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Review

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Volume transmission signalling via astrocytes

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The influence of astrocytes on synaptic function has been increasingly studied, owing to the discovery of both gliotransmission and morphological ensheathment of synapses. While astrocytes exhibit at best modest membrane potential fluctuations, activation of G-protein coupled receptors (GPCRs) leads to a prominent elevation of intracellular calcium which has been reported to correlate with gliotransmission. In this review, the possible role of astrocytic GPCR activation is discussed as a trigger to promote synaptic plasticity, by affecting synaptic receptors through gliotransmitters. Moreover, we suggest that volume transmission of neuromodulators could be a biological mechanism to activate astrocytic GPCRs and thereby to switch synaptic networks to the plastic mode during states of attention in cerebral cortical structures.

1. Introduction

With the advent of molecular genetics and cellular imaging techniques, our understanding of brain function has advanced substantially in the recent decade. Glial cell research has indisputably benefited from these techniques, as glial cells are generally electrically passive, and their dynamism resides most probably in biochemical and morphological changes. Among the glial cells, astrocytes occupy a significant proportion of the brain volume in mammals and are arguably the most numerous in primate cortical grey matter. The morphology of astrocytes is best described as an interface between vascular and neuronal networks. A typical protoplasmic astrocyte has a bushy organization of microprocesses that surround synapses and a few large processes that impinge on neighbouring vasculature (giant end-feet). For white matter fibrous astrocytes, the microprocesses extend around the nodal regions of myelinated axons. Such strategic positioning of astrocytes is indeed well matched with the classically supposed functions of astrocytes, including the clearance of synaptically released neurotransmitters, regulation of ionic concentrations and mediation of energy metabolism substrates. Since gliotransmission—the ability of astrocytes to secrete biochemical molecules to influence surrounding neurons—was discovered about two decades ago [1–3], astrocytes have been hypothesized to play active roles in neuronal network operations.

Membrane potential fluctuations recorded from the soma of mature astrocytes are quite modest (i.e. within several millivolts) at best. The resting membrane potential of a typical astrocyte is less than −80 mV, which is close to the reversal potential of potassium (K^+). Astrocytes have an order-of-magnitude lower input resistance than pyramidal cells owing to K^+ channels that are permeable at resting membrane potentials (e.g. TWIK-1, TREK-1 and $K_{ir}4.1$) [4,5] as well as the existence of hemichannels and gap junctions. While these properties and the lack of active conductance make astrocytes electrophysiologically quiescent, astrocytes have been reported to have cytosolic calcium (Ca^{2+}) elevations and intercellular Ca^{2+} waves [2]. These Ca^{2+} elevations occur without large membrane potential changes, because the Ca^{2+} is released from internal Ca^{2+} stores such as the

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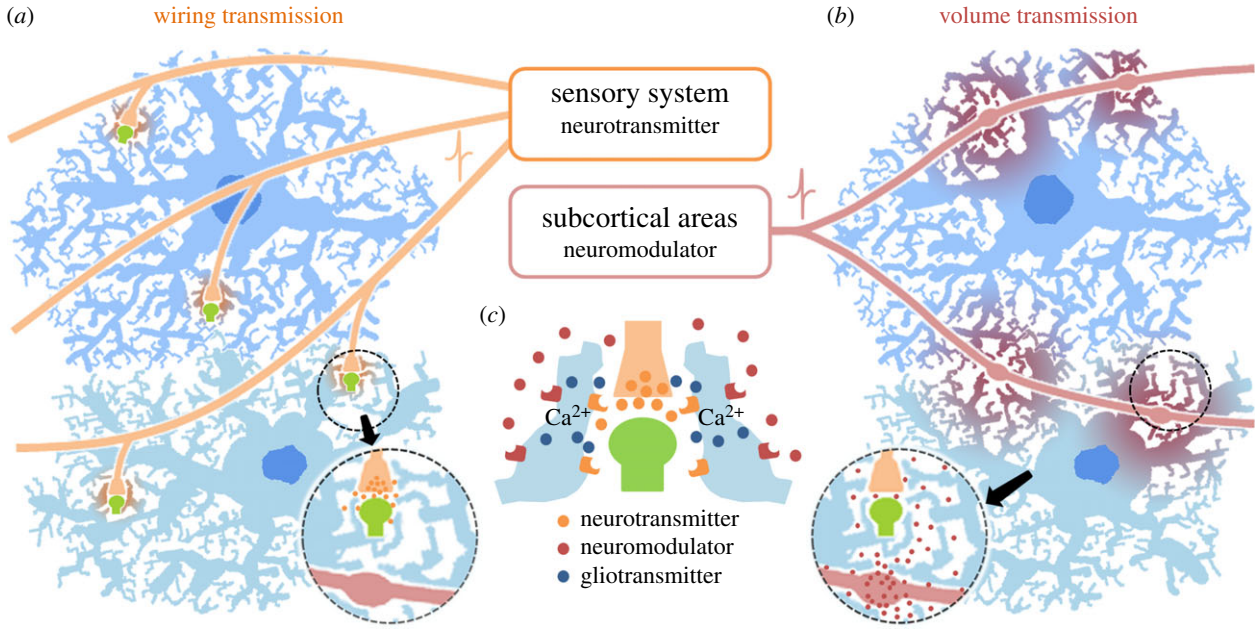

Figure 1. Wiring transmission versus volume transmission and their effects on astrocytes. (*a*) Wiring transmission targets designated synapses and produces localized responses in perisynaptic astrocytic processes. (*b*) In volume transmission, the neuromodulators diffuse into tortuous and convoluted ECS upon release from *en passant* varicosities. Such ECS diffusion results in activation of astrocytic GPCRs in larger areas than a synaptic component, resulting in synchronized and spatially spread astrocytic Ca^{2+} activities. (*c*) Volume transmission and synaptic transmission can occur simultaneously in brain states characterized by neuromodulator release, for instance, during attention.

endoplasmic reticulum (ER). Such cytosolic Ca^{2+} elevations in astrocytes have also been described *in vivo* in rodents [6]. Triggers that initiate astrocytic Ca^{2+} elevation are diverse, but common neurotransmitters and neuromodulators are potent agonists for astrocytic Ca^{2+} elevation through G-protein coupled receptors (GPCRs). One of the key questions in neuron–astrocyte interactions is whether astrocytic Ca^{2+} elevations play any role in brain operation, and to identify the circumstances under which such neuron–glia interactions occur. In this article, we focus on how subcortical neuromodulatory signals mediate astrocyte–neuron interactions in the context of synaptic plasticity in cerebral cortical structures.

