# *New roles for astrocytes*: Regulation of synaptic transmission

**Eric A. Newman**

Department of Neuroscience, University of Minnesota, 6-145 Jackson Hall, 321 Church Street SE, Minneapolis, MN 55455, USA

**Although glia often envelop synapses, they have traditionally been viewed as passive participants in synaptic function. Recent evidence has demonstrated, however, that there is a dynamic two-way communication between glia and neurons at the synapse. Neurotransmitters released from presynaptic neurons evoke $Ca^{2+}$ concentration increases in adjacent glia. Activated glia, in turn, release transmitters, including glutamate and ATP. These gliotransmitters feed back onto the presynaptic terminal either to enhance or to depress further release of neurotransmitter. Transmitters released from glia can also directly stimulate postsynaptic neurons, producing either excitatory or inhibitory responses. Based on these new findings, glia should be considered an active partner at the synapse, dynamically regulating synaptic transmission.**

The presynaptic terminal and the postsynaptic neuron have traditionally been viewed as the two functionally important elements of the synapse. Yet a third cellular component, the glial cell, is often associated with the synaptic structure. In the cortex, synapses are ensheathed by processes of astrocytes, the most common type of glial cell in the brain [1,2]. Synapses in the cerebellum are enveloped by Bergmann glia (specialized astrocytes) [3,4] and synapses in the retina are contacted by Müller cells (astrocyte-like radial glia) [5]. In the peripheral nervous system, the neuromuscular junction is tightly ensheathed by perisynaptic Schwann cells, which are specialized, non-myelinating peripheral glia [6,7].

These perisynaptic glia have traditionally been thought to play little role in synaptic function. Recent research has revealed, however, that a lively, bidirectional conversation is conducted between perisynaptic glia and the neuronal elements at the synapse. Release of neurotransmitter from the presynaptic terminal not only stimulates the postsynaptic neuron but also activates the perisynaptic glia. The activated glial cell, in turn, releases gliotransmitters that can directly stimulate the postsynaptic neuron and can feed back onto the presynaptic terminal either to enhance or to depress further release of neurotransmitter. (Transmitters released from glia will here be referred to as 'gliotransmitters', to distinguish them from neurotransmitters released from neurons [8].) Thus, the perisynaptic glial cell functions as an active partner in synaptic transmission. To understand synaptic function fully, the synapse must be considered a tripartite, rather than a bipartite, structure [9,10].

Many examples of bidirectional communication between neurons and glia at the synapse have now been documented. Experiments using culture and intact-tissue preparations demonstrate modulation of synaptic transmission by glia. Several of these studies will be highlighted in this review of glial regulation of synaptic function.

## Neuronal activation of glia

Astrocytes and other CNS glia express a wide variety of neurotransmitter receptors [11,12]. Activation of these receptors evokes a rich repertoire of responses in glia, including increases in intracellular $Ca^{2+}$ concentration ($[Ca^{2+}]$) and release of gliotransmitters, including glutamate and ATP.

Glial responses to neurotransmitters were initially characterized *in vitro*, using cultured astrocytes as a model system. These studies demonstrated that glial $[Ca^{2+}]$ increases could be evoked by a variety of neurotransmitters, including glutamate, GABA, adrenaline, ATP, serotonin, ACh and several peptides [11,12]. An essential question remained unanswered by these early studies, however: does the release of transmitters from neurons *in vivo* evoke glial responses? Recent experiments using brain slices have answered this question with a convincing yes, confirming findings from cell culture studies.

Release of several different neurotransmitters from active neurons can stimulate glia in intact tissue preparations, evoking increases in glial $[Ca^{2+}]$. In hippocampal slices, electrical stimulation of neurons evokes $[Ca^{2+}]$ increases in astrocytes. This response is mediated by several different astrocytic receptors, including metabotropic glutamate receptors [13,14], $GABA_B$ receptors [15] and muscarinic ACh receptors [16]. Similarly, in cerebellar slices, electrical stimulation of parallel fibers evokes $[Ca^{2+}]$ increases in Bergmann glia [3]. In this instance, the response is mediated by the release of nitric oxide (NO) from neurons [17]. Moderate neuronal stimulation of parallel fibers evokes $[Ca^{2+}]$ increases in small regions (microdomains) of individual Bergmann glia, suggesting that subcellular compartments of glia can function as independent units [3,4]. Neuronal activity can also evoke $[Ca^{2+}]$ increases in perisynaptic Schwann cells at the neuromuscular junction, with stimulation rates as low as 10 Hz evoking glial responses [6,18].

The capacity of glia to respond to neuronal activity has prompted suggestions that they might regulate local blood

*Corresponding author:* Eric A. Newman (ean@umn.edu).

