

GLIA: LISTENING AND TALKING TO THE SYNAPSE

Philip G. Haydon

Glial cells are emerging from the background to become more prominent in our thinking about integration in the nervous system. Given that glial cells associated with synapses integrate neuronal inputs and can release transmitters that modulate synaptic activity, it is time to rethink our understanding of the wiring diagram of the nervous system. It is no longer appropriate to consider solely neuron–neuron connections; we also need to develop a view of the intricate web of active connections among glial cells, and between glia and neurons. Without such a view, it might be impossible to decode the language of the brain.

Once overlooked as merely supportive elements in the nervous system, glial cells have recently come to centre stage in our efforts to understand the workings of the brain. This remarkable change in the status of these non-neuronal cells has resulted largely because of the appreciation that glia can integrate neuronal inputs and modulate synaptic activity^{1–4}. Here, my goal is to describe some of the recent breakthroughs that have changed our appreciation of how glial cells function. I discuss the emerging view that calcium (Ca^{2+}) levels are an important signal in glial cells, and that this intracellular signal causes the release of the transmitter glutamate. Once glial cells release glutamate, neuronal excitability and synaptic transmission can be locally modulated. So, in many respects, glia are similar to neurons: they respond to transmitters, integrate inputs and have long-range signalling systems that can lead to the release of transmitters at a distance. But a profound difference between the two cell types is timescale: glial Ca^{2+} signals propagate at rates of micrometres per second, whereas action potentials propagate at rates of metres per second. Given the cross-talk between glia and neurons, I propose that it will be essential to identify and understand their reciprocal connectivity as a prerequisite to appreciating the relevance of glia to brain function.

There are two general classes of glia in the central nervous system: the macroglia class, which consists of astrocytes and oligodendrocytes, and the microglia class, which has a macrophage-like role. Oligodendrocytes

are responsible for myelination in the central nervous system, and can be considered as the central equivalent of Schwann cells, the myelinating cells in the peripheral nervous system. Astrocytes, the focus of this article, are highly numerous and are likely to have many divergent roles. Morphological inspection of the nervous system shows that astrocytes are in close association with neurons, can enwrap synaptic terminals^{5,6} (FIG. 1) and have extensive contacts with endothelial cells from capillaries. Moreover, astrocytes are interconnected with one another by gap junctions. Astrocytes are therefore positioned in such a way that they have the potential to be a pathway for signalling between neurons, between astrocytes and between neurons and capillaries.

Neuronal control of astrocytic Ca^{2+}
For much of the past century, glia were thought to be passive cells that had few responses to electrical stimulation or synaptic activity. This apparent absence of cellular activity was probably the consequence of the choice of techniques that were used to explore the nervous system. As it was recognized that electrical activity was the language of neurons, electrical stimulation and recording methods were used to probe glial activity. But except for a few examples, astrocytes (and glia in general) show limited changes in their membrane potential. One early exception came from the observation that neuronal activity can depolarize astrocytes⁷. This depolarization results from the accumulation of extracellular K^+ , which

*Department of Zoology and Genetics, Iowa State University, Ames, Iowa 50011, USA.
e-mail: pghaydon@iastate.edu*