2. Volume transmission versus synaptic transmission

Chemical transmitters are released in two distinct transmission modes: wiring transmission and volume transmission (for classic reviews, see [7,8]). Wiring transmission is intercellular communication mediated via a physically defined connecting structure. Synaptic transmission is the primary mechanism of wiring transmission, and its primary feature is fast (millisecond-order) point-to-point communication. Glutamate and GABA are the predominant neurotransmitters for this in mammalian cortical structures. The potency and reliability of the synapse are the key determinants of information transmission. Astrocytic microprocesses that ensheath synapses are thought to increase the fidelity of synaptic transmission by rapid neurotransmitter clearance and insulation from other synapses [9].

Volume transmission is by non-synaptic release of neuromodulators diffusing through the extracellular space (ECS; figure 1), which is defined by an intricate and dense organization of synaptic and glial process morphology (for a review, see [10]). As a result, the manner of diffusion deviates significantly from free diffusion because of the tortuosity and limited volume fraction of the ECS. Subsequently, a relatively large number of cells sense neuromodulators via extrasynaptic receptors. In the cerebral cortex and hippocampus, volume-transmitted neuromodulators include acetylcholine and monoamines. The afferent fibres for neuromodulators are mainly of subcortical origin, and usually make asynaptic junctions in the cortex and hippocampus via terminal varicosities in stark contrast to glutamatergic and GABAergic innervation. For example, synaptic incidences are a mere 10–20% of the total varicosities for acetylcholine [11,12] and noradrenaline [13,14] and 20–30% for serotonin [15]. In addition to the complex ECS geometry, the true nature of ECS diffusion is complicated by the presence of diffusion obstacles (e.g. extracellular matrix and cell adhesion molecules) and active interference system (e.g. uptake by transporter or enzymatic degradation) [10,16]. Theoretical models and simulations have been compared with experimental data obtained by real-time iontophoresis or fluorescent macromolecule imaging [10].

As much as neurons receive this extrasynaptic neuromodulator transmission, astrocytes surrounding synapses are also the receivers. Serial reconstruction of the neuropil of rat hippocampal grey matter shows that glial processes occupy over 10% of all plasma membrane area [17]. This proportion is even higher when the analysis is confined to the extrasynaptic space, and thus glial surface represents a considerable target area for volume-transmitted neuromodulators. Moreover, astrocytes express receptors for subcortical neuromodulators [18].

Other remarkable differences between synaptic transmission and volume transmission are the time course and spatial range of signal transfer. While neurotransmitters travel 20–30 nm across the synaptic cleft, volume-transmitted neuromodulators travel on the scale of micrometres to reach their

receptors. At the receptor end, ionotropic receptors dominate in glutamatergic and GABAergic synapses of the cerebral cortex and hippocampus, ensuring millisecond-order signal transmission. Extrasynaptic receptors of neurons include both ionotropic receptors and metabotropic GPCRs. Literature suggests that neuromodulator receptors in astrocytes are predominantly GPCRs that have a much slower signal transduction (at least hundreds of milliseconds) [19]. Therefore, while affecting many targets, volume transmission is not expected to provide temporally precise signal transmission. Considering the tight coupling of neuromodulatory systems and behavioural states, and the slow time course of GPCR signalling, elucidation of the significance of astrocytic activation by neuromodulators may yield a new insight into understanding neuronal information processing in distinct behavioural states.

3. Astrocytic response to neurotransmitters and neuromodulators

Astrocytes respond to neurotransmitters and neuromodulators through a wide variety of GPCRs. Their activations trigger production of inositol 1,4,5-triphosphate (IP_3), which induces Ca^{2+} release from the ER. So far, several groups have reported that Ca^{2+} elevations in astrocytes lead to gliotransmission of glutamate, D-serine or ATP and in turn regulate neuronal activity and synaptic strength in brain slices [20–26]. D-Serine is an endogenous co-agonist of NMDA receptors (NMDARs), and several studies have suggested that astrocytes release D-serine by exocytosis [27–29]. D-Serine release from a single astrocyte can modulate neighbouring neuronal NMDAR currents [26], and basal astrocytic Ca^{2+} concentration and extracellular D-serine concentration are correlated [30]. Electron-microscopic analysis showed that glutamate and D-serine are localized in microvesicles near the ER within the perisynaptic processes of astrocytes [31], hinting at the significance of perisynaptic Ca^{2+} signalling in gliotransmission. Recent studies using high-resolution Ca^{2+} imaging of hippocampal slices suggested that astrocytic microprocesses respond to single synaptic activity with rapid and localized Ca^{2+} elevation [32,33]. Roles of gliotransmission from astrocytic processes in synaptic function have been studied in the hypothalamic nuclei, where the astrocytic coverage of synapses decreases during lactation. The availability of D-serine in the synapses was reduced in slices from lactating rats [34].

There is growing evidence that astrocyte-derived ATP, which was initially categorized as a paracrine messenger responsible for interglial propagation of Ca^{2+} waves [35–37], can regulate synaptic transmission [22,38]. Although several non-vesicular pathways have been identified, recent studies using transgenic mice selectively expressing a dominant-negative SNARE protein in astrocytes demonstrated the significance of vesicular release of astrocytic ATP [22,39]. Another study showed that electrical stimulation of excitatory input to the hypothalamus induces metabotropic glutamate receptor (mGluR)-dependent astrocytic Ca^{2+} elevation and release of ATP [40]. Notably, a rise in Ca^{2+} in the astrocyte compartments immediately adjacent to the postsynaptic neuron was necessary for ATP-mediated changes in synaptic transmission. Interestingly, noradrenaline application also led to astrocytic ATP release and similar synaptic transmission changes [41], implying that the astrocytes are capable of responding to and possibly integrating both neurotransmitters and neuromodulators (figure 1).