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flow in response to changes in neuronal activity [19]. As in other preparations, electrical stimulation of neurons in cortical brain slices evokes [$Ca^{2+}$] increases in astrocytes – a response mediated by the release of glutamate from neurons and activation of glial metabotropic receptors [20]. Stimulation of neurons in this preparation also induces dilation of blood vessels contacted by the glia. It is likely that dilation is mediated by the glia, as direct simulation of astrocytes induces vessel dilation. $Ca^{2+}$ chelators and inhibitors of cyclooxygenase block vessel dilation, suggesting that glia might regulate blood flow by a $Ca^{2+}$-dependent release of prostaglandins, which are cyclooxygenase products [20].

An intriguing aspect of glial $Ca^{2+}$ responses is that they sometimes trigger self-propagating waves. Activation of glia in the retina [21,22] and in brain slices [23], by mechanical stimulation or by application of neurotransmitters, evokes glial [$Ca^{2+}$] increases that propagate as $Ca^{2+}$ waves through neighboring glia at distances of up to several hundred μm. These intercellular $Ca^{2+}$ waves are propagated by two mechanisms: by diffusion of inositol (1,4,5)-trisphosphate [Ins(1,4,5)$P_3$] through gap junctions coupling the cells, and by release of ATP, which functions as an extracellular messenger [22]. A crucial and, as yet, unanswered question is whether transmitter release from active neurons can trigger propagated intercellular glial $Ca^{2+}$ waves. If propagated waves are evoked, they could represent a mechanism by which glia modulate synaptic transmission over long distances. Such propagated $Ca^{2+}$ waves might also contribute to the regulation of blood flow in the brain.

## Glutamate release from astrocytes

Glial [$Ca^{2+}$] increases evoked by neuronal activity might be written off as a curious epiphenomenon of little importance to brain function if it were not for the fact that they initiate additional glial responses. Perhaps the most significant of these $Ca^{2+}$-dependent responses is the release of glutamate from glia.

In both culture [24–27] and brain-slice [15,14,28,29] preparations, increases in [$Ca^{2+}$] result in a release of glutamate from astrocytes. The mechanism by which glutamate is released remains uncertain, although several lines of evidence indicate that glutamate can be released by a $Ca^{2+}$-dependent exocytotic process [28,30,31]. As described in the following paragraphs, glutamate release from astrocytes is an important means by which glia