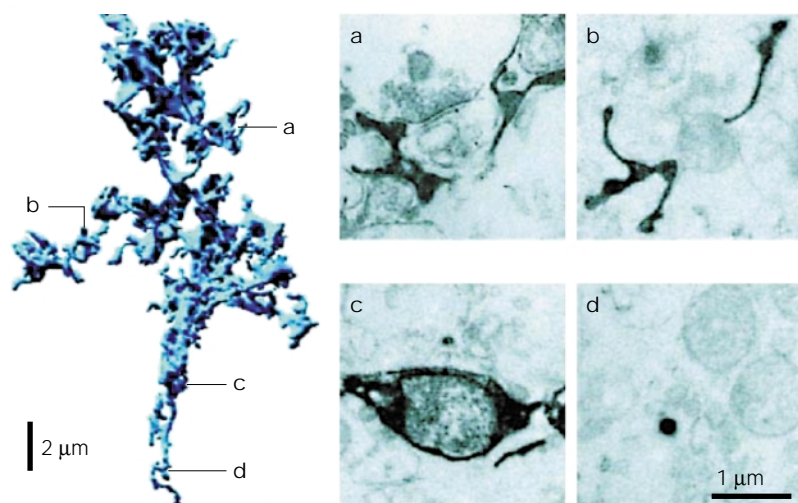


Figure 1 | **Glial cells make intimate contact with synaptic terminals.** A three-dimensional reconstruction of a portion of the arbour of a Bergmann glial cell is shown (left). Electron micrographs of four sections are shown on the right. The processes of the glial cell are black as a result of the photoconversion of injected dye. **a** | Glial process makes direct contact with a synapse. **b** | Glial compartment with no synaptic contact. **c** | Bulging glial structure containing a mitochondrion. **d** | The stalk of the glial appendage. (Modified with permission from REF. 6 © (1999) Macmillan Magazines Ltd.)

is thought to be siphoned away by astrocytes that express a high density of K^+ channels. More recently, oligodendrocyte precursors have been shown to receive direct synaptic inputs from glutamate-releasing neurons⁸.

The breakthrough discoveries that showed that astrocytes are dynamic and can respond to neuronal inputs came as the result of a series of technical advances. The development of ion-sensitive fluorescent indicators, such as fura-2 (REF. 9), together with the development of quantitative imaging techniques, patch-clamp recording from brain slices and confocal microscopy, provided the necessary tools to re-examine astrocyte responses. Studies in cell culture first, and later in brain slices, showed that transmitters that are released from neurons induce transient elevations of internal Ca^{2+} levels in astrocytes. For example, addition of glutamate to cultures of astrocytes¹⁰⁻¹³ evoked sustained or oscillating elevations of their internal Ca^{2+} level. Similarly, stimulation of premyelinated axons causes a Ca^{2+} elevation in cultured Schwann cells due to ATP that is released from the neuron¹⁴. Furthermore, glutamate-stimulated Ca^{2+} elevations spread from one astrocyte to another, indicating that Ca^{2+} signalling between astrocytes might form the basis of a long-range signalling pathway in the nervous system¹⁰.

Several studies done using brain slices have now shown that this Ca^{2+} -signalling pathway is present in nervous tissue, not just in cell culture. Initially, studies on organotypic hippocampal slices and later in acutely isolated hippocampus showed that axonal stimulation causes a glutamate-receptor-dependent increase in astrocytic Ca^{2+} (REFS 15,16) (FIG. 2), which might be mediated by the glutamate-receptor-dependent production of PROSTAGLANDINS¹⁷. Additionally, activation of axons in the cerebellum leads to elevations of the Ca^{2+} levels of

BERGMANN GLIA^{6,18}. So, the ability of neurotransmitters to mobilize Ca^{2+} levels in astrocytes is not just a property of cell culture, but is present in acutely isolated brain-slice preparations, indicating that it might be a physiologically relevant signalling pathway.

It is now clear that astrocytes respond to a variety of synaptically released transmitters in addition to glutamate. For example, noradrenaline^{18,19}, histamine²⁰, acetylcholine²⁰, ATP²¹⁻²³ and GABA (γ -aminobutyric acid)²⁴ can induce elevations of astrocytic Ca^{2+} . Indeed, the list of transmitters that can mobilize astrocytic Ca^{2+} is almost as long as the list of molecules that activate neuronal receptors²⁵⁻²⁷.

Long-range Ca^{2+} signalling between astrocytes
The initial observations of Cornell Bell *et al.*¹⁰ not only revealed glutamate-induced Ca^{2+} elevations in astrocytes but also pointed to the possibility that Ca^{2+} signals could spread between astrocytes in the form of Ca^{2+} waves. This possibility was supported by studies in which mechanical stimuli were delivered to single astrocytes in cell culture. Mechanical stimulation of an astrocyte caused a local elevation of astrocytic Ca^{2+} that subsequently spread to its neighbours in the form of a wave of

