, Gundersen V (2012b) A distinct set of synaptic-like microvesicles in astroglial cells contain VGLUT3. *Glia* 60:1289–1300. doi:10.1002/glia.22348
- Osikowicz M, Mika J, Przewlocka B (2013) The glutamatergic system as a target for neuropathic pain relief. *Exp Physiol* 98:372–384. doi:10.1113/expphysiol.2012.069922
- Ostroff LE, Manzur MK, Cain CK, Ledoux JE (2014) Synapses lacking astrocyte appear in the amygdala during consolidation of Pavlovian threat conditioning. *J Comp Neurol* 522:2152–2163. doi:10.1002/cne.23523
- Panatier A, Theodosis DT, Mothet J-P et al (2006) Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* 125:775–784. doi:10.1016/j.cell.2006.02.051
- Panatier A, Vallée J, Haber M et al (2011) Astrocytes are endogenous regulators of basal transmission at central synapses. *Cell* 146:785–798. doi:10.1016/j.cell.2011.07.022
- Pangrsic T, Potokar M, Stenovc M et al (2007) Exocytotic release of ATP from cultured astrocytes. *J Biol Chem* 282:28749–28758. doi:10.1074/jbc.M700290200
- Pannasch U, Rouach N (2013) Emerging role for astroglial networks in information processing: from synapse to behavior. *Trends Neurosci* 36:405–417. doi:10.1016/j.tins.2013.04.004
- Pannasch U, Freche D, Dallérac G et al (2014) Connexin 30 sets synaptic strength by controlling astroglial synapse invasion. *Nat Neurosci*. doi:10.1038/nn.3662
- Papouin T, Ladépêche L, Ruel J et al (2012) Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists. *Cell* 150:633–646. doi:10.1016/j.cell.2012.06.029
- Parpura V, Verkhratsky A (2012) The astrocyte excitability brief: from receptors to gliotransmission. *Neurochem Int* 61:610–621. doi:10.1016/j.neuint.2011.12.001
- Parpura V, Zorec R (2010) Gliotransmission: exocytotic release from astrocytes. *Brain Res Rev* 63:83–92. doi:10.1016/j.brainresrev.2009.11.008
- Parpura V, Basarsky TA, Liu F et al (1994) Glutamate-mediated astrocyte-neuron signalling. *Nature* 369:744–747. doi:10.1038/369744a0
- Perea G, Araque A (2005) Properties of synaptically evoked astrocyte calcium signal reveal synaptic information processing by astrocytes. *J Neurosci* 25:2192–2203. doi:10.1523/JNEUROSCI.3965-04.2005
- Perea G, Araque A (2007) Astrocytes potentiate transmitter release at single hippocampal synapses. *Science* 317:1083–1086. doi:10.1126/science.1144640
- Perez-Alvarez A, Navarrete M, Covelo A et al (2014) Structural and functional plasticity of astrocyte processes and dendritic spine interactions. *J Neurosci* 34:12738–12744. doi:10.1523/JNEUROSCI.2401-14.2014
- Piet R, Vargová L, Syková E et al (2004) Physiological contribution of the astrocytic environment of neurons to intersynaptic crosstalk. *Proc Natl Acad Sci USA* 101:2151–2155. doi:10.1073/pnas.0308408100
- Poitry-Yamate CL, Vutskits L, Rauen T (2002) Neuronal-induced and glutamate-dependent activation of glial glutamate transporter function. *J Neurochem* 82:987–997
- Puyal J, Ginet V, Clarke PGH (2013) Multiple interacting cell death mechanisms in the mediation of excitotoxicity and ischemic brain damage: a challenge for neuroprotection. *Prog Neurobiol* 105:24–48. doi:10.1016/j.pneurobio.2013.03.002
- Qiao Y, Chen J, Guo X et al (2005) Fabrication of nanoelectrodes for neurophysiology: cathodic electrophoretic paint insulation and focused ion beam milling. *Nanotechnology* 16:1598–1602. doi:10.1088/0957-4484/16/9/032
- Qu C, Gardner P, Schrijver I (2009) The role of the cytoskeleton in the formation of gap junctions by Connexin 30. *Exp Cell Res* 315:1683–1692. doi:10.1016/j.yexcr.2009.03.001

- Racchetti G, D'Alessandro R, Meldolesi J (2012) Astrocyte stellation, a process dependent on Rac1 is sustained by the regulated exocytosis of enlargeosomes. *Glia* 60:465–475. doi:10.1002/glia.22280
- Raghavachari S, Lisman JE (2004) Properties of quantal transmission at CA1 synapses. *J Neurophysiol* 92:2456–2467. doi:10.1152/jn.00258.2004