It has been shown that astrocytic Ca^{2+} signals in the adult brain are mediated by volume-transmitted neuromodulators. Upon electrical stimulation of locus coeruleus (LC; the sole source of noradrenergic input to cortex), astrocytes exhibit broad Ca^{2+} increases in somatosensory cortex [42]. Aversive stimulation, known to result in phasic LC activity, also led to widespread adrenergic astrocytic Ca^{2+} elevation throughout sensory cortex, which is more pronounced in awake conditions [43]. Acetylcholine also activates global astrocytic Ca^{2+} signalling *in vivo*. The predominant sources of cholinergic afferents for the cerebral cortex and hippocampus are the nucleus basalis of Meynert (NBM) and medial septum. We and others have demonstrated that stimulation of the respective cholinergic nuclei leads to muscarinic acetylcholine receptor (mAChR)-dependent astrocytic Ca^{2+} elevation in the cortex [44,45] and hippocampus [46]. Notably, NBM stimulation led to an increase in extracellular D-serine in the cortex of control mice, but not of mice lacking astrocyte-dominant IP_3 receptors (IP_3R2) [44]. Our results indicate that astrocytic Ca^{2+} responses by whisker or NBM stimulation differ in the following two aspects: (i) whisker stimulation induces mGluR-dependent weaker Ca^{2+} responses [47], whereas NBM stimulation produces mAChR-dependent robust responses; and (ii) while whisker-induced Ca^{2+} surges return to baseline even during stimulation, plateau Ca^{2+} increases persist throughout NBM stimulation.

GPCR signalling in astrocytes has also been suggested to regulate extracellular K^+ [48,49], neurotransmitter uptake [50] and neurovascular coupling [51–53] (but also see [54,55]). On the other hand, some recent studies that use molecular genetics have challenged the validity of gliotransmission. Astrocytic expression of and subsequent activation of a foreign GPCR (MrgA1) to selectively induce astrocytic Ca^{2+} elevation [56,57] or genetic deletion of IP_3R2s to diminish astrocytic Ca^{2+} elevations [57,58] did not result in a notable change in excitatory synaptic transmission in mouse hippocampal slices. This apparent contradiction may be due to the method used to stimulate astrocytes. For example, uncaging IP_3 in MrgA1-positive astrocytes increased the frequency of glutamatergic miniature excitatory postsynaptic currents (mEPSCs) in nearby neurons [56]. A recent study further addressed this issue and showed that Ca^{2+} uncaging in astrocytes triggers glutamate release, whereas agonist activation of MrgA1, PAR-1 or purinergic receptors does not [49]. Moreover, astrocytic glutamate release can be mediated by channels [59,60], and Gq-coupled GPCRs may also have IP_3-independent pathways [61]. Future investigation on neuromodulator-mediated Ca^{2+} signalling in astrocytic processes and their functional manipulation *in vivo* will advance our understanding of the role of astrocytes in normal brain function. Other key issues for future studies are to understand the functional significance of neuromodulator-driven global responses and neurotransmitter-driven individual localized transients and to identify the biological situation where these signalling modes are employed differentially or in synergy.

4. Neuromodulator activation and gamma oscillations

Distinct neuromodulators contribute to different modes of animals' behavioural states. Likewise, animals' behavioural states

and neuronal population dynamics are tightly correlated. For instance, large amplitude slow waves (0.5–2 Hz) appear in the electroencephalogram (EEG) during deep sleep, whereas faster and lower amplitude patterns are seen in waking states. Gamma oscillations (30–100 Hz) appear during states of attention [62], and this rhythm is thought to bind neural representation of different sensory modalities [63]. Detailed cellular mechanisms underlying gamma oscillations are yet to be fully elucidated, but reciprocal interactions between excitatory pyramidal neurons and inhibitory interneurons, particularly parvalbumin positive fast-spiking basket cells, probably play a key role [64]. As attention is naturally related to cognitive processing and learning efficiency, synaptic plasticity is probably induced during the gamma oscillation state. Indeed, repetitive phase locked activity of neurons at a gamma frequency provides a situation favourable for spike-timing-dependent plasticity [65]. Moreover, this type of plasticity is enhanced by activation of mAChRs [65].

As neuromodulator release and EEG states are both highly correlated to an animal's behaviour, they should naturally be closely linked. As a matter of fact, the gamma states also coincide with release of neuromodulators. For instance, gamma oscillations are induced by electrical stimulation of the NBM in anaesthetized rats [66] or optogenetic stimulation of cholinergic neurons in the basal forebrain in awake mice [67]. Moreover, noradrenergic transmission has been shown to be crucial for waking gamma that appears shortly after gas anaesthesia wears off [68]. During awake and REM sleep periods, higher amounts of acetylcholine are released in the cortex and hippocampus than during slow wave sleep [69]. In accordance with cortical activation, cholinergic neurons in the basal forebrain increase their firing rates, and alter their firing mode from single spike to rhythmic bursting [70].

Exposure to enriched environments (EE) has been known to boost animals' learning ability and its neural circuit remodelling effect has been studied for decades. We recently found that hippocampal gamma amplitude increases in rats raised in EE, which hints at a possible link between gamma oscillation and learning [71]. Increases of spine density and dendritic complexity are common effects of EE in the cortex and hippocampus. Although the mechanism for chronic gamma increase is most probably multifactorial, increased input to pyramidal cells is a conceivable factor, as gamma is a product of balanced excitatory and inhibitory synaptic input [72]. The requirement of NMDAR activation for chronic gamma enhancement [71] also suggests that a long-term potentiation (LTP)-like mechanism may be involved. Interestingly, GABAergic networks have also been reported to be altered by EE [73].