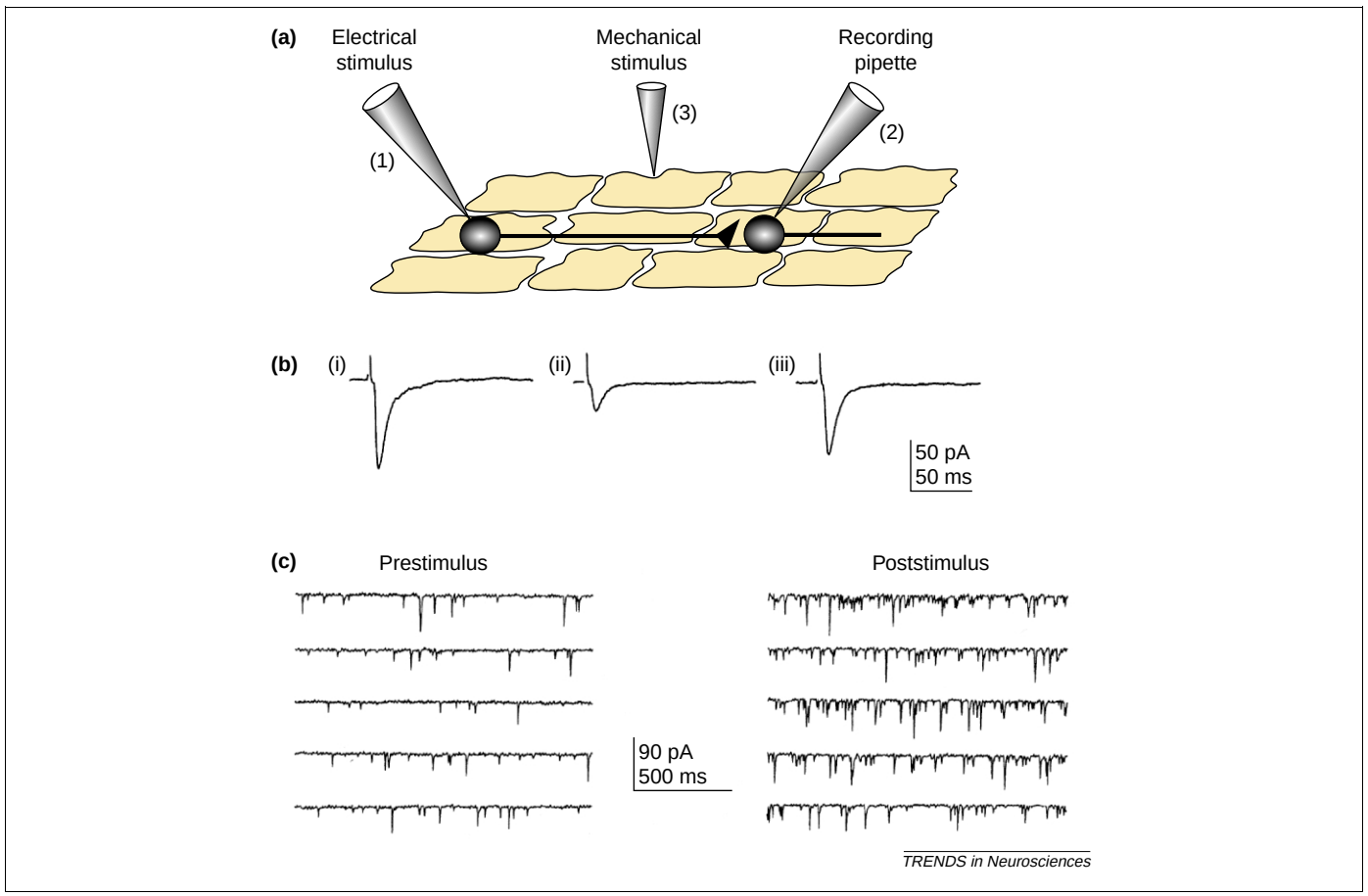


**Fig. 1**. Glial regulation of synaptic transmission in co-cultures of astrocytes and neurons. **(a)** The glial–neuronal culture preparation. A presynaptic neuron is stimulated with electrical pulses (1) while synaptic currents are recorded from a postsynaptic neuron (2). Astrocytes beneath the neurons (yellow) are activated with a mechanical stimulus (3). **(b)** Stimulation of the presynaptic neuron evokes an excitatory postsynaptic current (EPSC) in the postsynaptic neuron (i). Simultaneous stimulation of an adjacent astrocyte reduces the amplitude of the EPSC (ii). Following termination of glial stimulation, synaptic transmission recovers (iii). Glial depression of synaptic transmission was blocked by metabotropic-glutamate-receptor antagonists. Reproduced, with permission, from Ref. [25]. **(c)** Spontaneous miniature EPSCs (mEPSCs) recorded from a neuron (prestimulus). The frequency of mEPSCs increases following stimulation of a neighboring astrocyte (poststimulus). Glial enhancement of mEPSCs was blocked by NMDA antagonists. Reproduced, with permission, from Ref. [26], © (1998) the Society for Neuroscience.