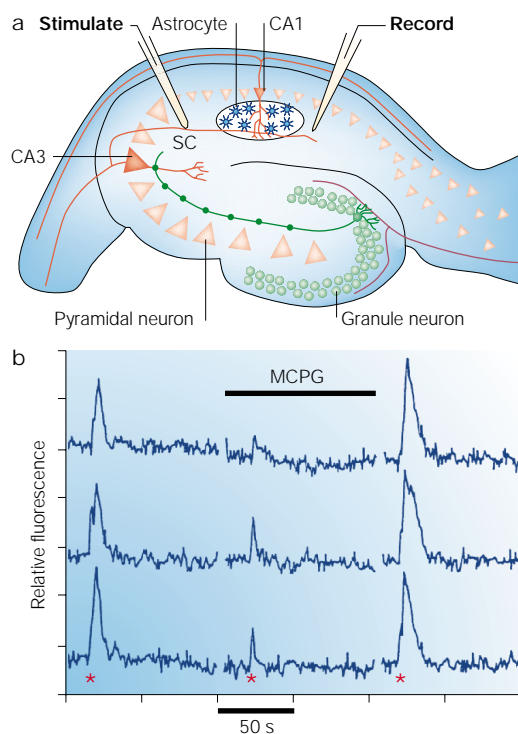


Figure 2 | **Neuronal stimulation causes a neurotransmitter-dependent elevation of astrocytic Ca^{2+} .** **a** | Representation of the hippocampus. A stimulating electrode was placed in the SCHAFFER COLLATERAL pathway (SC) to stimulate action potentials. **b** | Fluorescence imaging of the Ca^{2+} levels of astrocytes in area CA1 of the hippocampus revealed that SC stimulation (red stars) evoked Ca^{2+} elevations in individual astrocytes that were suppressed by the metabotropic glutamate receptor antagonist α -methyl-4-carboxyphenylglycine (MCPG). (Modified with permission from REF. 15 © (1996) Society for Neuroscience.)

PROSTAGLANDINS
Biologically active metabolites of arachidonic acid and other lipids. Prostaglandins have many functions; for example, they are involved in vasodilation, bronchodilation, inflammatory reactions and the regulation of cell proliferation.

BERGMANN GLIA
The main glial cell type present in the cerebellum.

SCHAFFER COLLATERALS
Axons of the CA3 pyramidal cells of the hippocampus that form synapses with the apical dendrites of CA1 neurons.

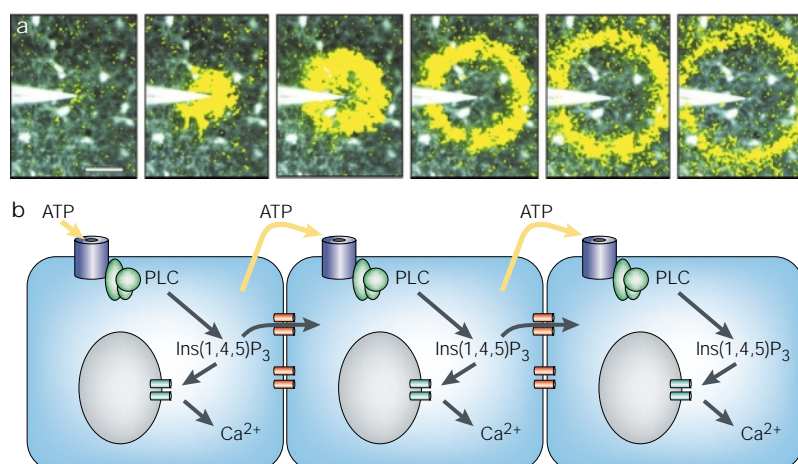


Figure 3 | Stimulation of glial cells from the retina evokes a radially propagating wave of elevated calcium. **a** | Mechanical stimulation of a glial cell in the centre of the field of view evoked a local elevation of Ca^{2+} . Subsequently, this Ca^{2+} elevation propagated to neighbouring cells. In this sequence, images were acquired at 0.93-s intervals and the yellow overlay image shows the leading edge of the wavefront. **b** | Putative mechanism for glial Ca^{2+} wave generation. Ca^{2+} is released from internal stores in response to elevated internal inositol-1,4,5-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$). $\text{Ins}(1,4,5)\text{P}_3$ can diffuse to neighbouring cells through gap junctions to cause short-range signalling. Longer-range calcium signalling requires the release of ATP, which causes the regenerative production of $\text{Ins}(1,4,5)\text{P}_3$ and further release of ATP from neighbouring astrocytes. (Panel **a** is modified with permission from REF. 30. © (1997) American Association for the advancement of Science)

Movies online

MÜLLER GLIA

The main glial cell type present in the retina.

GAP JUNCTIONS

Cellular specializations that allow the passage of small molecules between the cytoplasm of adjacent cells. They are formed by channels termed connexons, multimeric complexes of proteins known as connexins.

