components of the stretch-reflex system include: (1) dorsal-root-ganglion cells with their peripheral process that ends in striated-muscle stretch receptors and their central process that ends on ventral-horn motoneurons; and (2) the innervated ventral-horn motoneurons themselves. In other words, the stretch-reflex system itself consists of parts of two classical systems: the somatosensory (proprioceptive) and somatomotor systems.

Lanuza and colleagues suggest that because the basolateral amygdala and central amygdala are interconnected, and have been implicated in fear conditioning and emotional learning, these two brain areas form part of a fear-conditioning and emotional-learning system. This is certainly reasonable, as we indicated in our review[3]. However, it is important to acknowledge that this 'system' also includes the major sensory and motor systems as well. In a widely used fear-conditioning paradigm, auditory stimuli are used as conditioning stimuli, foot-shock (somatosensory) stimuli are used as unconditioned stimuli and the behavior of the animal that follows the presentation of such stimuli relies on the somatomotor system. In fact, very widespread parts of the nervous system must be active during fear conditioning and emotional learning in general.

Unfortunately, there is no general, systematic theory or taxonomy of the organization of mammalian neural systems. The development of one could be a major achievement of 21st-century neuroscience. Meanwhile, we have the classical sensory and motor systems[4], and an essentially infinite combination of circuits that are postulated to interconnect and integrate them for whatever behavior or function is under consideration.

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REVIEW

Tripartite synapses: glia, the unacknowledged partner

Alfonso Araque, Vladimir Parpura, Rita P. Sanzgiri and Philip G. Haydon

According to the classical view of the nervous system, the numerically superior glial cells have inferior roles in that they provide an ideal environment for neuronal-cell function. However, there is a wave of new information suggesting that glia are intimately involved in the active control of neuronal activity and synaptic neurotransmission. Recent evidence shows that glia respond to neuronal activity with an elevation of their internal Ca^{2+} concentration, which triggers the release of chemical transmitters from glia themselves and, in turn, causes feedback regulation of neuronal activity and synaptic strength. In view of these new insights, this article suggests that perisynaptic Schwann cells and synaptically associated astrocytes should be viewed as integral modulatory elements of tripartite synapses.

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GLIAL CELLS have a variety of roles in the nervous system[1]. Some of these roles place the glial cells in a subservient position, which supports the physiology of associated neurons. However, recent experimental evidence suggests that some glial cells also interact closely with neurons and participate in the regulation of synaptic neurotransmission. This article aims to discuss the emerging evidence that indicates such a role for glial cells and to propose that synapses are tripartite, consisting of synaptically associated glia as well as the presynaptic and postsynaptic nerve terminals.

In vertebrates, glia can be divided into four major categories. In the PNS are the Schwann cells, and in the CNS are microglia, oligodendrocytes and astrocytes. For the purposes of this article the discussion will be limited to glial cells that are intimately associated with synapses: the perisynaptic Schwann cells at the neuromuscular junction and the astrocytes of the CNS. From this point onwards the word 'glia' will be used to refer to these two types of cell.

In order to achieve the aims of this article it is unfortunately necessary to omit extensive discussions of particular aspects of glial physiology. However, recent reviews concerning astrocytes are available that focus on Ca^{2+} homeostasis and signaling[2–6], receptor distribution[2–6], metabolic support for neurons[7,8], the clearance of extracellular ions[9] and neurotransmitters[10,11], and the role of astrocytes in synaptogenesis[12,13].

The original demonstration of transmitter-induced elevations of intracellular Ca^{2+} concentration in glia, which can be either long-duration Ca^{2+} spikes or oscillations in Ca^{2+} levels[14,15], aroused the interest of several

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TABLE 1. Glia-induced neuronal modulation

Stimulus to glial cell	Assay	Cellular system	Consequences	Pharmacological sensitivity	Refs
Electrical	Calcium imaging	Cell-cultured forebrain (rat)	Increased neuronal Ca^{2+} levels (gap-junction mediated)	Octanol	33
Mechanical Photostimulation Bradykinin	Calcium imaging	Cell-cultured visual cortex (rat)	Increased neuronal Ca^{2+} levels (NMDA-receptor mediated)	AP5	23
Mechanical	Calcium imaging	Cell-cultured cortex (rat)	Increased neuronal Ca^{2+} levels	not determined	34
Mechanical Electrical	Calcium imaging and electro-physiology	Cell-cultured cortex and hippocampus (rat)	Increased neuronal Ca^{2+} levels and neuronal depolarization (iGluR mediated)	CNQX and AP5	32
ACPD	Calcium imaging	Hippocampal slice (rat)	Increased neuronal Ca^{2+} levels (iGluR mediated)	AP5 and NBQX	25
Prostaglandin E_2	Calcium imaging	Hippocampal slice (rat)	Increased neuronal Ca^{2+} levels (iGluR mediated)	AP5 and NBQX	24
Mechanical	Electrophysiology	Cell-cultured hippocampus (rat)	Presynaptic inhibition of elicited excitatory and inhibitory synaptic transmission (mGluR mediated)	MCPG and MAP4	35
Electrical Mechanical UV photolysis	Electrophysiology	Cell-cultured hippocampus (rat)	Increased frequency of spontaneous miniature synaptic currents (NMDA-receptor mediated)	AP5	36
Electrical Mechanical UV photolysis	Electrophysiology	Cell-cultured hippocampus (rat)	Neuronal slow-inward current and depolarization (iGluR mediated)	CNQX and AP5	26,35,36
Mechanical	Electrophysiology	Retina (rat)	Modulation of light-induced neuronal activity (iGluR mediated)	NBQX, AP7, bicuculline and strychnine	37
Guanine nucleotides	Electrophysiology	Neuromuscular junction (frog)	Presynaptic inhibition of neuromuscular transmission	not determined	38
Intracellular depolarization of astrocytes	Electrophysiology	Hippocampal slice (rat)	Facilitation of miniature inhibitory synaptic currents (iGluR mediated)	CNQX and AP5	39