As well as neurotransmission, there are notable changes in neuromodulation after EE. Rats raised in EE after weaning show increased hippocampal and anterior cortical choline acetyltransferase activity after maze training [74]. Similarly, the concentration of released acetylcholine is higher when rats solve more difficult tasks [75]. The causal relationship between chronic gamma increase and neuromodulator systems is not resolved at this time. However, it is remarkable that many studies report enhanced LTP by neuromodulators including acetylcholine and noradrenaline, suggesting a permissive role of GPCR for synaptic plasticity and learning [76]. As described in §3, GPCRs are not only expressed in neurons. Given the existence and functional response of GPCRs in astrocytes, it is logical to ask whether activation of astrocytic GPCR has a role in synaptic plasticity *in vivo*.

5. Astrocytic modulation of synaptic plasticity during gamma states

As gamma states coincide with volume transmission of neuromodulators including acetylcholine and noradrenaline, astrocytic Ca^{2+} dynamics are more active during these states (§3). Recently, three independent studies have investigated the role of gamma-state-induced astrocytic Ca^{2+} elevation in synaptic plasticity. These studies were performed in the somatosensory cortex [44], visual cortex [45] and hippocampus [46]. In each of these studies, the respective cholinergic nucleus was stimulated while sensory stimuli or electrical afferent stimulation was presented to anaesthetized animals. As a result, long-lasting enhancements (more than 1 h) in stimulus-evoked potential or neuronal firing rate were observed. These effects were diminished in IP_3R2 knockout (IP_3R2-KO) mice, in which astrocytic large Ca^{2+} elevations are deficient, suggesting the causal relationship between the astrocytic Ca^{2+} elevation and induction of the synaptic plasticity.

NBM-evoked cortical gamma oscillations seem to be uninfluenced by astrocytic Ca^{2+}, as the duration of gamma oscillations was similar between wild-type and IP_3R2-KO mice [44]. Neuronal activity is temporally coordinated in gamma rhythms by NBM stimulation and this synchronization could be a prevailing mechanism of augmented synaptic plasticity [77]. However, the deficiency of NBM-associated cortical plasticity in IP_3R2-KO mice strongly supports a role of astrocytic Ca^{2+} signalling in the synaptic plasticity.

In our investigation on plasticity in the somatosensory cortex, astrocytic Ca^{2+} activities were elevated during costimulation of whiskers and NBM. Similarly, the extracellular concentration of D-serine is elevated during NBM stimulation and returns to the baseline thereafter. Considering the major role of NMDARs in LTP [78], the lack of extracellular D-serine increase in IP_3R2-KO mice suggests a pivotal role of astrocytic Ca^{2+} signalling during the induction phase of the synaptic plasticity. Chen *et al.* [45] showed that single unit activities in the visual cortex are enhanced when visual orientation stimuli are combined with NBM stimulation. Importantly, the neuronal response is enhanced only for the orientation paired with NBM stimulation. As the orientation tunings of individual synapses are intermingled in mouse primary visual cortex [79], this result advocates the importance of sensory input as the determinant for specificity of plasticity. Further investigation on spatio-temporal relationship of active astrocytes and augmented synapses should characterize the effective range of gliotransmission.

While the synaptic plasticity in our study is NMDAR-dependent, a study by Navarrete *et al.* [46] investigated cholinergically augmented hippocampal CA3–CA1 plasticity in the presence of an NMDAR blocker. Their results suggest that glutamate acts as the gliotransmitter affecting neuronal mGluRs to express presynaptic plasticity, whereas a recent paper suggests other interpretations such as transient change of extracellular ionic composition [48]. These differences suggest that the molecular mechanisms of astrocyte-assisted synaptic plasticity may be diverse, but the common denominator of all *in vivo* experiments is the

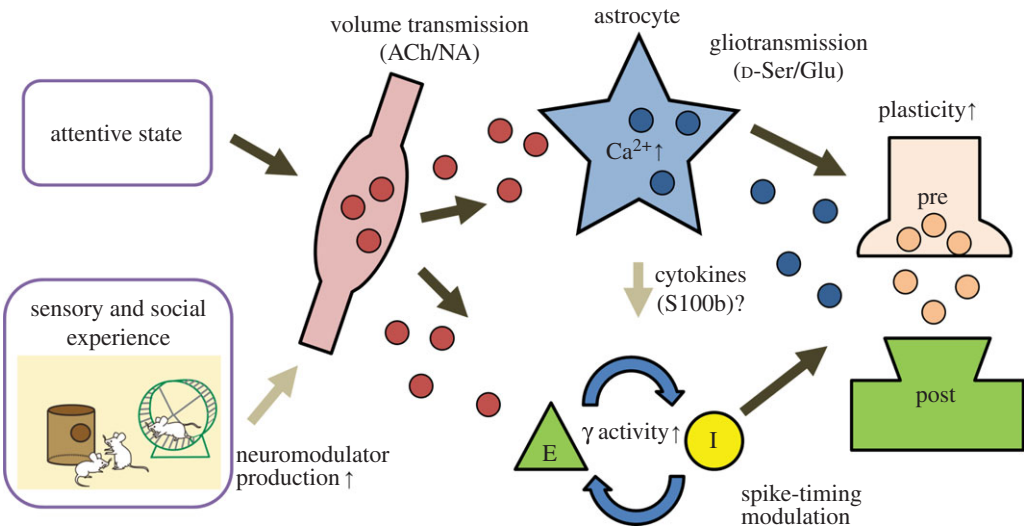

Figure 2. Schematic diagram for neuron–astrocyte interaction in the context of gamma-state-induced synaptic plasticity. Attentive states drive volume transmission of subcortical neuromodulators which in turn activates neuronal gamma oscillations and astrocytic gliotransmission to establish a state for synaptic plasticity induction. Sensory and social experience enhances neuromodulator production and gliotransmission of cytokines enhances gamma oscillations, although the exact mechanism remains to be elucidated. ACh, acetylcholine; NA, noradrenaline; E, excitatory neuron; I, inhibitory neuron.

activation of cholinergic volume transmission (figure 2). Notably, similar hippocampal plasticity was evoked when medial septum stimulation was replaced by tail pinch [46], and atropine could largely, but not completely, block the field response potentiation, suggesting that other neuromodulators such as noradrenaline could also be involved. Additionally, in prolonged gamma states, astrocytes can secrete cytokines including S100B and influence network synchronization [80] and synaptic plasticity [81].