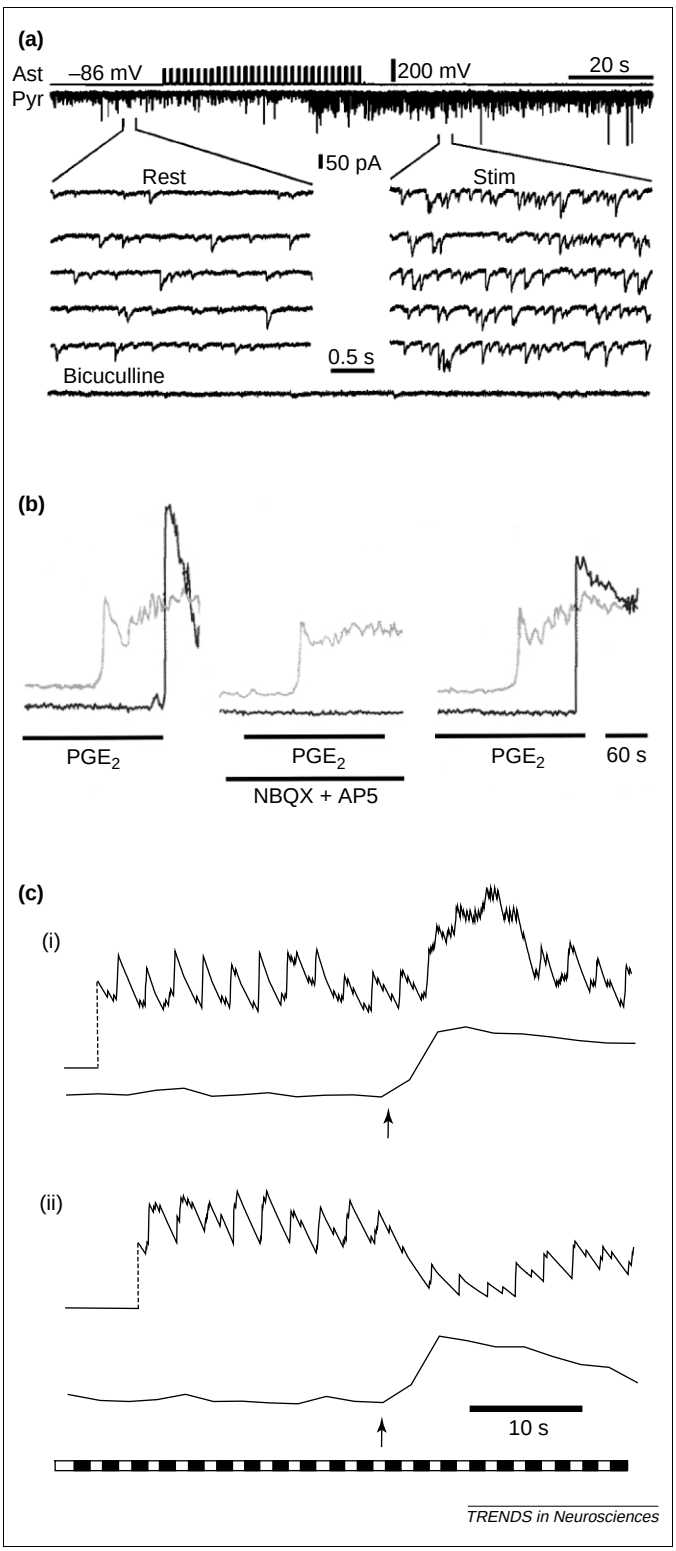


**Fig. 2**. Glial regulation of synaptic transmission in intact tissue preparations. **(a)** Spontaneous miniature inhibitory postsynaptic currents (mIPSCs) recorded from a pyramidal neuron (Pyr) in a hippocampal slice (Rest). Following stimulation of an astrocyte (Ast), the frequency of mIPSCs increases (Stim). The mIPSCs are blocked by the $GABA_A$-receptor antagonist bicuculline (bottom trace), whereas glial enhancement of mIPSC frequency was blocked by AMPA- and NMDA-receptor antagonists (not shown). Reproduced, with permission, from Ref. [15], © (1998) Nature (http://www.nature.com), Macmillan Magazines Limited. **(b)** Stimulation of astrocytes with prostaglandin $E_2$ ($PGE_2$) in a hippocampal slice evokes increases in $Ca^{2+}$ concentration ($[Ca^{2+}]$) in an astrocyte (gray traces) and in an adjacent neuron (black traces). Addition of the glutamate antagonists 6-nitro-7-sulfamoyl-benz(*f*)quinoxaline-2,3-dione (NBQX) and 2-amino-5-phosphonopentanoate (AP5) blocks the neuronal response but not the glial response, demonstrating that glia stimulate the neuron by releasing glutamate. Reproduced, with permission, from Ref. [28], © (1998) Nature (http://www.nature.com), Macmillan Magazines Limited. **(c)** Glial modulation of light-evoked spike activity recorded from ganglion cells in the retina. The top traces in (i) and (ii) show a running average of neuronal spike frequency and the bottom traces show glial $Ca^{2+}$ levels. (i) Neuron spike frequency increases when a stimulus (arrow) evokes a glial $[Ca^{2+}]$ increase. (ii) Spike frequency of a different neuron decreases when a glial $[Ca^{2+}]$ increase is evoked. Both excitatory and inhibitory modulation are mediated by presynaptic mechanisms. The bar at the bottom shows the repetitive light stimulus that evoked the spiking, with periods of light ON (open segments) and OFF (closed segments) indicated. Reproduced, with permission, from Ref. [35], © (1998) the Society for Neuroscience.

modulate the release of neurotransmitters from presynaptic terminals.

## Modulation of synaptic transmission in culture

Glial regulation of synaptic transmission has been best characterized in co-cultures of astrocytes and neurons, where $[Ca^{2+}]$ increases can be evoked in astrocytes by several stimuli, including electrical and mechanical stimulation and application of peptides and prostaglandin $E_2$ ($PGE_2$) [24–26,32,33]. Activation of astrocytes reduces the amplitude of excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs, respectively) evoked by electrical stimulation of presynaptic neurons [25] (Figure 1a,b). This depression of synaptic transmission is mediated by glutamate release from astrocytes and is blocked by metabotropic-glutamate-receptor antagonists.