Abbreviations: ACPD, 1-aminocyclopentane-2S,3R-dicarboxylate; AP5, D-2-amino-5-phosphonopentanoic acid; AP7, D-2-amino-7-phosphonoheptanoic acid; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione; iGluR, ionotropic glutamate receptor; MAP4, 2-amino-2-methyl-4-phosphonobutanoic acid; MCPG, α-methyl-4-carboxyphenylglycine; mGluR, metabotropic glutamate receptor; NBQX, 6-nitro-7-sulphamoylbenzo(*f*)quinoxaline-2,3-dione.

neurobiologists. As discussed elsewhere, astrocytes possess a large array of neurotransmitter receptors[2–4]. Many of these are coupled to second-messenger systems that cause the release of Ca^{2+} from IP_3-sensitive stores[2–4,14]. The propensity of ligands that cause Ca^{2+} elevations raises the question of their role in astrocyte function.

Astrocytes release neurotransmitters

It has been known for more than two decades that glial cells can release chemical transmitters[16–22]. An important question is whether transmitter substances can be released in response to elevations in Ca^{2+} concentration. Indeed, Ca^{2+}-dependent glutamate release has been demonstrated in cultured astrocytes[23,24] and in acutely isolated hippocampal slices[24,25]. Bradykinin, or co-activation of glutamate receptors by AMPA and 1-aminocyclopentane-1S,3R-dicarboxylate (ACPD), causes an elevation of Ca^{2+} levels in astrocytes, which is followed by the release of glutamate[23,24]. This release is Ca^{2+}-dependent: experimental manipulations that block the ligand-induced Ca^{2+} concentration elevation or that elevate Ca^{2+} concentration independently of ligand show that an elevation of Ca^{2+} concentration is both necessary and sufficient to produce glutamate release from astrocytes[23,24].

The mechanism of Ca^{2+}-dependent glutamate release is not fully defined. However, it is unlikely to be mediated by glutamate-transporter reversal because transport inhibitors do not affect Ca^{2+}-dependent release[23,24,26]. The ability of α-latrotoxin[27] and tetanus toxin[24], respectively, to stimulate and inhibit the release of endogenous glutamate from astrocytes, points to an exocytotic pathway underlying Ca^{2+}-dependent glutamate release. However, a more thorough evaluation and careful ultrastructural studies are required before such a mechanism can be attributed to this release pathway.

