Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Neurobiology

Glial regulation of synapse maturation and stabilization in the developing nervous system

Marion R Van Horn and Edward S Ruthazer

The dynamic interaction between neurons and glia is a fundamental aspect of developmental neurobiology. Astrocytic processes are extremely complex and can physically surround neuronal synapses where they are involved in regulating neuronal activity and synaptic plasticity. This review describes important roles glial cells play in synapse maturation and stabilization in the developing central nervous system. We highlight recent evidence showing that the motility of astrocytic and radial glial processes is modulated by neuronal signals and is important for normal synapse maturation and function. Examples of glia-derived molecules that influence synapse maturation and stabilization are presented. We close by touching on recent and future trends in neuron-glia research.

Address
Montreal Neurological Institute, McGill University, Montreal, QC, H3A 2B4, Canada

Corresponding author: Ruthazer, Edward S (edward.ruthazer@mcgill.ca)

Current Opinion in Neurobiology 2019, **54**:113–119

This review comes from a themed issue on **Neurobiology of learning and plasticity**

Edited by **Scott Waddell** and **Jesper Sjotrom**

https://doi.org/10.1016/j.conb.2018.10.002

Introduction

Measuring changes in electrical activity of neurons has provided an important foundation for understanding how information is processed and encoded throughout the brain. However, advanced imaging techniques, in particular those based on intracellular calcium dynamics and intravital imaging of cellular morphology, have begun to provide experimental evidence that glial cells are also dynamic cells that respond to changes in neuronal activity and engage in bi-directional communication with neurons and other glial cells. In some cases these interactions comprise a physical 'tripartite synapse' consisting of presynaptic, postsynaptic and glia components [1]. Accordingly, in order to have a complete picture of how information is processed in the brain it is important to jointly consider the contributions of both neurons and glia.

Structural plasticity of the tripartite synapse promotes neural circuit development

Synapses are the primary points of communication between neurons. During development, appropriate synapses are strengthened and inappropriate synapses are lost. This process of synapse selection is essential for the formation of neural circuits, effective information processing and overall brain function.

Astrocytes have diffuse, arborized branches with a myriad of nanoscopic protusions, known as perisynaptic astrocytic processes (PAPs) that extend and associate with synapses. While PAPs are found in all brain areas, the number of synapses covered by PAPs, and the degree to which an individual synapse is covered, varies significantly. During development, astrocyte process motility and as well as coverage of synapses has been found to be quite dynamic [2•,3]. In the cerebellum, ensheathment of dendritic spines by Bergmann glia increases during periods of synaptogenesis [3]. Furthermore, in the developing *Xenopus* tadpole, two-photon *in vivo* time-lapse imaging of radial glial cells, which are thought to be functionally analogous to mammalian stellate astrocytes [4,5], shows radial glia processes are significantly more dynamic during early periods of development when circuits are undergoing extensive synaptic remodeling [2•].

We recently investigated the functional significance of glial motility on the development of neural circuits and neuronal function. We found that suppressing the filopodial dynamics of radial glia during a period of extensive synapse development, by interfering with cGMP-dependent protein kinase 1, Rac1 and RhoA signaling, impaired normal synaptic maturation. In particular, miniature excitatory postsynaptic current (mEPSC) frequencies were significantly decreased in optic tectal neurons neighboring glia with impaired filopodial dynamics. This could indicate either a decrease in the total number of synapses formed or a failure to traffic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) to the synaptic cleft of immature *N*-methyl-D-aspartate receptor (NMDAR)-only 'silent' synapses, suggesting that glial process motility plays an important role in promoting normal synaptic maturation of these neurons during development.

The functional significance of astrocyte process coverage of synapses during development has also been reported in the cerebellum. When glia sheath retraction was induced by expressing GluA2 subunits to render AMPARs

Ca^{2+}-impermeable in Bergmann glia, there was a significant increase in synaptic puncta and dendritic spine density [6]. This observation is consistent with an earlier study that reported a deficit in the developmental pruning of climbing fiber inputs onto Purkinje cells, resulting in greater poly-innervation [7].

Neuronal signals promote structural plasticity of astrocyte processes and influences synapse stabilization

Examining the morphology and motility of astrocytic and radial glial processes has shown that the plasticity of PAPs and their proximity to synapses is influenced by a number of physiological conditions. A particularly striking demonstration of the functional consequences of the remodeling of astrocytic coverage takes place at synapses in the hypothalamus during lactation and dehydration [5]. Astrocyte processes are physically retracted from synapses, resulting in reduced clearance of glutamate [6], changes in the diffusion properties of the extracellular space [7], as well as significant changes in synaptic transmission [8•].