6. Concluding remarks

We have discussed a possible role of cortical astrocytes as an element enhancing cortical plasticity via gliotransmission. Volume transmission of subcortical neuromodulators serves as the drive for activation of astrocytes and gamma oscillations. Gamma oscillations appear during attentive states and provide temporally synchronized activation of groups of neurons (i.e. cell assembly), association of which will lead to formation of memory and learning. Considering the astrocytic expression of functional GPCRs for neuromodulators and the tight relationship between the subcortical neuromodulator system and the cognitive states of an animal, the framework of this model is sound. Recently, multiple groups have shown that the cholinergic system can mediate such a mechanism in rodent cortex and hippocampus [44–46,82]. Remarkably, noradrenergic transmission is reported to provide a dominant drive for astrocytic Ca^{2+} elevations during awake states [43]. Cholinergic volume transmission may provide an additional input to enhance Ca^{2+} elevations in astrocytes during attention. Indeed, a synergistic effect of acetylcholine and noradrenaline in synaptic plasticity has been described [83,84]. It is conceivable that similar operating principles are in effect in extracortical areas. For instance, the basal ganglia system is under the strong control of dopaminergic innervation, whereas the cerebellar cortex receives significant serotonergic and noradrenergic innervations. Molecular and physiological investigations on the heterogeneity of astrocytes will be important to understand the regional operational characteristics of astrocyte–neuron interactions. Response to neuromodulators is widespread across the astrocytic syncytium owing to the nature of volume transmission, and possibly owing to interastrocytic Ca^{2+} wave propagation [85]. Synaptic activity-driven elevation of focal Ca^{2+} rise in astrocytes [32,33] may provide us an additional mechanism to promote synaptic efficacy.

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References

1. Parpura V, Basarsky TA, Liu F, Jeftinija K, Jeftinija S, Haydon PG. 1994 Glutamate-mediated astrocyte–neuron signalling. *Nature* **369**, 744–747. (doi:10.1038/369744a0)
2. Nedergaard M. 1994 Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* **263**, 1768–1771. (doi:10.1126/science.8134839)
3. Hassinger TD, Guthrie PB, Atkinson PB, Bennett MV, Kater SB. 1996 An extracellular signaling component in propagation of astrocytic calcium waves. *Proc. Natl Acad. Sci. USA* **93**, 13 268–13 273. (doi:10.1073/pnas.93.23.13268)
4. Zhou M, Xu G, Xie M, Zhang X, Schools GP, Ma L, Kimelberg HK, Chen H. 2009 TWIK-1 and TREK-1 are potassium channels contributing significantly to astrocyte passive conductance in rat hippocampal slices. *J. Neurosci.* **29**, 8551–8564. (doi:10.1523/JNEUROSCI.5784-08.2009)
5. Djukic B, Casper KB, Philpot BD, Chin L-S, McCarthy KD. 2007 Conditional knock-out of Kir4.1 leads to glial membrane depolarization, inhibition of potassium and glutamate uptake, and enhanced short-term synaptic potentiation. *J. Neurosci.* **27**, 11 354–11 365. (doi:10.1523/JNEUROSCI.0723-07.2007)
6. Hirase H, Qian L, Bartho P, Buzsaki G. 2004 Calcium dynamics of cortical astrocytic networks *in vivo*. *PLoS Biol.* **2**, E96. (doi:10.1371/journal.pbio.0020096)
7. Agnati LF, Zoli M, Strömberg I, Fuxe K. 1995 Intercellular communication in the brain: wiring versus volume transmission. *Neuroscience* **69**, 711–726. (doi:10.1016/0306-4522(95)00308-6)