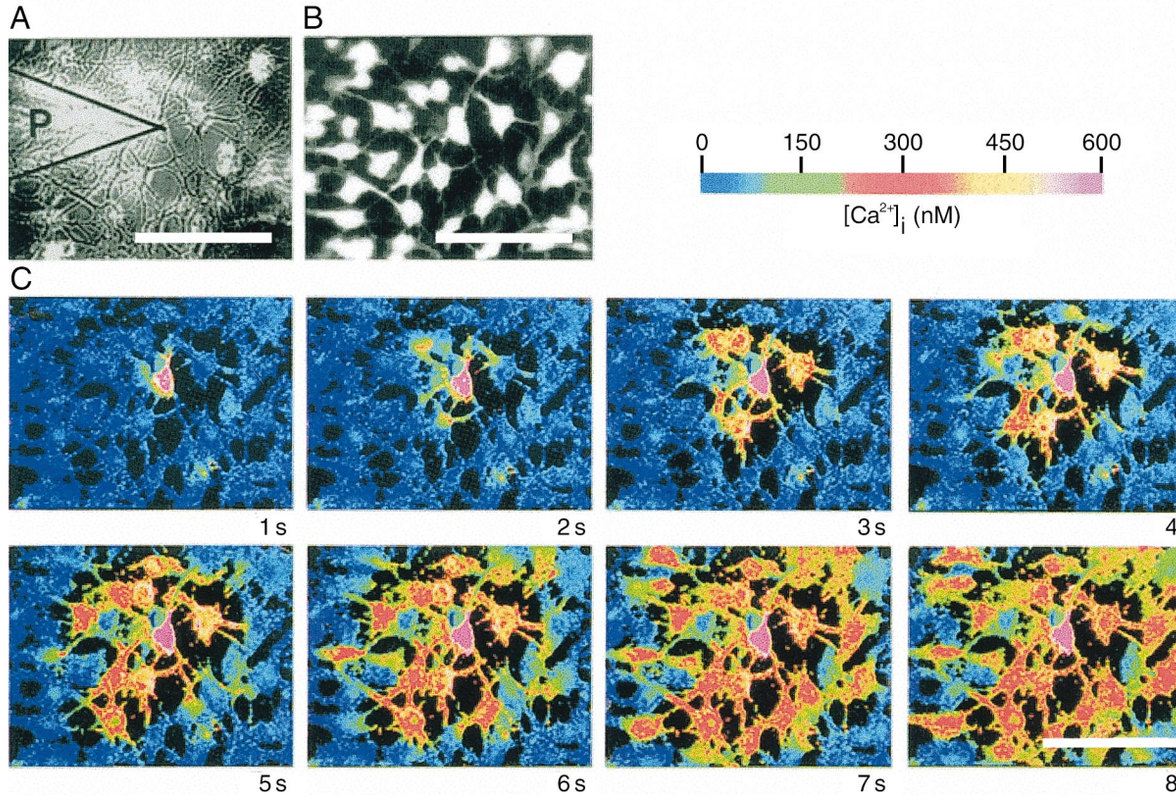
Astrocytes isolated from the hippocampus and visual cortex[23,24], as well as Schwann cells derived from dorsal-root ganglia[28], all show Ca^{2+}-dependent glutamate release, which suggests that it might be a widespread property of glia. Whether other neurotransmitters are subject to Ca^{2+}-regulated release in astrocytes, is unknown. For example, it is not known whether astrocytes only release glutamate or whether they release the transmitter used by neighboring synaptic nerve terminals preferentially. However, recent cell-culture studies suggest that astrocytes can also release ATP, which acts in intercellular signaling between neighboring astrocytes[29,30] (see below). Given that there are different subtypes of astrocytes[31], an important future area

Box 1. Techniques for the selective stimulation of glial cells

For most of the past century, glia were considered to be non-excitable cells. However, the demonstration that astrocytes do exhibit internal Ca^{2+} excitability[a–d] suggested they could have important roles in the CNS. Despite this recognition, it proved to be difficult to provide selective stimuli to astrocytes when they were in the presence of neurons. This problem was overcome by the observation that localized mechanical stimuli mobilize Ca^{2+} within astrocytes[e]. Contact between the tip of a micropipette and a cultured astrocyte causes an elevation of Ca^{2+} levels within the stimulated cell (Fig. I). Furthermore, because of gap-junction communication between astrocytes as well as the release of endogenous transmitters[f], this elevation of internal Ca^{2+} levels in astrocytes propagates among the astrocyte networks (Fig. I). The spatio–temporal control over mechanical stimuli has permitted selective stimulation of astrocytes in mixed cultures of astrocytes and neurons. From the time of the original publication of the selectivity of mechanical stimuli, it took just three years for four groups to use this stimulus (or a minor modification thereof, where the pipette is used to provide local electrical stimuli) to demonstrate that astrocytes signal to adjacent neurons[g–j].

While selective mechanical or electrical stimulation of glial cells is easily applicable in cell-culture experiments, its use with *in vivo* or *in situ* preparations is more difficult. Nevertheless, mechanical stimulation of astrocytes in the eye-cup preparation[k] has been performed. Other approaches that are being used are more arduous and require single-cell recordings or microinjection. For example, in the frog neuromuscular junction, microinjection of guanine nucleotide analogs into perisynaptic glial cells has been applied successfully to the study of glia–neuron signaling[l]. While these microelectrode–patch-pipette methods permit a high degree of experimental precision in delivering a drug or stimulus to an astrocyte, an alternative approach is to take advantage of the fact that astrocytes possess a large number of neurotransmitter receptors. However, one of the obvious concerns with this approach is that neurons also have receptors, which makes selective stimulation of the astrocyte difficult. Nonetheless, such problems have been overcome by performing a spatio–temporal analysis of the sequence of actions of neuroligands on astrocytes and neurons[m], together with the use of pharmacological tools that prevent neuronal activity (for example, tetrodotoxin), glutamate receptor activation (for example, AMPA- and NMDA-receptor antagonists)[m,n] or neuronal exocytosis (for example, tetanus toxin)[n].

Following the demonstration of astrocyte-to-neuron signaling that was initiated by mechanical stimuli, the field of



of research will be to determine their heterogeneity in neurotransmitter-release properties.