While there is accumulating evidence showing that astrocyte proximity to synapses is critical for synaptic function, we are just beginning to understand the mechanisms mediating astrocyte process plasticity. Recent experiments have begun to examine how neuronal activity influences astrocyte process motility [8•,9–11,12•]. *In vivo* live imaging of radial glial cell processes in the neuropil of the developing tadpole optic tectum has revealed that their dynamics is significantly increased when measured 5 min after visual stimulation, a phenomenon that was prevented by MK801 blockade of NMDARs, which are expressed on tectal neurons but not on radial glial cells [10]. Furthermore, *in vivo* imaging of somatosensory cortex in mice has found that a greater proportion of astrocytic processes are displaced after whisker stimulation compared to unstimulated mice [8•,12•]. Notably, the increase in stimulation-induced motility was prevented by group I metabotropic glutamate receptor (mGluR) antagonists, but not by NMDAR blockade. It was also absent in $IP_3R2^{-/-}$ mice, which have impairments in G-protein-mediated calcium transients in astrocytes [12•]. This last experiment raised important questions about the possible relationship between intracellular calcium release and astrocytic motility.

To further examine the mechanisms mediating astrocyte plasticity, activation of a single astrocytic process was induced using a mouse hippocampal slice model. Expression of a photoactivatable G-protein-coupled receptor in astrocytes, which was used to increase local Ca^{2+} transients, induced processes to move towards a neighboring synapse, leading to an increase in spine coverage [8•]. Notably, astrocytic process motility was reduced once a process was associated with a stable spine. This compliments previous research in hippocampal slice cultures showing that astrocytic contacts with dendritic spines promote both the lifetime and morphological maturation of dendritic protrusions [13].

While these results highlight that the fact that neuronal activity can signal to astrocytes and influence interactions with synapses, the mechanism and signaling pathways mediating these changes need to be further investigated. Notably, expression of mGluR5, which has been identified in a number of studies as an important regulator of astrocyte motility, has been found to be developmentally regulated and not expressed in the adult brain [14]. Accordingly, mechanisms mediating astrocyte process motility during development may differ from those in adulthood.

Astrocytic motility may regulate synaptic availability of glutamate and gliotransmitters

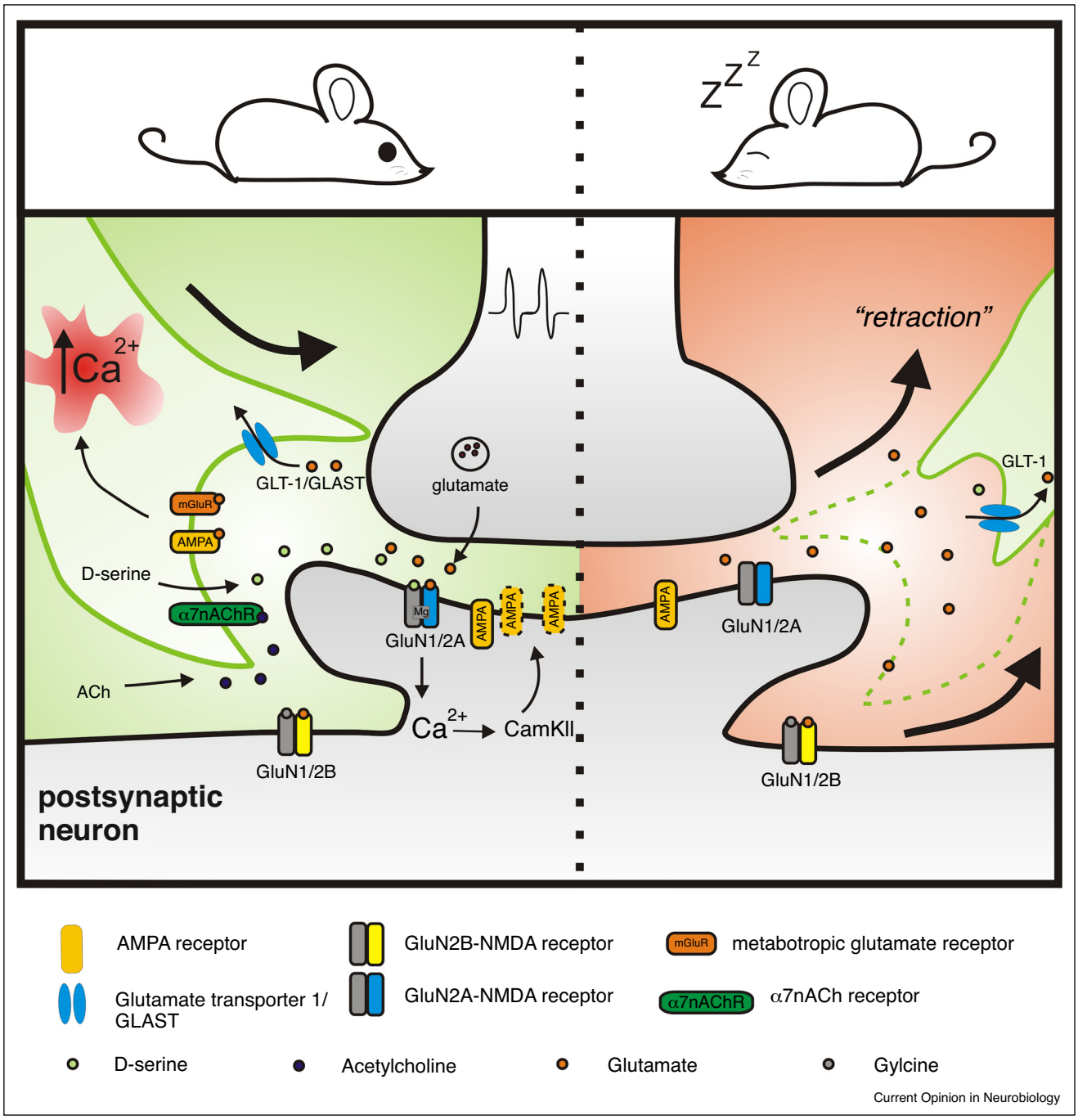
Synaptic remodeling that occurs during development relies on changes in synaptic strength akin to those implicated in learning and memory such as long-term potentiation (LTP) [15,16]. A fundamental mechanism underlying changes in synaptic strength is the delivery and removal of AMPARs at synapses through a process that has been shown to be in part dependent on the activation of NMDARs [17,18].