- Reeves AMB, Shigetomi E, Khakh BS (2011) Bulk loading of calcium indicator dyes to study astrocyte physiology: key limitations and improvements using morphological maps. *J Neurosci* 31:9353–9358. doi:10.1523/JNEUROSCI.0127-11.2011
- Regan MR, Huang YH, Kim YS et al (2007) Variations in promoter activity reveal a differential expression and physiology of glutamate transporters by glia in the developing and mature CNS. *J Neurosci* 27:6607–6619. doi:10.1523/JNEUROSCI.0790-07.2007
- Reichenbach A, Derouiche A, Kirchhoff F (2010) Morphology and dynamics of perisynaptic glia. *Brain Res Rev* 63:11–25. doi:10.1016/j.brainresrev.2010.02.003
- Reissner KJ, Kalivas PW (2010) Using glutamate homeostasis as a target for treating addictive disorders. *Behav Pharmacol* 21:514–522. doi:10.1097/FBP.0b013e32833d41b2
- Rosenberg D, Kartvelishvili E, Shleper M et al (2010) Neuronal release of D-serine: a physiological pathway controlling extracellular D-serine concentration. *FASEB J* 24:2951–2961. doi:10.1096/fj.09-147967
- Rothstein JD, Martin L, Levey AI et al (1994) Localization of neuronal and glial glutamate transporters. *Neuron* 13:713–725
- Safavi-Abbasi S, Wolff JR, Missler M (2001) Rapid morphological changes in astrocytes are accompanied by redistribution but not by quantitative changes of cytoskeletal proteins. *Glia* 36:102–115
- Santucci DM, Raghavachari S (2008) The effects of NR2 subunit-dependent NMDA receptor kinetics on synaptic transmission and CaMKII activation. *PLoS Comput Biol* 4:e1000208. doi:10.1371/journal.pcbi.1000208
- Sarthy PV, Fu M, Huang J (1989) Subcellular localization of an intermediate filament protein and its mRNA in glial cells. *Mol Cell Biol* 9:4556–4559
- Scanziani M, Häusser M (2009) Electrophysiology in the age of light. *Nature* 461:930–939. doi:10.1038/nature08540
- Schell MJ, Molliver ME, Snyder SH (1995) D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc Natl Acad Sci USA* 92:3948–3952
- Schools GP, Kimelberg HK (1999) mGluR3 and mGluR5 are the predominant metabotropic glutamate receptor mRNAs expressed in hippocampal astrocytes acutely isolated from young rats. *J Neurosci Res* 58:533–543
- Semyanov A, Kullmann DM (2001) Kainate receptor-dependent axonal depolarization and action potential initiation in interneurons. *Nat Neurosci* 4:718–723. doi:10.1038/89506
- Shamah SM, Lin MZ, Goldberg JL et al (2001) EphA receptors regulate growth cone dynamics through the novel guanine nucleotide exchange factor ephexin. *Cell* 105:233–244
- Sheppard CA, Simpson PB, Sharp AH et al (1997) Comparison of type 2 inositol 1,4,5-trisphosphate receptor distribution and subcellular Ca²⁺ release sites that support Ca²⁺ waves in cultured astrocytes. *J Neurochem* 68:2317–2327
- Shigetomi E, Kracun S, Sofroniew MV, Khakh BS (2010) A genetically targeted optical sensor to monitor calcium signals in astrocyte processes. *Nat Neurosci* 13:759–766. doi:10.1038/nn.2557
- Shigetomi E, Bushong EA, Hausteiner MD et al (2013) Imaging calcium microdomains within entire astrocyte territories and endfeet with GCaMPs expressed using adeno-associated viruses. *J Gen Physiol* 141:633–647. doi:10.1085/jgp.201210949
- Spacek J (1985) Three-dimensional analysis of dendritic spines. III. Glial sheath. *Anat Embryol (Berl)* 171:245–252
- Syková E, Nicholson C (2008) Diffusion in brain extracellular space. *Physiol Rev* 88:1277–1340. doi:10.1152/physrev.00027.2007
- Tanaka M, Ohashi R, Nakamura R et al (2004) Tiam1 mediates neurite outgrowth induced by ephrin-B1 and EphA2. *EMBO J* 23:1075–1088. doi:10.1038/sj.emboj.7600128
- Tanaka M, Shih P-Y, Gomi H et al (2013) Astrocytic Ca²⁺ signals are required for the functional integrity of tripartite synapses. *Mol Brain* 6:6. doi:10.1186/1756-6606-6-6