CAGED $\text{Ins}(1,4,5)\text{P}_3$

In general terms, a caged molecule is a labile derivative of a biologically active molecule that will break down after appropriate (commonly luminous) stimulation to yield the bioactive compound.

LUCIFERIN–LUCIFERASE ASSAY

Method to detect the presence of ATP, which is based on the ability of the firefly enzyme luciferase to catalyse a reaction between its substrate luciferin and ATP and release the two terminal phosphate groups of ATP. Luciferin becomes excited during the process but, on return to its basal state, it releases energy in the form of light.

elevated Ca^{2+} (REFS 22,28,29) that propagated at a rate of about $20 \mu\text{m s}^{-1}$. Although the mechanical stimulus is not physiological, it permits focal stimulation at a controlled location and time, and it reproduces the waves of Ca^{2+} increases originally identified in studies in which glutamate was applied to cultures^{10,11}. Moreover, Ca^{2+} waves have also been detected in more intact tissue. Ca^{2+} -imaging studies in the acutely isolated retina have shown that waves of elevated Ca^{2+} can spread between retinal astrocytes and MÜLLER GLIA³⁰ (FIG. 3). Although such Ca^{2+} waves can be detected in the retina and in organotypic cultures of the hippocampus^{30,31}, it is not yet certain whether they exist in acutely isolated brain tissue.

The events underlying astrocytic Ca^{2+} wave propagation have been the focus of several studies, which have led to some general conclusions about its possible cellular mechanisms. Ca^{2+} elevations in astrocytes result from the release of Ca^{2+} from intracellular stores, which are activated by elevations of the second messenger inositol-1,4,5-trisphosphate ($\text{Ins}(1,4,5)\text{P}_3$). This messenger is generated as a consequence of the activity of phospholipase C (PLC), which is in turn activated by certain G-protein-coupled receptors. Although it is clear that Ca^{2+} release from $\text{Ins}(1,4,5)\text{P}_3$ -regulated intracellular Ca^{2+} stores causes the elevation of Ca^{2+} , there has been considerable debate about the mechanism by which the signal can spread to neighbouring astrocytes. Two prominent hypotheses have emerged, and it is likely that both can account for different components of the intercellular spread of the Ca^{2+} wave.

Initially, Ca^{2+} waves were thought to spread as a result of gap-junction-mediated metabolic coupling between astrocytes^{32–34}. According to this hypothesis, $\text{Ins}(1,4,5)\text{P}_3$

diffuses between astrocytes through GAP JUNCTIONS to stimulate the release of Ca^{2+} from internal stores of neighbouring cells and give rise to an intercellular wave of Ca^{2+} (REFS 33–36). In support of this possibility, C6 glioma cells, which express few gap junctions, do not show Ca^{2+} waves unless connexins are artificially expressed³⁴. $\text{Ins}(1,4,5)\text{P}_3$ is likely to diffuse between astrocytes, as indicated by experiments using CAGED $\text{Ins}(1,4,5)\text{P}_3$. Injection of this molecule and its subsequent focal photolysis liberates $\text{Ins}(1,4,5)\text{P}_3$ and evokes a Ca^{2+} wave. This result was also observed when photolysis was elicited in non-injected cells adjacent to those that were injected with the caged compound³⁰. However, modelling studies have indicated that passive diffusion of $\text{Ins}(1,4,5)\text{P}_3$ would not be sufficient by itself to account for the spread of the Ca^{2+} wave. Therefore, some form of regeneration of the source of $\text{Ins}(1,4,5)\text{P}_3$ would be required to explain it^{35,37}. Although the partially regenerative nature of the signal could involve the Ca^{2+} -dependent activation of PLC, an alternative possibility is that an extracellular signal causes the receptor-dependent activation of PLC.

Studies during the past few years have supported the involvement of an extracellular component to the intercellular Ca^{2+} wave. Enkvist and McCarthy³⁸, and Hassinger *et al.*²⁸ showed that a wave of elevated intracellular Ca^{2+} can ‘jump’ the gap between two groups of astrocytes that are separated by cell-free lanes. Ca^{2+} waves can frequently pass between disconnected cells as long as the gap between them does not exceed $\sim 120 \mu\text{m}$ (REF. 28). Such an observation clearly supports the presence of an extracellular signal that coordinates the intercellular propagation of the Ca^{2+} wave. Further support of this idea is provided by the observation that Ca^{2+} waves can follow the direction of extracellular perfusion^{28,39}. Additionally, extracellular medium collected from cells that are participating in Ca^{2+} waves can induce Ca^{2+} elevations in others²².