Astrocytic processes contain glutamate transporters, which are necessary for clearing synaptic glutamate and preventing widespread excitotoxicity [19]. A recent study has shown that connexin 30, an astrocyte gap-junction protein, is important for controlling astrocyte process motility. Mice that are deficient in connexin 30 have an increase in astrocytic coverage at synapses resulting in enhanced glutamate clearance and a decrease in synaptic glutamate concentration [20] (Figure 1).

Interestingly, the mobility of glutatmate transporters on the astroglial cell membrane has been found to be driven by neuronal activity [21,22]. Diffusion of GLT-1 (also known as Excitatory Amino Acid Transporter 2) in astrocytic membranes has been found to slow down near synapses, and impairing GLT-1 diffusion mobility results in slowed kinetics of excitatory postsynaptic currents [21] (Figure 1). Glutamate uncaging at synapses increased GLT-1 diffusion, suggesting rapid turnover of transporter at actively releasing synapses. This highlights the fact that astrocytic processes at synapses play an active role in the clearance of glutamate, which ultimately influences postsynaptic, as well as extrasynaptic, NMDAR activation and contributes to overall synaptic plasticity and function.

In addition to clearing glutamate, astrocytic processes release a number of glia-derived molecules, including gliotransmitters like D-serine, which has been shown to modify NMDAR signaling leading to changes in synaptic strength and stabilization. D-serine is an endogenous

Figure 1



Schematic representation of a synapse surrounded by astrocytic processes. Astrocytic coverage is increased during periods of wakefulness and during increased neural activity (left). Neuronal activity increases glutamate release from the presynaptic terminal which activates glutamate receptors on neighboring astrocytic processes and leads to an elevation in astrocytic calcium and subsequent release of gliotransmitters, such as D-serine. During wakefulness, increased cholinergic tone is sensed by α7nAChRs on astrocytes and leads to an increase in D-serine release. D-serine enhances NMDAR activity on nearby postsynaptic neurons. Enhanced synaptic activation in the presence of D-serine can promote synapse maturation, increasing postsynaptic AMPAR insertion and eventually modifying presynaptic release efficacy. In contrast, astrocytic coverage is decreased during periods of rest and during other physiological conditions, such as lactation and dehydration (right). Decreases in astrocytic coverage has been associated with decreased D-serine levels and reduced NMDAR activation, as well as deficits in glutamate clearance and an increase in synaptic glutamate concentration.

co-agonist for NMDARs, and has been shown to be instrumental in modulating synaptic transmission and plasticity in many brain areas [23–25,26••,27,28•].

We recently studied whether D-serine plays a role in facilitating synapse maturation in the developing visual system [28•]. We found that extracellular D-serine levels are increased by AMPAR activation in the developing optic tectum of the tadpole. Moreover, chronically elevating levels of D-serine during development resulted in increases in synaptic AMPAR/NMDAR ratios and in the number of functional synapses, suggesting that D-serine, by potentiating NMDAR currents may promote synaptic maturation by increasing synaptic insertion of AMPARs (Figure 1). Conversely, enzymatically degrading endogenous D-serine resulted in impaired synaptic maturation. Notably, elevating D-serine levels for two days to promote synaptic maturation, but not acute D-serine administration, resulted in an increase in the probability of release at retinotectal synapses, suggesting that the postsynaptic modifications induced by D-serine may induce subsequent changes in presynaptic release efficacy.