Glutamate-dependent astrocyte-to-neuron signaling

In coincidence with the demonstration of Ca^{2+}-dependent glutamate release from astrocytes, four independent laboratories showed that elevations of Ca^{2+} concentration in cell-cultured astrocytes could lead to neuronal responses[23,32–34] (Table 1). Stimuli that elevate Ca^{2+} levels in astrocytes (mechanical, electrical, photostimulation or the addition of bradykinin; see Box 1) all resulted in elevations of neuronal Ca^{2+} levels following the stimulated elevation of astrocyte Ca^{2+} levels. Furthermore, neuronal, but not astrocyte, elevations in Ca^{2+} concentration could be blocked by ionotropic-glutamate-receptor antagonists[23,32]. Additional support for glutamate-dependent signaling between astrocytes and neurons is provided by electrophysiological methods, which have demonstrated

astrocyte–neuron investigations has expanded rapidly. Now, additional manipulations have been made that can test for a role for astrocytes in the control of neuronal function. For example, photolysis of caged-Ca^{2+} compounds injected into astrocytes has been used to elevate Ca^{2+} levels in astrocytes selectively[o]. Microinjection of Ca^{2+} chelators into astrocytes has also been used to prevent intracellular increases in Ca^{2+} levels[o,p]. This type of manipulation, together with a careful understanding of astrocyte signal-transduction pathways[n] is likely to permit an elucidation of the role of astrocyte-ligand receptors in the regulation of synaptic neurotransmission and neuronal function.

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Fig. I. ***A communicated Ca^{2+} wave in response to mechanical stimulation.*** **(A)** *A phase-contrast image of a field of cells in a 7-day-old mixed glial culture. A micropipette (P) is positioned over a single cell and is used to stimulate the cell mechanically.* **(B)** *A fluorescence image of the same field of cells loaded with fura-2 at an excitation wavelength of 380 nm.* **(C)** *Intracellular Ca^{2+} concentration {$[Ca^{2+}]_i$} maps of the same field of cells at sequential time points following mechanical stimulation of a single cell. Time (in seconds) after mechanical stimulation is indicated below each panel. A wave of increased $[Ca^{2+}]_i$ is communicated cell by cell in all directions from the stimulated cell. The peak $[Ca^{2+}]_i$ increase in this example varies from 150–600 nM, as indicated by the pseudocolor scale bar. The wave is initiated at a specific point on each cell and spreads across the cell body and along its processes until it reaches the cell boundary. A delay time between the arrival of a Ca^{2+} wave at the borders of one cell and the communication of the wave to an adjacent cell can be seen in some cells; this intercellular delay time generally increases as the response spreads to more-distal cells. Scale bars, 100 μm.* Reproduced, with permission, from Ref. q.

an astrocyte-induced NMDA-receptor-dependent and non-NMDA-receptor-dependent depolarization[26,32], and a slow-inward current in hippocampal neurons co-cultured with astrocytes[35]. Since these initial cell culture studies, neurotransmitter-dependent astrocyte–neuron signaling has been demonstrated elegantly in the retina[37] and in acutely isolated hippocampal slices[24,25], which has removed doubts about whether this signaling pathway is merely a curiosity of cell culture.

Mechanical stimulation of retinal astrocytes and Müller cells, in order to elicit a wave of Ca^{2+} between these cells, leads to a modulation of light-induced excitation of ganglion cells[37]. When the Ca^{2+}-wave front within the glial cells reached the ganglion cell that was being recorded from, neuronal activity was frequently inhibited (although in some cells excitation could result). The Ca^{2+} concentration elevation in the glial cells is important for this neuronal modulation, as addition of thapsigargin (which blocks glial Ca^{2+} waves) attenuated the glial-induced modulation of neuronal activity. On the basis of previous cell-culture studies, which have measured glutamate release from astrocytes in response to elevated internal Ca^{2+} levels[23], it is likely that a similar pathway mediates glial–neuron signaling in the retina. In agreement with this possibility, the modulatory action of glial Ca^{2+} waves was blocked by the glutamate-receptor antagonists 6-nitro-7-sulphmoyl-benz(*f*)quinoxalone-2,3-dione (NBQX) and D-2-amino-7-phosphoheptanoic acid (AP7). However, in addition, the inhibitory GABA-receptor antagonists, strychnine and bicuculline, also blocked glia-induced neuronal modulation. A logical interpretation of these data is that the elevation of Ca^{2+} levels in the astrocytes causes a glutamate-dependent excitation of inhibitory interneurons, which in turn reduces ganglion-cell activity.

In the hippocampal-slice preparation, the study of astrocyte–neuron signaling has been more challenging because the ability to provide selective stimuli directly to an astrocyte has been more difficult (Box 1). Nonetheless, pharmacological methods have demonstrated the presence of astrocyte-to-neuron signaling. Addition of the metabotropic-glutamate-receptor (mGluR) agonist, ACPD, elevates Ca^{2+} levels in astrocytes and is followed by a delayed elevation of neuronal Ca^{2+} levels. Consistent with the Ca^{2+}-dependent release of glutamate from astrocytes that mediate an astrocyte-to-neuron signaling pathway, the elevation in neuronal Ca^{2+} levels is sensitive to the ionotropic-glutamate-receptor antagonists NBQX and D(–)-amino-5-phosphopentanoate (AP5)[24,25]. Prostoglandin E_2 (PGE_2) is a second messenger that is responsible for mediating some actions of glutamate-receptor activation in astrocytes[24]. Accordingly, addition of PGE_2 to the hippocampal-slice preparation causes an elevation of Ca^{2+} levels in astrocytes followed by a delayed elevation of Ca^{2+} levels in neurons that is blocked by ionotropic-glutamate-receptor antagonists[24]. Taken together, the studies that were performed in cell culture, acutely isolated hippocampal slices and in the retina, build a strong case for the existence of a Ca^{2+}-dependent glutamate-release pathway from astrocytes that can signal to neighboring neurons to elevate internal Ca^{2+} levels and modulate neuronal activity.