8. Zoli M, Agnati LF. 1996 Wiring and volume transmission in the central nervous system: the concept of closed and open synapses. *Prog. Neurobiol.* **49**, 363–380. (doi:10.1016/0301-0082(96)00020-2)
9. Nedergaard M, Verkhratsky A. 2012 Artifact versus reality: how astrocytes contribute to synaptic events. *Glia* **60**, 1013–1023. (doi:10.1002/glia.22288)
10. Sykova E, Nicholson C. 2008 Diffusion in brain extracellular space. *Physiol. Rev.* **88**, 1277–1340. (doi:10.1152/physrev.00027.2007)
11. Mechawar N, Cozzari C, Descarries L. 2000 Cholinergic innervation in adult rat cerebral cortex: a quantitative immunocytochemical description. *J. Comp. Neurol.* **428**, 305–318. (doi:10.1002/1096-9861(20001211)428:2<305::AID-CNE9>3.0.CO;2-Y)
12. Umbriaco D, Watkins KC, Descarries L, Cozzari C, Hartman BK. 1994 Ultrastructural and morphometric features of the acetylcholine innervation in adult rat parietal cortex: an electron microscopic study in serial sections. *J. Comp. Neurol.* **348**, 351–373. (doi:10.1002/cne.903480304)
13. Séguéla P, Watkins KC, Geffard M, Descarries L. 1990 Noradrenaline axon terminals in adult rat neocortex: an immunocytochemical analysis in serial thin sections. *Neuroscience* **35**, 249–264. (doi:10.1016/0306-4522(90)90079-J)
14. Cohen Z, Molinatti G, Hamel E. 1997 Astroglial and vascular interactions of noradrenaline terminals in the rat cerebral cortex. *J. Cereb. Blood Flow Metab.* **17**, 894–904. (doi:10.1097/00004647-199708000-00008)
15. Umbriaco D, Garcia S, Beaulieu C, Descarries L. 1995 Relational features of acetylcholine, noradrenaline, serotonin and GABA axon terminals in the stratum radiatum of adult rat hippocampus (CA1). *Hippocampus* **5**, 605–620. (doi:10.1002/hipo.450050611)
16. Vizi ES, Fekete A, Karoly R, Mike A. 2010 Non-synaptic receptors and transporters involved in brain functions and targets of drug treatment. *Br. J. Pharmacol.* **160**, 785–809. (doi:10.1111/j.1476-5381.2009.00624.x)
17. Mishchenko Y, Hu T, Spacek J, Mendenhall J, Harris KM, Chklovskii DB. 2010 Ultrastructural analysis of hippocampal neuropil from the connectomics perspective. *Neuron* **67**, 1009–1020. (doi:10.1016/j.neuron.2010.08.014)
18. Hösli E, Hösli L. 1993 Receptors for neurotransmitters on astrocytes in the mammalian central nervous system. *Prog. Neurobiol.* **40**, 477–506. (doi:10.1016/0301-0082(93)90019-O)
19. Agulhon C, Petravicz J, McMullen AB, Sweger EJ, Minton SK, Taves SR, Casper KB, Fiacco TA, McCarthy KD. 2008 What is the role of astrocyte calcium in neurophysiology? *Neuron* **59**, 932–946. (doi:10.1016/j.neuron.2008.09.004)
20. Fellin T, Pascual O, Gobbo S, Pozzan T, Haydon PG, Carmignoto G. 2004 Neuronal synchrony mediated by astrocytic glutamate through activation of extrasynaptic NMDA receptors. *Neuron* **43**, 729–743. (doi:10.1016/j.neuron.2004.08.011)
21. Fiacco TA, McCarthy KD. 2004 Intracellular astrocyte calcium waves in situ increase the frequency of spontaneous AMPA receptor currents in CA1 pyramidal neurons. *J. Neurosci.* **24**, 722–732. (doi:10.1523/JNEUROSCI.2859-03.2004)
22. Pascual O *et al*. 2005 Astrocytic purinergic signaling coordinates synaptic networks. *Science* **310**, 113–116. (doi:10.1126/science.1116916)
23. Serrano A, Haddjeri N, Lacaille JC, Robitaille R. 2006 GABAergic network activation of glial cells underlies hippocampal heterosynaptic depression. *J. Neurosci.* **26**, 5370–5382. (doi:10.1523/JNEUROSCI.5255-05.2006)
24. Perea G, Araque A. 2007 Astrocytes potentiate transmitter release at single hippocampal synapses. *Science* **317**, 1083–1086. (doi:10.1126/science.1144640)
25. Jourdain P *et al*. 2007 Glutamate exocytosis from astrocytes controls synaptic strength. *Nat. Neurosci.* **10**, 331–339. (doi:10.1038/nn1849)
26. Henneberger C, Papouin T, Oliet SH, Rusakov DA. 2010 Long-term potentiation depends on release of D-serine from astrocytes. *Nature* **463**, 232–236. (doi:10.1038/nature08673)
27. 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. (doi:10.1073/pnas.92.9.3948)
28. Mothet JP, Pollegioni L, Ouanounou G, Martineau M, Fossier P, Baux G. 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)