It has been proposed that the proximity of an astrocytic process to a synapse could facilitate the precise delivery of gliotransmitters to the synapse. In line with this hypothesis, the retraction of astrocytic processes in the supraoptic nucleus of the hypothalamus of rats during periods of lactation is associated with a decrease in D-serine levels at the synaptic cleft, which results in reduced NMDAR-dependent synaptic plasticity [23].

Changes in astrocytic coverage of synapses have also been associated with changes in wakefulness [29]. In particular, electron microscopy of dendritic spines in layer II of the prefrontal cortex has shown increased coverage of spines during periods of wakefulness compared to periods of sleep. Notably, increased expression during wakefulness of genes that are important for regulating astrocytic process motility has been reported. Additional *in vivo* imaging of astrocytic process dynamics during different periods of wakefulness may be an interesting approach for further understading the roles played by glia in circadian and attentional regulation of plasticity.

Recent studies have found that D-serine levels in the hippocampus fluctuate across a 24 h period depending on activation of astrocytic α7-nicotinic acetylcholine receptors (α7-nAChRs) In particular, D-serine levels were found to be the highest during wakefulness and changes in available D-serine significantly affected synaptic transmission [25] (Figure 1). It will be important to confirm whether increased D-serine release during wakefulness is also correlated with increased coverage of synapses in the hippocampus. This finding has implications for future experiments because it suggests the time of day can seriously impact synaptic plasticity.

Astrocytes may also exert control over neuronal NMDAR currents through metabolic coupling via the 'astrocyte-neuron lactate shuttle'. Lactate released from astrocytes provides an important energy substrate for neurons that is essential for the formation and the maintenance of synapses *in vivo*, and contributes to hippocampal LTP [30]. It is believed to act through the production of NADH which enhances postsynaptic NMDAR currents to promote calcium influx, leading to the expression of a number of plasticity related genes including Arc, Zif268, cFos, and BDNF [31] (Figure 2).

TNFα induces synaptic scaling by increasing synaptic AMPARs

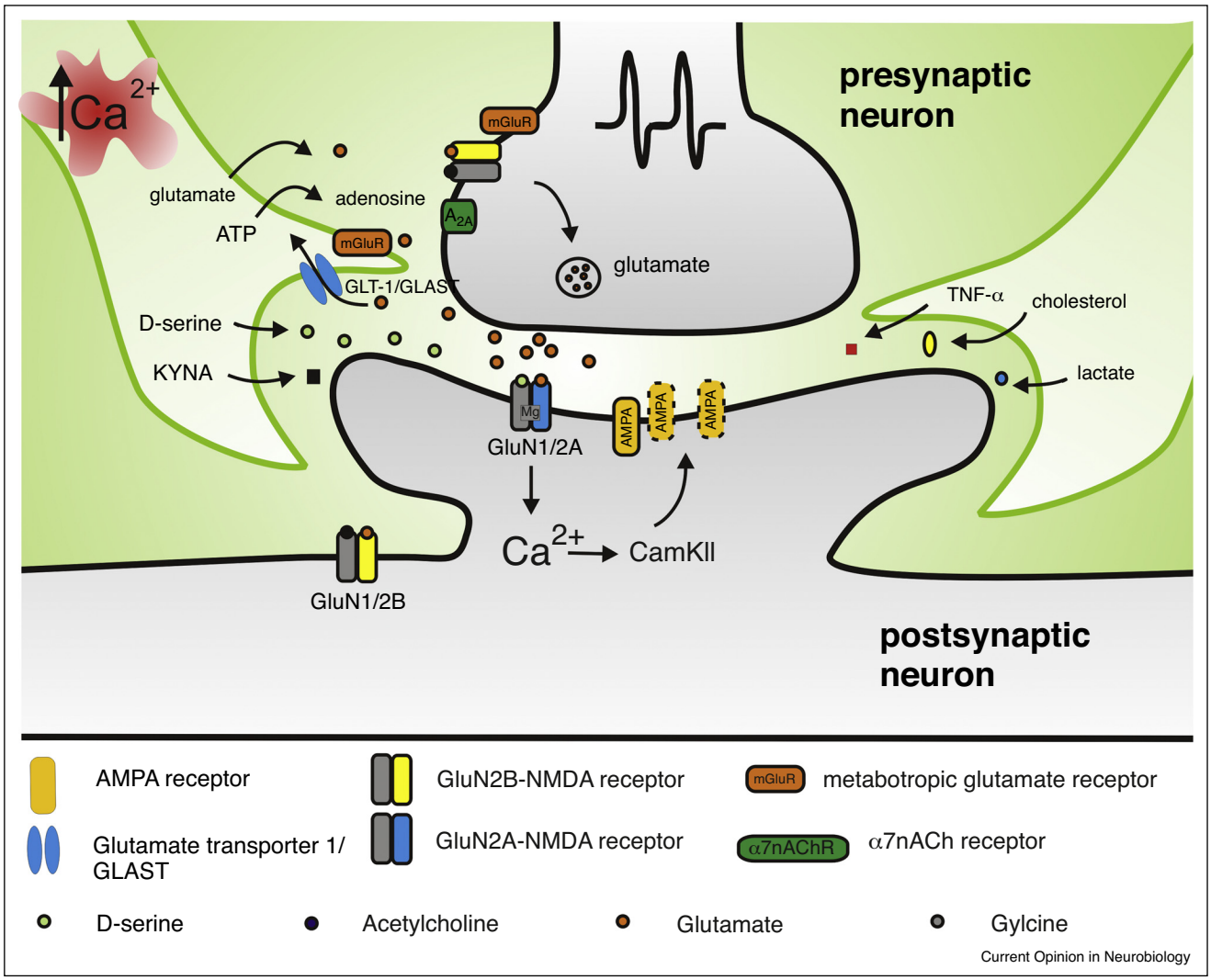
Activity-dependent release of the cytokine tumor necrosis factor alpha (TNFα) from astrocytes has been found to be critical for inducing homeostatic synaptic scaling [32]. In particular, decreased neuronal activity triggers the release of TNFα which increases the levels of AMPARs at the synaptic cleft (Figure 2). TNFα has also been found to play a role in experience-dependent synaptic plasticity in the developing visual cortex. Monocular deprivation during the critical period is associated with a decrease in response of cortical neurons to stimulation of the closed eye, shortly followed by an increased response to vision through the open eye. However, in mice deficient in TNFα, the increased response to the open eye does not occur, suggesting that the strengthening of the non-deprived eye response may constitute a form of homeostatic plasticity [33••].