Astrocytes modulate synaptic neurotransmission

The ultrastructure of the CNS suggests that in addition to modulating neuronal activity, astrocytes might regulate synaptic neurotransmission. Astrocytes enwrap nerve terminals[40], which makes them perfectly positioned to 'listen' and 'talk' to synapses. Several studies have demonstrated that glia can respond to synaptic activation (Table 2), but it is not clear whether they 'talk back'.

Using the high-resolution technique of cell culture, the issue of whether astrocytes can modulate synaptic

TABLE 2. Neurotransmitter-mediated neuron-to-glial signaling

Cellular system	Consequences of neuronal activity	Pharmacological sensitivity	Interpretation	Refs
Squid giant axon to Schwann cell	Glial-cell hyperpolarization	2-APB and tubocurarine	Non-synaptic release of glutamate which in turn leads to ACh release from glial cells	18,41
Retinal ganglion cell axons to optic nerve astrocytes	Elevation of internal Ca^{2+} levels in astrocytes	not determined	Non-synaptic release of glutamate	42
Nerve terminal to perisynaptic Schwann cell	Elevation of Ca^{2+} levels in Schwann cells	ω-conotoxin GVIA	Nerve-terminal release of unidentified transmitter	43,44
Area CA3 of organotypic cultured hippocampal slice	Elevation of Ca^{2+} levels in astrocytes	Kynurenic acid	Synaptically released glutamate reaches adjacent astrocytes (glutamate-receptor mediated)	45
Area CA1 of hippocampal slice	Elevation of Ca^{2+} levels in astrocytes	MCPG	Synaptically released glutamate reaches adjacent astrocytes (mGluR mediated)	25,46
		CGP55845A	GABA released from interneurons, which acts on astrocytes ($GABA_B$-receptor mediated)	39

Abbreviations: 2-APB, 2-amino-4-phosphonobutyrate; MCPG, α-methyl-4-carboxyphenylglycine; mGluR, metabotropic glutamate receptor.

neurotransmission between cultured rat hippocampal neurons has been investigated (Fig. 1). While recording synaptic neurotransmission between pairs of neurons, a Ca^{2+} wave was induced in adjacent astrocytes. A reduction in the magnitude of action-potential elicited synaptic neurotransmission following astrocyte stimulation was detected reliably, with no effect on the amplitude of spontaneous miniature synaptic currents[35]. This suggested a presynaptic site of modulation of the synapse. Similar actions were detected at both glutamatergic and GABAergic synapses. Previous studies have shown that glutamate causes a mGluR-dependent inhibition of synaptic neurotransmission at hippocampal synapses[47–51]. As the astrocyte-induced modulation of synaptic neurotransmission was sensitive to mGluR antagonists, it was concluded that the increases in levels of Ca^{2+} in astrocytes led to the release of glutamate, which causes an mGluR-dependent presynaptic inhibition of neurotransmitter release at hippocampal synapses[35]. Additionally, these data provide the first clear demonstration that glia can modulate synaptic neurotransmission.

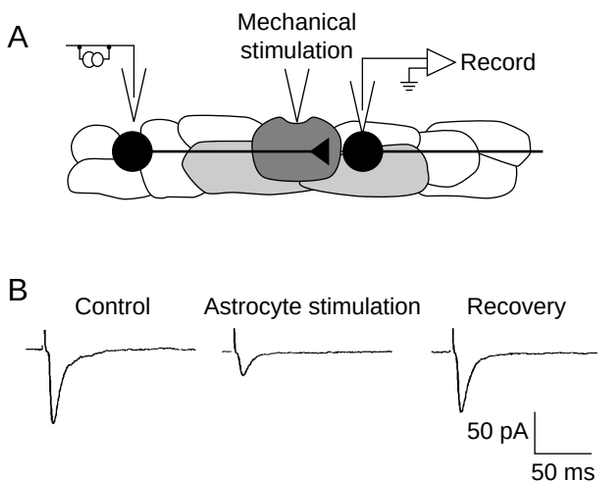

Fig. 1. ***Astrocytes and synaptic neurotransmission.*** **(A)** *Schematic representation of the cultured-hippocampal-cell experimental arrangement. While the postsynaptic currents elicited by extracellular presynaptic stimulation were recorded from a neuron (black), an adjacent astrocyte (dark gray) was stimulated mechanically to elicit a Ca^{2+} wave between neighboring astrocytes (light gray).* **(B)** *After astrocyte stimulation, the amplitude of excitatory postsynaptic currents was decreased transiently for about 1 min. Average excitatory postsynaptic currents elicited at 1 Hz are shown.* Reproduced, with permission, from Ref. 35.