Spontaneous synaptic events recorded from postsynaptic neurons are also modulated by astrocytes. The frequency of both miniature EPSCs (mEPSCs) and miniature IPSCs (mIPSCs) rises following astrocyte activation [26] (Figure 1c). This response, opposite in sign to glial modulation of evoked synaptic potentials, is mediated by glutamate release from astrocytes and stimulation of presynaptic NMDA receptors. Glutamate release from astrocytes can also directly stimulate postsynaptic neurons, evoking depolarizing 'slow inward currents' by activating neuronal AMPA and NMDA receptors [24,32,33]. In summary, experiments utilizing co-cultures of astrocytes and neurons demonstrate that glia are able to modulate synaptic transmission by release of glutamate.

## Modulation in brain slices

Recent experiments employing brain slices and other preparations in which the morphological relationship between neurons and glia is preserved also reveal glial regulation of synaptic transmission. In the hippocampus, repetitive firing of inhibitory interneurons leads to potentiation of synaptic transmission between the interneurons and pyramidal cells. This potentiation is mediated by glutamate release from neighboring astrocytes [15]. Potentiation arises when GABA, released from the interneurons, activates astrocytic $GABA_B$ receptors, evoking a $Ca^{2+}$-dependent glutamate release from the glia. The released glutamate activates interneuron AMPA and NMDA receptors, potentiating transmitter release. Direct electrical stimulation of hippocampal astrocytes leads to increased mIPSC frequency (Figure 2a) and raises the success rate of synaptic responses recorded from pyramidal neurons, most likely by $Ca^{2+}$-dependent glutamate release from the glia [15].

Additional experiments in brain slices of the hippocampus [14,28] and thalamus [34] demonstrate that activated astrocytes can directly stimulate neurons by release of glutamate. Application of glutamate agonists and $PGE_2$ in addition to neuronal stimulation evokes a $Ca^{2+}$-dependent glutamate release from astrocytes. Released glutamate activates neighboring neurons and results in increases in neuronal $Ca^{2+}$ levels (Figure 2b). The evoked neuronal responses are blocked by antagonists of AMPA and NMDA receptors.

## Modulation in the retina

Glia can also modulate synaptic activity driven by natural, physiological stimuli. This has been demonstrated in the mammalian retina, where glial activation modulates ganglion cell spike activity that is driven by light stimulation [35]. (Ganglion cells are the output neurons of the retina that project to the brain.) For some ganglion cells, light-evoked spiking is enhanced when neighboring glia are activated; in other ganglion cells, spiking is inhibited (Figure 2c). The precise mechanism of glial modulation of ganglion cell activity is not known, but recent experiments monitoring ganglion cell EPSCs demonstrate that both excitatory and inhibitory glial modulation is mediated by presynaptic mechanisms (E.A. Newman, unpublished).

In addition to the excitatory and inhibitory glial modulation of synaptic transmission, glia can influence neuronal activity in the retina by a third mechanism: direct inhibition of postsynaptic ganglion cells [36]. When Müller cells, the specialized radial glia of the retina, are activated, they release ATP into the inner synaptic layer of the retina. The released ATP is rapidly converted in the extracellular space to adenosine, which activates ganglion cell $A_1$ receptors and inhibits the neurons by increasing $K^+$ conductance.

Release of ATP from glia could have widespread modulatory effects in the CNS. Activation of astrocytes as well as Müller cells results in ATP release [22,37,38] (although the mechanism responsible for ATP release from glia is a matter of debate). Released ATP could excite neurons directly through activation of P2X receptors. Alternatively, released ATP, once converted to adenosine, could inhibit neurons by activating $A_1$ receptors, as it does in the retina [36]. Adenosine could also act presynaptically via $A_1$ and $A_2$ receptors to either depress or potentiate synaptic transmission. In addition, released ATP can activate additional glia via glial purinergic receptors [11,12,21,22].