TNFα was further found to be involved in mediating astrocytic glutamate release, which may be an important mediator of presynaptic NMDAR activation [34]. Notably, astrocytic glutamate has been shown to induce NMDAR-mediated responses mainly via GluN2B-containing NMDARs, which are typically extrasynaptic. This is in contrast to D-serine which has been associated more closely with postsynaptic GluN2A-containing NMDARs [26••].

Kynurenic acid may be an endogenous inhibitor of NMDARs

Astrocytes can also release factors that are associated with decreases in NMDAR activation. For example, kynurenic acid (KYNA), a tryptophan metabolite that is synthesized and released in the brain by astrocytes, is an antagonist of NMDARs, as well as nicotinic acetylcholine receptors [35]. While endogenous KYNA has not been extensively studied, levels are elevated during periods of inflammation and a recent study showed that increased KYNA induces changes in NMDAR-neurogranin-CaMKII signalling [36]. Additional studies are required to determine the role of endogenous KYNA during periods of synaptic remodeling.

Figure 2



Schematic representation of the tripartite synapse illustrating many of the releasable factors and contact mediated signals astrocytes use to influence synapse maturation and stabilization during development.

Astrocyte factors influence presynaptic maturation and function

Astrocytes also contribute to synaptic maturation by modulating vesicular release at presynaptic terminals. For example, single synaptic events have been found to be sufficient to induce local astrocytic calcium transients via mGluR5 activation which in turn enhance the efficiency of basal synaptic transmission via presynaptic adenosine A_{2A} receptors [37••] (Figure 2).

Notably, norepinephrine has been found to trigger the release of ATP from cultured glial cells. In the hypothalamus, stimulation of astrocytes with norepinephrine leads to activation of postsynaptic P2 × 7 receptors and promotes the insertion of AMPAR [38]. These results highlight the fact that the release of astrocytic neuromodulators can be triggered by different neurotransmitters and through different mechanisms. Moreover, the same neuromodulator has the potential to influence synapse strength through presynaptic or postsynaptic mechanisms.

Synaptic membranes are enriched in cholesterol, which is an important element for regulating many aspects of synaptic function [39]. Lipid metabolism in neurons is considered inefficient and neurons rely on astrocytes as a source of cholesterol. A recent *in vivo* study showed that disrupting cholesterol synthesis in astrocytes results in immature synapses with impaired presynaptic structure and a significant decrease in levels of SNAP25, an important t-SNARE protein required for presynaptic vesicular

release [40]. Previous work has shown that astrocytic cholesterol is also important for regulating postsynaptic neurotransmitter receptor clustering and stability [41–43].

Heterogeneity of astrocytes

While it has become increasingly clear that astrocytes play an instrumental role in establishing functional neuronal circuits, we are just beginning to unravel the complex interactions that exist between neurons and glia. Future research is needed to investigate how astrocyte properties vary across brain areas. For example, a recent analysis of the synaptogenic properties of astrocytes revealed distinct gene expression profiles across different regions of the central nervous system [44–46]. Furthermore, not all synapses are enwrapped by astrocytic processes and different neuron subtypes interact with different types of glial cells. For example, a recent study in the cerebellum has shown that neurons expressing high levels of sonic hedgehog are more likely to associate with Bergmann glia whereas neurons expressing low levels of sonic hedgehog are more likely to associate with velate astrocytes [47]. Accordingly, it will be important to determine what other signals and mechanisms influence glial cells to be associated with a particular synapse.