29. Yang Y, Ge W, Chen Y, Zhang Z, Shen W, Wu C, Poo M, Duan S. 2003 Contribution of astrocytes to hippocampal long-term potentiation through release of D-serine. *Proc. Natl Acad. Sci. USA* **100**, 15 194–15 199. (doi:10.1073/pnas.2431073100)
30. Shigetomi E, Jackson-Weaver O, Huckstepp RT, O'Dell TJ, Khakh BS. 2013 TRPA1 channels are regulators of astrocyte basal calcium levels and long-term potentiation via constitutive D-serine release. *J. Neurosci.* **33**, 10 143–10 153. (doi:10.1523/JNEUROSCI.5779-12.2013)
31. Bergersen LH *et al*. 2011 Immunogold detection of L-glutamate and D-serine in small synaptic-like microvesicles in adult hippocampal astrocytes. *Cereb. Cortex* **22**, 1690–1697. (doi:10.1093/cercor/bhr254)
32. Di Castro MA, Chuquet J, Liaudet N, Bhaukaurally K, Santello M, Bouvier D, Tiret P, Volterra A. 2011 Local Ca^{2+} detection and modulation of synaptic release by astrocytes. *Nat. Neurosci.* **14**, 1276–1284. (doi:10.1038/nn.2929)
33. Panatier A, Vallee J, Haber M, Murai KK, Lacaille JC, Robitaille R. 2011 Astrocytes are endogenous regulators of basal transmission at central synapses. *Cell* **146**, 785–798. (doi:10.1016/j.cell.2011.07.022)
34. Panatier A, Theodosis DT, Mothet JP, Touquet B, Pollegioni L, Poulain DA, Oliet SH. 2006 Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* **125**, 775–784. (doi:10.1016/j.cell.2006.02.051)
35. Cotrina ML, Lin JH, Alves-Rodrigues A, Liu S, Li J, Azmi-Ghadimi H, Kang J, Naus CC, Nedergaard M. 1998 Connexins regulate calcium signaling by controlling ATP release. *Proc. Natl Acad. Sci. USA* **95**, 15 735–15 740. (doi:10.1073/pnas.95.26.15735)
36. Guthrie PB, Knappenberger J, Segal M, Bennett MV, Charles AC, Kater SB. 1999 ATP released from astrocytes mediates glial calcium waves. *J. Neurosci.* **19**, 520–528.
37. Newman EA. 2001 Propagation of intercellular calcium waves in retinal astrocytes and Müller cells. *J. Neurosci.* **21**, 2215–2223.
38. Zhang JM, Wang HK, Ye CQ, Ge W, Chen Y, Jiang ZL, Wu CP, Poo MM, Duan S. 2003 ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* **40**, 971–982. (doi:10.1016/S0896-6273(03)00717-7)
39. Lalo U, Palygin O, Rasooli-Nejad S, Andrew J, Haydon PG, Pankratov Y. 2014 Exocytosis of ATP from astrocytes modulates phasic and tonic inhibition in the neocortex. *PLoS Biol.* **12**, e1001747. (doi:10.1371/journal.pbio.1001747)
40. Gordon GRJ, Baimoukhametova DV, Hewitt SA, Rajapaksha WR, Fisher TE, Bains JS. 2005 Norepinephrine triggers release of glial ATP to increase postsynaptic efficacy. *Nat. Neurosci.* **8**, 1078–1086. (doi:10.1038/nn1498)
41. Gordon GR, Iremonger KJ, Kantevari S, Ellis-Davies GC, MacVicar BA, Bains JS. 2009 Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. *Neuron* **64**, 391–403. (doi:10.1016/j.neuron.2009.10.021)
42. 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)
43. Ding F, O'Donnell J, Thrane AS, Zeppenfeld D, Kang H, Xie L, Wang F, Nedergaard M. 2013 α1-Adrenergic receptors mediate coordinated Ca^{2+} signaling of cortical astrocytes in awake, behaving mice. *Cell Calcium* **54**, 387–394. (doi:10.1016/j.ceca.2013.09.001)
44. Takata N, Mishima T, Hisatsune C, Nagai T, Ebisui E, Mikoshiba K, Hirase H. 2011 Astrocyte calcium signaling transforms cholinergic modulation to cortical plasticity *in vivo*. *J. Neurosci.* **31**, 18 155–18 165. (doi:10.1523/JNEUROSCI.5289-11.2011)
45. Chen N, Sugihara H, Sharma J, Perea G, Petravicz J, Le C, Sur M. 2012 Nucleus basalis-enabled stimulus-specific plasticity in the visual cortex is mediated by astrocytes. *Proc. Natl Acad. Sci. USA* **109**, E2832–E2841. (doi:10.1073/pnas.1206557109)
46. Navarrete M, Perea G, Fernandez de Sevilla D, Gómez-Gonzalo M, Núñez A, Martín ED, Araque A. 2012 Astrocytes mediate *in vivo* cholinergic-induced synaptic plasticity. *PLoS Biol.* **10**, e1001259. (doi:10.1371/journal.pbio.1001259)
47. Wang X, Lou N, Xu Q, Tian GF, Peng WG, Han X, Kang J, Takano T, Nedergaard M. 2006 Astrocytic Ca^{2+} signaling evoked by sensory stimulation *in vivo*. *Nat. Neurosci.* **9**, 816–823. (doi:10.1038/nn1703)
48. Wang F, Smith NA, Xu Q, Fujita T, Baba A, Matsuda T, Takano T, Bekar L, Nedergaard M. 2012 Astrocytes modulate neural network activity by Ca^{2+}-