Perisynaptic Schwann cells modulate neuromuscular neurotransmission

Glia-induced modulation of synaptic neurotransmission is not an exclusive property of synapses in the CNS as perisynaptic Schwann cells modulate transmitter release at the frog neuromuscular junction[38]. Microinjection of the non-hydrolysable analog of GTP, GTPγS, into perisynaptic Schwann cells causes a reduction in transmitter release at the frog neuromuscular junction. Furthermore, the typical depression of neuromuscular neurotransmission elicited by high-frequency stimulation of the axon was prevented by G-protein inactivation in the Schwann cell, which was brought about by microinjection of GDPβS. This is a striking and surprising result, with significant implications. This depression of synaptic neurotransmission was often attributed to an activity-dependent depletion of vesicles in the nerve terminal, so that fewer vesicles were available for release in response to subsequent action potentials[52]. However, these recent data suggest that during high-frequency stimulation, an undefined signal is transmitted from the presynaptic nerve terminal to the associated Schwann cell, which leads to a feedback inhibition of neurotransmission between the nerve and the muscle (Fig. 2)[38]. Although glia are not necessary for such functional neurotransmission[53], Robitaille's observation[38] indicates that, as he suggests, '…glia are active partners of the synapse…'.

Astrocytes serve as feedback elements at synapses in the CNS

In synaptic modulation in the CNS, astrocytes have, potentially, a similar role to perisynaptic Schwann cells. Several reports have demonstrated that neuronal activity can signal to Schwann cells and astrocytes[42–44,54–56] (see also Table 2). In the hippocampus, stimulation of presynaptic pathways can cause elevations of Ca^{2+} levels in astrocytes[45,46]. Under high-frequency stimulation, glutamate 'spills over' from

synapses and activates glutamate receptors on astrocytes, which causes an elevation of their intracellular Ca^{2+} levels[46]. This leads to the release of glutamate from the astrocyte which can feedback to the neuron[35]. Given that glutamate released from astrocytes can lead to an inhibition of synaptic neurotransmission[35], a similar negative-feedback loop, as described at the nerve–muscle–Schwann-cell synapse, is likely to exist at hippocampal synapses (Fig. 2).

In the CNS, however, the potential modulatory roles of astrocytes can extend beyond negative feedback. In addition to activating presynaptic mGluR receptors, astrocytes can, under appropriate conditions, increase the frequency of spontaneous miniature inhibitory and excitatory postsynaptic currents[36]. For example, by manipulating the intracellular Ca^{2+} levels in single-cell cultured astrocytes {by microinjection of the Ca^{2+} chelator BAPTA [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid], or by photolysis of the UV-sensitive Ca^{2+}-cage, NP-EGTA (*o*-nitrophenyl-EGTA)} it has been demonstrated that a rise in Ca^{2+} concentration in astrocytes is both necessary and sufficient to modulate spontaneous neurotransmitter release from nerve terminals. This Ca^{2+}-dependent astrocyte-induced enhancement of miniature-postsynaptic-current frequency is mediated by activation of NMDA receptors[36]. Similar results have now been reported in hippocampal slices where repeated activation of GABAergic interneurons can cause an increase in the frequency of miniature IPSCs (mIPSCs) recorded in CA1 pyramidal neurons that is thought to require astrocytes as a key modulatory element[39].

Thus, astrocytes can have opposing actions on the synapse: they can elicit an mGluR-dependent depression[35] or an NMDA-receptor-dependent increase in neurotransmitter release[36]. These exciting studies have shown the potential for astrocytes in the control of synaptic neurotransmission and plasticity. It will now be crucial to identify the conditions that lead to elevations in Ca^{2+} levels in astrocytes, and to determine under which situations the resulting glutamate that is released from the astrocyte can access either NMDA receptors or mGluRs in order to facilitate or depress the synapse.

Ca^{2+} waves in astrocytes and synaptic circuits

Two unique properties of astrocytes might impart novel capabilities on synaptic circuits. First, in response to brief stimuli, Ca^{2+} levels are elevated in astrocytes[57], which leads to synaptic modulation that lasts for a minute or more[35,36]. Thus, transmitter 'spillover' from the synapse could lead to a prolonged elevation of extracellular glutamate levels through an astrocyte intermediate. Given the importance of this transmitter in the induction of synaptic plasticity it will be interesting to determine whether glutamate supplied by astrocytes contributes significantly to this process. Second, an elevation of Ca^{2+} levels in one astrocyte can propagate to its neighbors[14,15,33,57,58]. While Ca^{2+}-wave coordination among astrocytes was believed initially to be ubiquitously due to the passage of an intracellular signal through gap junctions, recent experiments have demonstrated that, at least in some cases, it might also be due to a signal that is released from astrocytes. For example, in cell culture, an increase in Ca^{2+} levels in one astrocyte has been shown to cause an increase in Ca^{2+} levels in physically disconnected cortical astrocytes[58]. In this case, ATP is necessary for Ca^{2+}-wave propagation[29,30].