Recent studies have identified fundamental functional, morphological and transcriptomic differences between human and rodent astrocytes [48]. Significant progress in differentiating astrocytes and microglia from induced pluripotent stem cells offers promise for studying the interactions between human neurons and glial cells across development, both *in vitro* and *in vivo* [49–51]. These developments are particularly relevant for developing better models to study human neurodevelopmental disorders, such as autism and schizophrenia, as well as overall glial contributions to healthy brain connectivity.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by the Canadian Institutes for Health Research [grant number FDN - 143238]; and the Fonds de recherche du Québec - santé [grant number 31036].

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Araque A, Parpura V, Sanzgiri RP, Haydon PG: **Tripartite synapses: glia, the unacknowledged partner**. *Trends Neurosci* 1999, **22**:208-215.

2. • Sild M, Van Horn MR, Schohl A, Jia D, Ruthazer ES: **Neural activity-dependent regulation of radial glial filopodial motility is mediated by glial cGMP-dependent protein kinase 1 and contributes to synapse maturation in the developing visual system**. *J Neurosci* 2016, **36**:5279-5288.

In vivo manipulation of PKG, Rac1 and RhoA signaling in Xenopus tadpole radial glia significantly alters radial glia motility and causes deficits in synaptic maturation in neighboring neurons.

3. Lippman JJ, Lordkipanidze T, Buell ME, Yoon SO, Dunaevsky A: **Morphogenesis and regulation of Bergmann glial processes during Purkinje cell dendritic spine ensheathment and synaptogenesis**. *Glia* 2008, **56**:1463-1477.

4. Sild M, Ruthazer ES: **Radial glia: progenitor, pathway, and partner**. *Neuroscientist* 2011, **17**:288-302.

5. Lyons DA, Talbot WS: **Glial cell development and function in zebrafish**. *Cold Spring Harb Perspect Biol* 2014, **7**:a020586.

6. Lippman Bell JJ, Lordkipanidze T, Cobb N, Dunaevsky A: **Bergmann glial ensheathment of dendritic spines regulates synapse number without affecting spine motility**. *Neuron Glia Biol* 2010, **6**:193-200.

7. Iino M, Goto K, Kakegawa W, Okado H, Sudo M, Ishiuchi S, Miwa A, Takayasu Y, Saito I, Tsuzuki K *et al.*: **Glia-synapse interaction through Ca2+-permeable AMPA receptors in Bergmann glia**. *Science* 2001, **292**:926-929.

8. • Bernardinelli Y, Randall J, Janett E, Nikonenko I, Konig S, Jones EV, Flores CE, Murai KK, Bochet CG, Holtmaat A *et al.*: **Activity-dependent structural plasticity of perisynaptic astrocytic domains promotes excitatory synapse stability**. *Curr Biol* 2014, **24**:1679-1688.

One of the first studies to show how synaptic activity regulates the structural plasticity of astrocyte processes. Using time-lapse confocal imaging they illustrate how the proximity of astrocytic processes to dendrite spines is dynamic and that more stable spines have greater astrocytic coverage and the astrocyte processes that contact larger spines are less motile.

9. Haber M, Zhou L, Murai KK: **Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses**. *J Neurosci* 2006, **26**:8881-8891.

10. Tremblay M, Fugere V, Tsui J, Schohl A, Tavakoli A, Travencolo BA, Costa Lda F, Ruthazer ES: **Regulation of radial glial motility by visual experience**. *J Neurosci* 2009, **29**:14066-14076.

11. Lavialle M, Aumann G, Anlauf E, Prols F, Arpin M, Derouiche A: **Structural plasticity of perisynaptic astrocyte processes involves ezrin and metabotropic glutamate receptors**. *Proc Natl Acad Sci U S A* 2011, **108**:12915-12919.

12. • Perez-Alvarez A, Navarrete M, Covelo A, Martin ED, Araque A: **Structural and functional plasticity of astrocyte processes and dendritic spine interactions**. *J Neurosci* 2014, **34**:12738-12744.

LTP paradigm increased the motility of synapse-associated astrocytic processes within 5 min. Motility was found to be mGluR-dependent and required changes in intracellular Ca2+ in astrocytes.

13. Nishida H, Okabe S: **Direct astrocytic contacts regulate local maturation of dendritic spines**. *J Neurosci* 2007, **27**:331-340.

14. Sun W, McConnell E, Pare JF, Xu Q, Chen M, Peng W, Lovatt D, Han X, Smith Y, Nedergaard M: **Glutamate-dependent neuroglial calcium signaling differs between young and adult brain**. *Science* 2013, **339**:197-200.