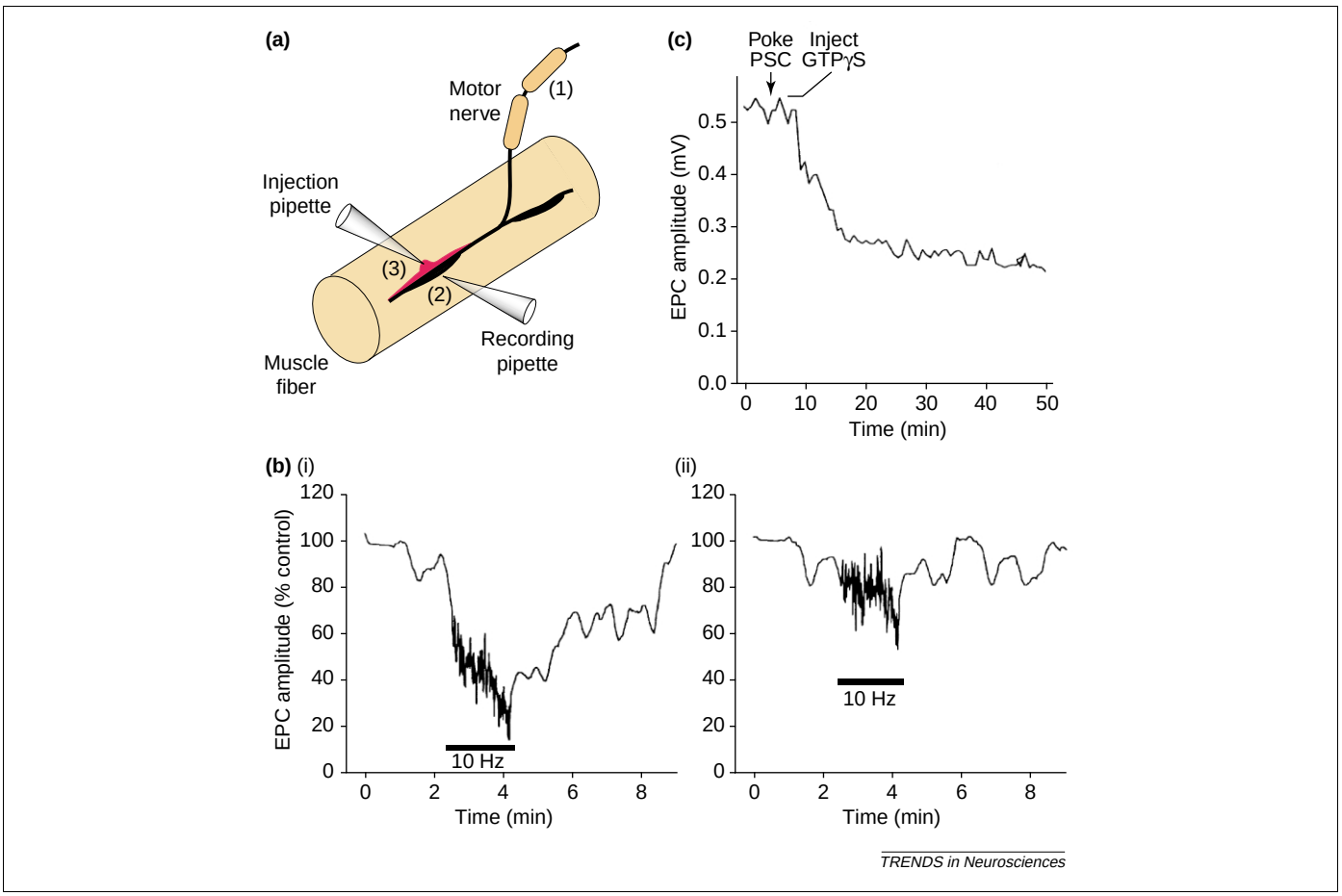


**Fig. 3.** Glial depression of synaptic transmission at the neuromuscular junction. **(a)** The neuromuscular junction preparation. A motor nerve axon (1) synapses onto a muscle fiber. The synaptic junction (2) is ensheathed by a perisynaptic Schwann cell (red, 3). Stimulation of the motor nerve evokes an endplate current (EPC), which is recorded from the muscle fiber. EPC amplitudes are graphed in (b) and (c). **(b)** Synaptic transmission is depressed when the motor nerve is stimulated by a train of pulses (i). The activity-dependent depression is blocked following injection of the GDP analog GDPβS into the perisynaptic Schwann cell (ii). **(c)** Injection of the GTP analog GTPγS into a perisynaptic Schwann cell induces depression at the synapse ensheathed by the glial cell. The arrow (Poke PSC) indicates when the injection pipette penetrates the perisynaptic Schwann cell. (b) and (c) reproduced, with permission, from Ref. [7].

## Modulation at the neuromuscular junction

Glial modulation of synaptic transmission is not limited to the CNS. A dramatic example of such modulation occurs at the neuromuscular junction, where perisynaptic Schwann cells are responsible for half of the synaptic depression seen during repetitive stimulation of the motor nerve [6,18,39,40]. Release of ACh and ATP from the presynaptic terminal activates the perisynaptic Schwann cell, evoking a $Ca^{2+}$-independent release of a gliotransmitter, thought to be glutamate, from the glial cell [6,18,39,41]. The released gliotransmitter, through production of NO, feeds back onto the presynaptic terminal, decreasing ACh release [39]. Perisynaptic Schwann cell depression of synaptic transmission is mediated by activation of a G-protein pathway in the glial cell. Synaptic depression is blocked by the GDP analog GDPβS and is stimulated by the GTP analog GTPγS injected into the glial cell [7] (Figure 3).

Glial regulation of synaptic transmission at the neuromuscular junction is complex, as perisynaptic Schwann cells possess a second, $Ca^{2+}$-dependent mechanism by which they can enhance transmitter release [42]. This excitatory action of the glial cell is weaker than is the inhibitory action and is observed only when inhibition is blocked.