- Tashiro A, Minden A, Yuste R (2000) Regulation of dendritic spine morphology by the rho family of small GTPases: antagonistic roles of Rac and Rho. *Cereb Cortex* 10:927–938
- Thomsen R, Lade Nielsen A (2011) A Boyden chamber-based method for characterization of astrocyte protrusion localized RNA and protein. *Glia* 59:1782–1792. doi:10.1002/glia.21223
- Tønnesen J, Katona G, Rózsa B, Nägerl UV (2014) Spine neck plasticity regulates compartmentalization of synapses. *Nat Neurosci* 17:678–685. doi:10.1038/nn.3682
- Tzingounis AV, Wadiche JI (2007) Glutamate transporters: confining runaway excitation by shaping synaptic transmission. *Nat Rev Neurosci* 8:935–947. doi:10.1038/nrn2274
- Ventura R, Harris KM (1999) Three-dimensional relationships between hippocampal synapses and astrocytes. *J Neurosci* 19:6897–6906
- Verbich D, Prenosil GA, Chang PK-Y et al (2012) Glial glutamate transport modulates dendritic spine head protrusions in the hippocampus. *Glia* 60:1067–1077. doi:10.1002/glia.22335
- Volterra A, Meldolesi J (2005) Astrocytes, from brain glue to communication elements: the revolution continues. *Nat Rev Neurosci* 6:626–640. doi:10.1038/nrn1722
- Wang X, Lou N, Xu Q et al (2006) Astrocytic Ca²⁺ signaling evoked by sensory stimulation in vivo. *Nat Neurosci* 9:816–823. doi:10.1038/nn1703
- Wenzel J, Lammert G, Meyer U, Krug M (1991) The influence of long-term potentiation on the spatial relationship between astrocyte processes and potentiated synapses in the dentate gyrus neuropil of rat brain. *Brain Res* 560:122–131
- Westin L, Reuss M, Lindskog M et al (2014) Nanoscopic spine localization of Norbin, an mGluR5 accessory protein. *BMC Neurosci* 15:45. doi:10.1186/1471-2202-15-45
- Willig KI, Rizzoli SO, Westphal V et al (2006) STED microscopy reveals that synaptotagmin remains clustered after synaptic vesicle exocytosis. *Nature* 440:935–939. doi:10.1038/nature04592
- Witcher MR, Kirov SA, Harris KM (2007) Plasticity of perisynaptic astroglia during synaptogenesis in the mature rat hippocampus. *Glia* 55:13–23. doi:10.1002/glia.20415
- Witcher MR, Park YD, Lee MR et al (2010) Three-dimensional relationships between perisynaptic astroglia and human hippocampal synapses. *Glia* 58:572–587. doi:10.1002/glia.20946
- Wolff JR, Chao TI (2004) Non-neuronal cells of the nervous system: function and dysfunction. doi: 10.1016/S1569-2558(03)31001-X
- Woo S, Gomez TM (2006) Rac1 and RhoA promote neurite outgrowth through formation and stabilization of growth cone point contacts. *J Neurosci* 26:1418–1428. doi:10.1523/JNEUROSCI.4209-05.2006
- Woo DH, Han K-S, Shim JW et al (2012) TREK-1 and Best1 channels mediate fast and slow glutamate release in astrocytes upon GPCR activation. *Cell* 151:25–40. doi:10.1016/j.cell.2012.09.005
- Xu J, Xiao N, Xia J (2010) Thrombospondin 1 accelerates synaptogenesis in hippocampal neurons through neuroligin 1. *Nat Neurosci* 13:22–24. doi:10.1038/nn.2459
- Yagodin SV, Holtzclaw L, Sheppard CA, Russell JT (1994) Nonlinear propagation of agonist-induced cytoplasmic calcium waves in

- single astrocytes. *J Neurobiol* 25:265–280. doi:[10.1002/neu.480250307](https://doi.org/10.1002/neu.480250307)
- Yizhar O, Fenno LE, Prigge M et al (2011) Neocortical excitation/inhibition balance in information processing and social dysfunction. *Nature* 477:171–178. doi:[10.1038/nature10360](https://doi.org/10.1038/nature10360)
- Zhang Z, Gong N, Wang W et al (2008) Bell-shaped D-serine actions on hippocampal long-term depression and spatial memory retrieval. *Cereb Cortex* 18:2391–2401. doi:[10.1093/cercor/bhn008](https://doi.org/10.1093/cercor/bhn008)
- Zhou J, Sutherland ML (2004) Glutamate transporter cluster formation in astrocytic processes regulates glutamate uptake activity. *J Neurosci* 24:6301–6306. doi:[10.1523/JNEUROSCI.1404-04.2004](https://doi.org/10.1523/JNEUROSCI.1404-04.2004)