dependent uptake of extracellular K^+. *Sci. Signal.* **5**, ra26. (doi:10.1126/scisignal.2002334)

49. Wang F, Smith NA, Xu Q, Goldman S, Peng W, Huang JH, Takano T, Nedergaard M. 2013 Photolysis of caged Ca^{2+} but not receptor-mediated Ca^{2+} signaling triggers astrocytic glutamate release. *J. Neurosci.* **33**, 17 404–17 412. (doi:10.1523/JNEUROSCI.2178-13.2013)
50. Hertz L, Lovatt D, Goldman SA, Nedergaard M. 2010 Adrenoceptors in brain: cellular gene expression and effects on astrocytic metabolism and $[Ca^{2+}]i$. *Neurochem. Int.* **57**, 411–420. (doi:10.1016/j.neuint.2010.03.019)
51. Mulligan SJ, MacVicar BA. 2004 Calcium transients in astrocyte endfeet cause cerebrovascular constrictions. *Nature* **431**, 195–199. (doi:10.1038/nature02827)
52. Takano T, Tian GF, Peng W, Lou N, Libionka W, Han X, Nedergaard M. 2006 Astrocyte-mediated control of cerebral blood flow. *Nat. Neurosci.* **9**, 260–267. (doi:10.1038/nn1623)
53. Bekar LK, Wei HS, Nedergaard M. 2012 The locus coeruleus-norepinephrine network optimizes coupling of cerebral blood volume with oxygen demand. *J. Cereb. Blood Flow Metab.* **32**, 2135–2145. (doi:10.1038/jcbfm.2012.115)
54. Nizar K *et al*. 2013 *In vivo* stimulus-induced vasodilation occurs without IP3 receptor activation and may precede astrocytic calcium increase. *J. Neurosci.* **33**, 8411–8422. (doi:10.1523/JNEUROSCI.3285-12.2013)
55. Takata N, Nagai T, Ozawa K, Oe Y, Mikoshiba K, Hirase H. 2013 Cerebral blood flow modulation by basal forebrain or whisker stimulation can occur independently of large cytosolic Ca^{2+} signaling in astrocytes. *PLoS ONE* **8**, e66525. (doi:10.1371/journal.pone.0066525)
56. Fiacco TA, Agulhon C, Taves SR, Petravicz J, Casper KB, Dong X, Chen J, McCarthy KD. 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)
57. 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)
58. Petravicz J, Fiacco TA, McCarthy KD. 2008 Loss of IP3 receptor-dependent Ca^{2+} increases in hippocampal astrocytes does not affect baseline CA1 pyramidal neuron synaptic activity. *J. Neurosci.* **28**, 4967–4973. (doi:10.1523/JNEUROSCI.5572-07.2008)
59. Woo DH *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)
60. Sasaki T, Beppu K, Tanaka KF, Fukazawa Y, Shigemoto R, Matsui K. 2012 Application of an optogenetic byway for perturbing neuronal activity via glial photostimulation. *Proc. Natl Acad. Sci. USA* **109**, 20 720–20 725. (doi:10.1073/pnas.1213458109)
61. Agulhon C, Boyt KM, Xie AX, Friocourt F, Roth BL, McCarthy KD. 2013 Modulation of the autonomic nervous system and behaviour by acute glial cell Gq protein-coupled receptor activation *in vivo*. *J. Physiol.* **591**, 5599–5609. (doi:10.1113/jphysiol.2013.261289)
62. Jensen O, Kaiser J, Lachaux J-P. 2007 Human gamma-frequency oscillations associated with attention and memory. *Trends Neurosci.* **30**, 317–324. (doi:10.1016/j.tins.2007.05.001)
63. Singer W, Gray CM. 1995 Visual feature integration and the temporal correlation hypothesis. *Annu. Rev. Neurosci.* **18**, 555–586. (doi:10.1146/annurev.ne.18.030195.003011)
64. Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI. 2009 Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* **459**, 663–667. (doi:10.1038/nature08002)
65. Wespatat V, Tennigkeit F, Singer W. 2004 Phase sensitivity of synaptic modifications in oscillating cells of rat visual cortex. *J. Neurosci.* **24**, 9067–9075. (doi:10.1523/JNEUROSCI.2221-04.2004)
66. Metherate R, Cox CL, Ashe JH. 1992 Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J. Neurosci.* **12**, 4701–4711.
67. Pinto L, Goard MJ, Estandian D, Xu M, Kwan AC, Lee S-H, Harrison TC, Feng G, Dan Y. 2013 Fast modulation of visual perception by basal forebrain cholinergic neurons. *Nat. Neurosci.* **16**, 1857–1863. (doi:10.1038/nn.3552)
68. Constantinople CM, Bruno RM. 2011 Effects and mechanisms of wakefulness on local cortical networks. *Neuron* **69**, 1061–1068. (doi:10.1016/j.neuron.2011.02.040)
69. Marrosu F, Portas C, Mascia MS, Casu MA, Fà M, Giagheddu M, Imperato A, Gessa GL. 1995 Microdialysis measurement of cortical and hippocampal acetylcholine release during sleep–wake cycle in freely moving cats. *Brain Res.* **671**, 329–332. (doi:10.1016/0006-8993(94)01399-3)
70. Lee MG, Hassani OK, Alonso A, Jones BE. 2005 Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. *J. Neurosci.* **25**, 4365–4369. (doi:10.1523/JNEUROSCI.0178-05.2005)
71. Shinohara Y, Hosoya A, Hirase H. 2013 Experience enhances gamma oscillations and interhemispheric asymmetry in the hippocampus. *Nat. Commun.* **4**, 1652. (doi:10.1038/ncomms2658)
72. Atallah BV, Scanziani M. 2009 Instantaneous modulation of gamma oscillation frequency by balancing excitation with inhibition. *Neuron* **62**, 566–577. (doi:10.1016/j.neuron.2009.04.027)
73. Donato F, Rompani SB, Caroni P. 2013 Parvalbumin-expressing basket-cell network plasticity induced by experience regulates adult learning. *Nature* **504**, 272–276. (doi:10.1038/nature12866)
74. Park GAS, Pappas BA, Murtha SM, Ally A. 1992 Enriched environment primes forebrain choline acetyltransferase activity to respond to learning experience. *Neurosci. Lett.* **143**, 259–262. (doi:10.1016/0304-3940(92)90278-F)
75. Pych JC, Chang Q, Colon-Rivera C, Haag R, Gold PE. 2014 Acetylcholine release in the hippocampus and striatum during place and response training. *Learn. Mem.* **12**, 564–572. (doi:10.1101/lm.33105)
76. Gu Q. 2002 Neuromodulatory transmitter systems in the cortex and their role in cortical plasticity. *Neuroscience* **111**, 815–835. (doi:10.1016/S0306-4522(02)00026-X)
77. Weinberger NM, Miasnikov AA, Bieszczad KM, Chen JC. 2013 Gamma band plasticity in sensory cortex is a signature of the strongest memory rather than memory of the training stimulus. *Neurobiol. Learn. Mem.* **104**, 49–63. (doi:10.1016/j.nlm.2013.05.001)
78. Bliss TV, Collingridge GL. 1993 A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* **361**, 31–39. (doi:10.1038/361031a0)
79. Jia H, Rochefort NL, Chen X, Konnerth A. 2010 Dendritic organization of sensory input to cortical neurons *in vivo*. *Nature* **464**, 1307–1312. (doi:10.1038/nature08947)
80. Sakatani S, Seto-Ohshima A, Shinohara Y, Yamamoto Y, Yamamoto H, Itohara S, Hirase H. 2008 Neural-activity-dependent release of S100B from astrocytes enhances kainate-induced gamma oscillations *in vivo*. *J. Neurosci.* **28**, 10 928–10 936. (doi:10.1523/JNEUROSCI.3693-08.2008)
81. Nishiyama H, Knopfel T, Endo S, Itohara S. 2002 Glial protein S100B modulates long-term neuronal synaptic plasticity. *Proc. Natl Acad. Sci. USA* **99**, 4037–4042. (doi:10.1073/pnas.052020999)
82. López-Hidalgo M, Salgado-Puga K, Alvarado-Martínez R, Medina AC, Prado-Alcalá RA, García-Colunga J. 2012 Nicotine uses neuron–glia communication to enhance hippocampal synaptic transmission and long-term memory. *PLoS ONE* **7**, e49998. (doi:10.1371/journal.pone.0049998)
83. Bear MF, Singer W. 1986 Modulation of visual cortical plasticity by acetylcholine and noradrenaline. *Nature* **320**, 172–176. (doi:10.1038/320172a0)
84. Bröcher S, Artola A, Singer W. 1992 Agonists of cholinergic and noradrenergic receptors facilitate synergistically the induction of long-term potentiation in slices of rat visual cortex. *Brain Res.* **573**, 27–36. (doi:10.1016/0006-8993(92)90110-U)
85. Kuga N, Sasaki T, Takahara Y, Matsuki N, Ikegaya Y. 2011 Large-scale calcium waves traveling through astrocytic networks *in vivo*. *J. Neurosci.* **31**, 2607–2614. (doi:10.1523/JNEUROSCI.5319-10.2011)