Perisynaptic Schwann cell modulation of synaptic transmission at the neuromuscular junction provides perhaps the most convincing evidence to date that glia regulate synapses *in vivo*. Morphologically, the neuromuscular junction preparation closely reproduces conditions *in vivo*. In addition, levels of motor nerve stimulation that reliably evoke glial depression of synaptic transmission (10 Hz) are well within the range that occurs *in vivo* [18,40].

## Other forms of synaptic modulation

As reviewed in preceding sections, release of gliotransmitters from glia can modulate the release of neurotransmitters from the presynaptic terminal and can stimulate postsynaptic neurons. This mechanism of synaptic regulation can be termed 'direct modulation'. Glia can also regulate synaptic transmission by 'indirect' mechanisms.

The best characterized of the indirect regulatory mechanisms is the uptake of glutamate by glia via excitatory amino acid transporters [43,44]. Synaptic transmission at glutamatergic synapses is terminated by removal of glutamate from the synaptic cleft. Glia (primarily astrocytes in the brain and Müller cells in the retina) account for the bulk of glutamate uptake at the synapse [45–47]. Glial uptake of glutamate can significantly affect synaptic transmission. In the retina, for instance, the amplitude and duration of ganglion cell EPSCs are dramatically increased when the Müller glial glutamate transporter is blocked [48]. Glial glutamate transport can also modulate short-term synaptic plasticity [49].

Glia also modulate synaptic transmission by releasing chemical cofactors. The best-documented example of such modulation is activation of the NMDA receptor, which