15. Wu G, Malinow R, Cline HT: **Maturation of a central glutamatergic synapse**. *Science* 1996, **274**:972-976.

16. Heynen AJ, Yoon BJ, Liu CH, Chung HJ, Huganir RL, Bear MF: **Molecular mechanism for loss of visual cortical responsiveness following brief monocular deprivation**. *Nat Neurosci* 2003, **6**:854-862.

17. Petralia RS, Esteban JA, Wang YX, Partridge JG, Zhao HM, Wenthold RJ, Malinow R: **Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses**. *Nat Neurosci* 1999, **2**:31-36.

18. Huganir RL, Nicoll RA: **AMPARs and synaptic plasticity: the last 25 years**. *Neuron* 2013, **80**:704-717.

19. Rose CR, Felix L, Zeug A, Dietrich D, Reiner A, Henneberger C: **Astroglial glutamate signaling and uptake in the hippocampus**. *Front Mol Neurosci* 2017, **10**:451.

20. Pannasch U, Freche D, Dallerac G, Ghezali G, Escartin C, Ezan P, Cohen-Salmon M, Benchenane K, Abudara V, Dufour A *et al.*: **Connexin 30 sets synaptic strength by controlling astroglial synapse invasion**. *Nat Neurosci* 2014, **17**:549-558.

21. Murphy-Royal C, Dupuis JP, Varela JA, Panatier A, Pinson B, Baufreton J, Groc L, Oliet SH: **Surface diffusion of astrocytic glutamate transporters shapes synaptic transmission**. *Nat Neurosci* 2015, **18**:219-226.

22. Al Awabdh S, Gupta-Agarwal S, Sheehan DF, Muir J, Norkett R, Twelvetrees AE, Griffin LD, Kittler JT: **Neuronal activity mediated regulation of glutamate transporter GLT-1 surface diffusion in rat astrocytes in dissociated and slice cultures**. *Glia* 2016, **64**:1252-1264.

23. Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, Oliet SH: **Glia-derived D-serine controls NMDA receptor activity and synaptic memory**. *Cell* 2006, **125**:775-784.

24. Henneberger C, Papouin T, Oliet SH, Rusakov DA: **Long-term potentiation depends on release of D-serine from astrocytes**. *Nature* 2010, **463**:232-236.

25. Papouin T, Dunphy JM, Tolman M, Dineley KT, Haydon PG: **Septal cholinergic neuromodulation tunes the astrocyte-dependent gating of hippocampal NMDA receptors to wakefulness**. *Neuron* 2017, **94**:840-854 e847.

26. •• Papouin T, Ladepeche L, Ruel J, Sacchi S, Labasque M, Hanini M, Groc L, Pollegioni L, Mothet JP, Oliet SH: **Synaptic and extrasynaptic NMDA receptors are gated by different endogenous coagonists**. *Cell* 2012, **150**:633-646.

First study to show that the levels of the gliotransmitter D-serine oscillate as a function of wakefulness and are driven by cholinergic activation through the α7- acetylcholine nicotinic receptor.

27. Fossat P, Turpin FR, Sacchi S, Dulong J, Shi T, Rivet JM, Sweedler JV, Pollegioni L, Millan MJ, Oliet SH *et al.*: **Glial D-serine gates NMDA receptors at excitatory synapses in prefrontal cortex**. *Cereb Cortex* 2012, **22**:595-606.

28. • Van Horn MR, Strasser A, Miraucourt LS, Pollegioni L, Ruthazer ES: **The gliotransmitter d-serine promotes synapse maturation and axonal stabilization in vivo**. *J Neurosci* 2017, **37**:6277-6288.

In vivo study showing that D-serine promotes synapse maturation and stabilization during development.

29. Bellesi M, de Vivo L, Tononi G, Cirelli C: **Effects of sleep and wake on astrocytes: clues from molecular and ultrastructural studies**. *BMC Biol* 2015, **13**:66.

30. Suzuki A, Stern SA, Bozdagi O, Huntley GW, Walker RH, Magistretti PJ, Alberini CM: **Astrocyte-neuron lactate transport is required for long-term memory formation**. *Cell* 2011, **144**:810-823.

31. Yang J, Ruchti E, Petit JM, Jourdain P, Grenningloh G, Allaman I, Magistretti PJ: **Lactate promotes plasticity gene expression by potentiating NMDA signaling in neurons**. *Proc Natl Acad Sci U S A* 2014, **111**:12228-12233.

32. Stellwagen D, Malenka RC: **Synaptic scaling mediated by glial TNF-alpha**. *Nature* 2006, **440**:1054-1059.

33. •• Kaneko M, Stellwagen D, Malenka RC, Stryker MP: **Tumor necrosis factor-alpha mediates one component of competitive, experience-dependent plasticity in developing visual cortex**. *Neuron* 2008, **58**:673-680.