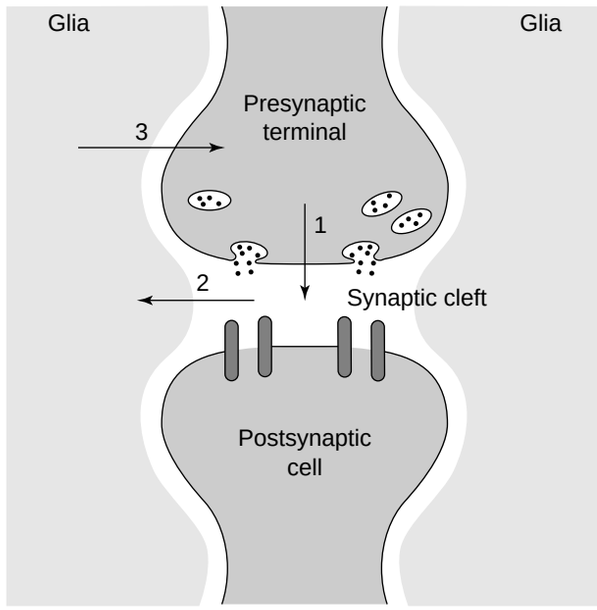

Fig 2. ***Signaling pathways at the tripartite synapse.*** *During synaptic neurotransmission, neurons release neurotransmitters from synaptic nerve terminals into the synaptic cleft to communicate with other neurons or effector cells such muscle fibers (1). The neurotransmitter released from the synapse (or other co-released neurotransmitter) can, under certain circumstances, 'spill over' from the synaptic cleft and reach neurotransmitter receptors in adjacent glial cells (astrocytes or perisynaptic Schwann cells), eliciting intracellular increases in Ca^{2+} concentrations in the glial cells (2). The increase in the glial-cell Ca^{2+} concentration causes it to release a chemical neurotransmitter from the glial cell, which in the case of astrocytes is glutamate (3), that feeds back to the presynaptic nerve terminal to modulate synaptic neurotransmission. (Glutamate released from the astrocyte can also cause elevations in neuronal Ca^{2+} levels and a slow-inward current. These actions are likely to be extrasynaptic[35,36] and consequently are not shown in this figure.)*

While the importance of Ca^{2+} waves in astrocytes is not yet fully appreciated, they might permit the effective spread of a modulatory signal to neighboring synapses (Fig. 3). For example, local spillover of neurotransmitter from one synapse will elevate Ca^{2+} levels in neighboring astrocytes, which will lead to local-feedback modulatory actions. Additionally, through a propagation of the Ca^{2+} wave between astrocytes, Ca^{2+}-dependent glutamate release will modulate synapses that are distant from the synapse that was initially active. In this manner, the active synapse, as well as adjacent inactive synapses, will be subject to modulation by the glutamate released from astrocytes. This could lead to lateral information transfer between synapses. Whatever the endpoint of the Ca^{2+} waves in astrocytes, it is clear that the addition of this signaling component to synaptic networks provides additional complexity in synaptic modulation.

Recruitment of glia into synaptic communication

Given the evidence that glial cells can respond to neuronal activity, and can in turn regulate synaptic strength, a question that begs to be answered is what are the conditions under which glial cells are activated to cause feedback release of neurotransmitter? For example, are glia able to respond to single action potentials in adjacent neurons, or is high-frequency

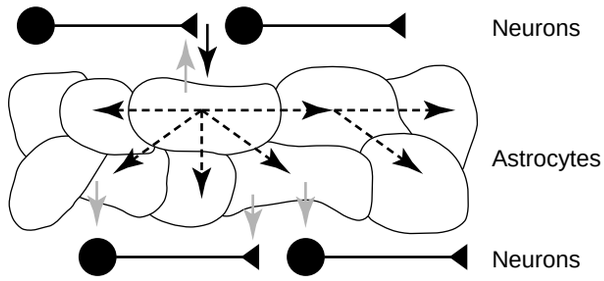

Fig. 3. ***Bi-directional communication between astrocytes and neurons.*** *Neurotransmitters released from the synapse might activate receptors in astrocytes that increase their intracellular Ca^{2+} concentration, providing the neuron-to-astrocyte signaling (black arrow). This neuronal-induced increase in the Ca^{2+} concentration in astrocytes can spread to neighboring astrocytes, which forms a Ca^{2+} wave that represents a form of communication between astrocytes (astrocyte-to-astrocyte signaling, broken lines). In response to this Ca^{2+}-concentration elevation, astrocytes might release glutamate that can increase the Ca^{2+} levels in adjacent neurons, influence the electrical activity of neurons and modulate synaptic neurotransmission (astrocyte-to-neuron signaling, gray arrows).*

activity required? While a systematic study has not been performed, data suggest that trains of action potentials are required to activate glia. In the frog neuromuscular junction, GDPβS injection into perisynaptic Schwann cells did not affect the magnitude of endplate currents when axons were stimulated at low frequency (0.2 Hz)[38]. However, inactivation of G proteins in Schwann cells did affect endplate currents when axons were stimulated at 10 Hz (Ref. 38), a frequency that has been shown to be sufficient to elevate Ca^{2+} levels in these Schwann cells[44]. In the hippocampal-slice preparation, single action potentials do not elicit Ca^{2+} concentration rises in astrocytes[25,46], although stimulation at higher frequencies was able to elicit delayed Ca^{2+} responses in astrocytes[25]. Furthermore, the magnitude of the glial Ca^{2+} response and the latency to onset appear to be graded, depending on the frequency of presynaptic neuronal activity[25,46]. These graded glial Ca^{2+} responses suggest a graded feedback modulation of the synapse.