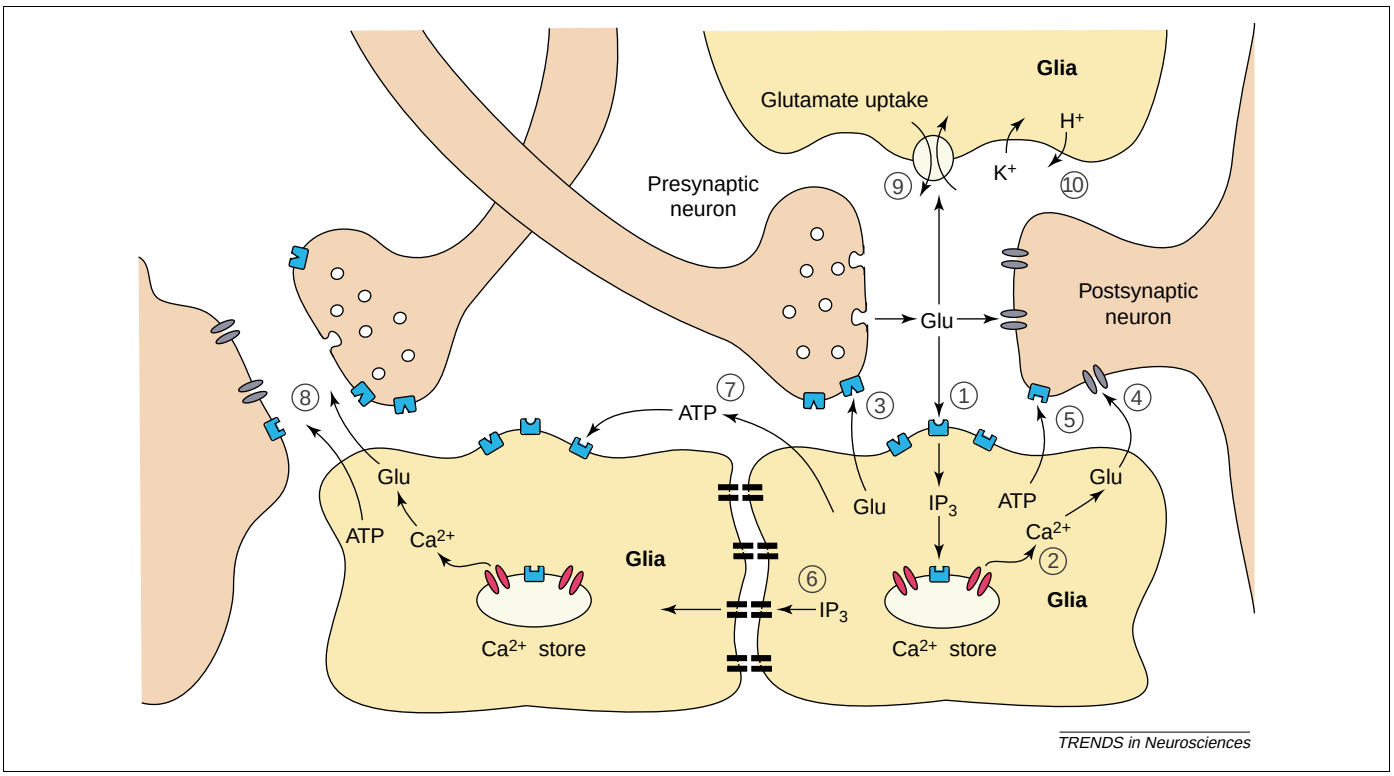


**Fig. 4**. Summary of proposed mechanisms of glial regulation of synaptic transmission. Release of glutamate (Glu) from the presynaptic terminal activates glial receptors (1), evoking an increase in $Ca^{2+}$ levels (2) and the release of glutamate from glia. Glutamate activation of presynaptic receptors (3) regulates transmitter release, while activation of postsynaptic receptors (4) directly depolarizes neurons. Stimulation of glia also elicits the release of ATP, which inhibits postsynaptic neurons by activating $A_1$ receptors (5). Activation of glia might also evoke an intercellular $Ca^{2+}$ wave, which propagates between glia by diffusion of inositol (1,4,5)-trisphosphate ($IP_3$) through gap junctions (6) and by release of ATP (7), and results in the modulation of distant synapses (8). Glia also modulate synaptic transmission by uptake of glutamate (9) and by regulating extracellular $K^+$ and $H^+$ levels (10). Other glial–neuronal interactions are not illustrated. These include glial activation by neurotransmitters other than glutamate and glial regulation of NMDA-receptor-containing synapses by release of D-serine.

requires the presence of glutamate as well as a cofactor that binds to the glycine-binding site of the receptor. Recent evidence indicates that D-serine, not glycine, is the endogenous agonist that activates the glycine-binding site [50,51]. Glia (astrocytes in the brain and Müller cells in the retina) are the sole source of D-serine in the CNS and most likely modulate synaptic transmission at NMDA synapses by releasing D-serine [52,53].

A third indirect mechanism of modulation is via glial regulation of extracellular ion levels. Neuronal activity leads to substantial variations in the concentrations of $K^+$ and $H^+$ in the extracellular space [54–57]. These variations can alter synaptic transmission; increases in $K^+$ levels depolarize synaptic terminals [58], whereas $H^+$ block presynaptic $Ca^{2+}$ channels [59,60] and NMDA receptors [61]. Glia play an essential role in regulating extracellular $K^+$ and $H^+$ concentrations [55,62,63], thus influencing the effect of these ions on synaptic transmission.

Glia can also modulate synaptic transmission by directly controlling synaptogenesis. Retinal ganglion cells, when cultured in the absence of glia, display little synaptic activity. Addition of astrocytes to these cultures substantially increases the frequency of mEPSCs, decreases the failure rate of evoked transmission, and increases the number of morphologically identified synapses [64,65]. Glial enhancement of synapse formation is mediated, at least in part, by the release from astrocytes of cholesterol complexed to apolipoprotein-E-containing lipoproteins [66].

## Concluding remarks

There is increasing evidence that glia play a dynamic role in regulating synaptic transmission. Experiments conducted in both culture and intact-tissue preparations demonstrate that transmitters released from neurons can stimulate glia, leading to the release of glutama4te, ATP and other neuroactive substances from the glia. These gliotransmitters can feed back onto the presynaptic terminal to either enhance or depress the further release of neurotransmitter. Gliotransmitters released from glia can directly stimulate postsynaptic neurons as well, evoking either excitatory or inhibitory responses in these cells. These glial–neuronal interactions are summarized in Figure 4.

**Box 1. Key questions for future research**

- What mechanisms are responsible for the release of gliotransmitters, such as glutamate and ATP, from glia?
- Does the activity of neurons *in vivo* evoke increases in $Ca^{2+}$ concentration in glia at synapses? What patterns of neuronal activity induce glial activation?
- Do these glial increases in $Ca^{2+}$ concentration trigger $Ca^{2+}$ waves that propagate between glial cells?
- Do activated glia release gliotransmitters at synapses *in vivo*? Which gliotransmitters modulate the release of neurotransmitters from presynaptic terminals? Which gliotransmitters directly stimulate postsynaptic neurons?
- What role do glia play in information processing, learning and memory in the brain?

Despite recent advances, many important questions remain to be answered concerning glial regulation of synaptic transmission (Key questions for future research). Are glia activated by neurons *in vivo*, as *in situ* experiments suggest? Does the release of gliotransmitters from glia *in vivo* modulate the release of neurotransmitters at synapses and directly stimulate postsynaptic neurons, as it does in culture and in intact-tissue preparations? What role do glia play in information processing, learning and memory? These questions must be answered before we can fully understand the function of glia at the synapse.

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