Using monocular deprivation during development this study showed that the increased neuronal response to the non-deprived eye does not occur if TNF-α is not present in astrocytes.

34. Santello M, Bezzi P, Volterra A: **TNFalpha controls glutamatergic gliotransmission in the hippocampal dentate gyrus**. *Neuron* 2011, **69**:988-1001.

35. Guillemin GJ, Kerr SJ, Smythe GA, Smith DG, Kapoor V, Armati PJ, Croitoru J, Brew BJ: **Kynurenine pathway metabolism in human astrocytes: a paradox for neuronal protection**. *J Neurochem* 2001, **78**:842-853.

36. Oliveros A, Wininger K, Sens J, Larsson MK, Liu XC, Choi S, Faka A, Schwieler L, Engberg G, Erhardt S *et al.*: **LPS-induced cortical kynurenic acid and neurogranin-NFAT signaling is associated with deficits in stimulus processing during Pavlovian conditioning**. *J Neuroimmunol* 2017, **313**:1-9.

37. •• Panatier A, Vallee J, Haber M, Murai KK, Lacaille JC, Robitaille R: **Astrocytes are endogenous regulators of basal transmission at central synapses**. *Cell* 2011, **146**:785-798.

Using fast, confocal calcium imaging this study was one of the first to show local and rapid Ca2+ changes in fine astrocytic processes could be elicited by evoking single action potentials in presynaptic axons or by using local two-photon uncaging of glutamate at specific areas in the astrocytic compartment.

38. Gordon GR, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS: **Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy**. *Nat Neurosci* 2005, **8**:1078-1086.

39. Allen JA, Halverson-Tamboli RA, Rasenick MM: **Lipid raft microdomains and neurotransmitter signalling**. *Nat Rev Neurosci* 2007, **8**:128-140.

40. van Deijk AF, Camargo N, Timmerman J, Heistek T, Brouwers JF, Mogavero F, Mansvelder HD, Smit AB, Verheijen MH: **Astrocyte lipid metabolism is critical for synapse development and function in vivo**. *Glia* 2017, **65**:670-682.

41. Mauch DH, Nagler K, Schumacher S, Goritz C, Muller EC, Otto A, Pfrieger FW: **CNS synaptogenesis promoted by glia-derived cholesterol**. *Science* 2001, **294**:1354-1357.

42. Goritz C, Mauch DH, Pfrieger FW: **Multiple mechanisms mediate cholesterol-induced synaptogenesis in a CNS neuron**. *Mol Cell Neurosci* 2005, **29**:190-201.

43. Hering H, Lin CC, Sheng M: **Lipid rafts in the maintenance of synapses, dendritic spines, and surface AMPA receptor stability**. *J Neurosci* 2003, **23**:3262-3271.

44. Buosi AS, Matias I, Araujo APB, Batista C, Gomes FCA: **Heterogeneity in Synaptogenic Profile of Astrocytes from Different Brain Regions**. *Mol Neurobiol* 2018, **55**:751-762.

45. Zhang Y, Barres BA: **Astrocyte heterogeneity: an underappreciated topic in neurobiology**. *Curr Opin Neurobiol* 2010, **20**:588-594.

46. Khakh BS, Sofroniew MV: **Diversity of astrocyte functions and phenotypes in neural circuits**. *Nat Neurosci* 2015, **18**:942-952.

47. Farmer WT, Abrahamsson T, Chierzi S, Lui C, Zaelzer C, Jones EV, Bally BP, Chen GG, Theroux JF, Peng J *et al.*: **Neurons diversify astrocytes in the adult brain through sonic hedgehog signaling**. *Science* 2016, **351**:849-854.

48. Zhang Y, Sloan SA, Clarke LE, Caneda C, Plaza CA, Blumenthal PD, Vogel H, Steinberg GK, Edwards MS, Li G *et al.*: **Purification and characterization of progenitor and mature human astrocytes reveals transcriptional and functional differences with mouse**. *Neuron* 2016, **89**:37-53.

49. Chandrasekaran A, Avci HX, Leist M, Kobolak J, Dinnyes A: **Astrocyte differentiation of human pluripotent stem cells: new tools for neurological disorder research**. *Front Cell Neurosci* 2016, **10**:215.

50. Sloan SA, Darmanis S, Huber N, Khan TA, Birey F, Caneda C, Reimer R, Quake SR, Barres BA, Pasca SP: **Human astrocyte maturation captured in 3D cerebral cortical spheroids derived from pluripotent stem cells**. *Neuron* 2017, **95**:779-790 e776.

51. Abud EM, Ramirez RN, Martinez ES, Healy LM, Nguyen CHH, Newman SA, Yeromin AV, Scarfone VM, Marsh SE, Fimbres C *et al.*: **iPSC-derived human microglia-like cells to study neurological diseases**. *Neuron* 2017, **94**:278-293 e279.