So far this article has only discussed glial cells being activated by neurotransmitter released from immediately adjacent synapses or through propagating Ca^{2+} waves within networks of astrocytes. As astrocytes respond to a rich array of transmitters and humoral ligands, other inputs might also regulate astrocytes. For example, the hippocampus receives noradrenergic input and hippocampal astrocytes, which respond to noradrenaline by elevating Ca^{2+} levels[59], appear to be targets for noradrenergic inputs from the locus coeruleus[60,61]. Thus, extrinsic neurotransmitters have the potential to regulate Ca^{2+} levels in astrocytes, which in turn could then modulate local synaptic circuits.

Concluding remarks

From the foregoing discussion, it is clear that there is much excitement about the potential roles of glial cells in the nervous system. In addition to their well-known role in the uptake of neurotransmitters[10,11] the recent observations that stimuli producing an elevation in Ca^{2+} levels in glia lead to the release of glutamate, which in turn regulates neuronal Ca^{2+} levels and synaptic neurotransmission, have forced a reconsideration of the role of glial cells in the nervous system. As astrocytes and perisynaptic Schwann cells are associated intimately with the synapse, receive signals from the presynaptic neuron and respond by releasing feedback signals, it is proposed that these glial cells should be considered as an integral modulatory component of the synapse. Consequently, this article suggests that glial cells are active partners in this tripartite synapse.

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Seeing what is coming: building collision-sensitive neurones

F. Claire Rind and Peter J. Simmons

The image of a rapidly approaching object has to elicit a quick response. An animal needs to know that the object is approaching on a collision course and how imminent a collision is. The relevant information can be computed from the way that the image of the object grows on the retina of one eye. Firm data about the types of neurones that react to such looming stimuli and trigger avoidance reactions come from recent studies on the pigeon and the locust. The neurones responsible are tightly tuned to detect objects that are approaching on a direct collision course. In the pigeon these neurones signal the time remaining before collision whereas in the locust they have a crucial role in the simple strategy this animal uses to detect an object approaching on a collision course.

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INFORMATION about approaching objects is highly significant to many animal species and can signal the approach of a predator, or imminent collision with another object as the animal moves around. Detecting imminent collision is challenging to the visual system because of the speed at which the information must be extracted from the changing image on the retina and used to guide behaviour. As an object moves directly towards the head, both the size of the image on the retina and the disparity between the images on the two retinas increase. In humans, both these features contribute to the perception that an object is moving towards the observer and psychophysical observations indicate that this depth perception involves combinations of inputs from separate neural mechanisms that detect changes in image size and changes in binocular disparity[1–3]. These findings are supported by single-unit studies in primates, which have reported neurones in the medial superior temporal cortex that respond both to a change in image size and to a change in binocular disparity[4]. However, the temporal resolution of the stereoscopic pathway in humans is low and it is, therefore, not well suited to the task of tracking objects moving rapidly towards the subject[1,5]. Similarly, cat binocular neurones in area 18 that signal motion in depth[6] are more responsive to low velocities of retinal motion, between 10° and 40° per second. In humans, vergence eye movements in response to the radial expansion of a pattern can be induced monocularly and can occur with a latency of 80 ms, shorter than expected for stereoscopic processing[7]. In invertebrates measurements of motion in depth that involve binocular interactions are extremely rare[8,9], mainly because there is little overlap between the visual fields of the left and right eyes and the absolute distances separating the two eyes are small[10].

Looming stimuli

In humans a compelling illusion of motion in depth is given by a retinal image that changes in size[11]. Images that grow in size trigger avoidance reactions in a number of species[12]. Gibson suggested that the crucial feature for determining whether an object is on a direct collision course with an observer, is the symmetrical expansion of the retinal image[13]. Consistent with this hypothesis, psychophysical investigations by Regan and Hamstra have revealed that the human visual system uses separate channels for tracking the leading and trailing edges in the image of an approaching object[14]. Regan and Hamstra argue that this, combined with specific information about the size or rate of change of an object's image, provides the basis for collision avoidance when driving on the motorway and during ball hitting. As these are important operations, knowing how a nervous system extracts such information would represent a significant step forward in this area of research.

Estimating time to collision

In order to time its reactions appropriately, an animal needs to gain an estimate of the time remaining before colliding with an approaching object. This could be derived from a knowledge of the distance and speed of movement of the object, neither of which would be readily available. An alternative strategy, suggested by Lee, is to determine the ratio between retinal image size at a given instant and the rate of expansion of the image[15] (Fig. 1A). Both of these measurements can be extracted from the pattern of image motion that flows over the retina of each eye, for example, during locomotion (flow field). This particular ratio, τ, gives an accurate measure of time to collision as long as the image is not too large and

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