The extracellular message involved in wave propagation is likely to be ATP. Addition of pharmacological agents that either degrade ATP or block purine receptors reduces or abolishes inter-astrocytic Ca^{2+} waves^{22,23}. Expression of the metabotropic ATP receptor subtype P2Y_1 in an astrocytoma cell line permitted the propagation of intercellular Ca^{2+} waves⁴⁰. Chemiluminescence studies using the LUCIFERIN–LUCIFERASE ASSAY to detect extracellular ATP have shown the appearance of extracellular ATP during Ca^{2+} waves^{22,23}, and an imaging study has shown waves of extracellular ATP during astrocytic Ca^{2+} waves²¹. Eric Newman has recently added to these data by showing that the extracellular pathway of Ca^{2+} wave propagation and ATP release is used by glia of the retina⁴¹. It must be kept in mind, however, that the mechanism of ATP release is likely to be distinct from that mediating glutamate release. Initial studies indicate that although ATP release is dependent on PLC, Ca^{2+} is neither necessary nor sufficient to evoke it^{21,42}.

Therefore, compelling data support the idea that gap junctions and ATP release are important for Ca^{2+} wave propagation. This conclusion is not as contradictory as it might look at first sight. Cotrina *et al.*²³ did elegant experiments that help to bring together these apparently

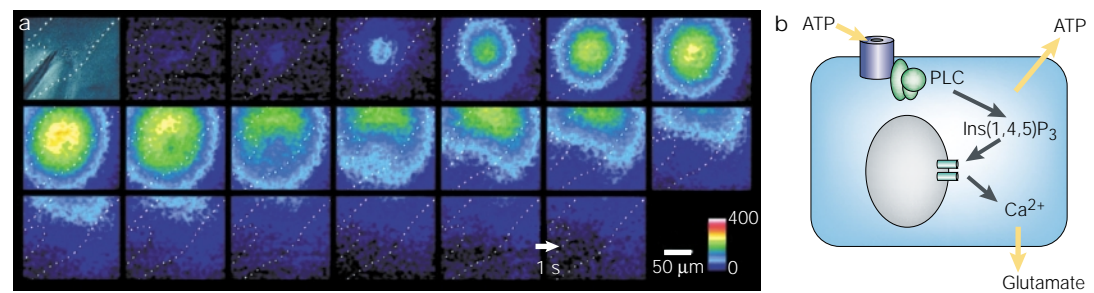


Figure 4 | **Astrocytic calcium waves cause the calcium-dependent release of glutamate.** **a** | The extracellular fluorescence of NADH is monitored as an indicator of released glutamate. Stimulation of a Ca^{2+} wave causes the radial release of glutamate (linear pseudocolour image presentation). **b** | The likely signalling pathway involved in glutamate release. Note that although ATP release depends on phospholipase C (PLC), it is Ca^{2+} -independent, in contrast to the mechanism of glutamate release. (Panel **a** is modified with permission from REF. 55 © (2000) Society for Neuroscience.) (Ins (1,4,5) P_3 , inositol-1,4,5-triphosphate.)

[Movie online](#)

conflicting results. They used cells that normally have low levels of connexins to test whether ATP release was augmented after forced expression of connexins. ATP release induced by UTP (a purine-receptor agonist that is not detected by ATP assays) increased 5–15-fold after expression of connexins, which implies that expression of connexins causes an upregulation of the ATP release pathway. It is not likely that this is a direct relationship, however, because the addition of connexin inhibitors did not block the ATP-release-dependent Ca^{2+} waves.

So, it is now thought that ATP release is an important component of long-range Ca^{2+} signalling. However, shorter-range Ca^{2+} signalling might still be mediated by metabolic coupling through gap junctions. Indeed, several observations support this idea. For example, although the flow of extracellular perfusion can control the direction of the Ca^{2+} wave (see above), a small component of the wave propagated in the opposite direction³⁹. Additionally, several studies have shown that purine receptor antagonists do not fully block the Ca^{2+} wave^{43–45}. It is likely that this smaller short-range Ca^{2+} signal results from the diffusion of $\text{Ins}(1,4,5)\text{P}_3$ through gap junctions. Of particular interest, the exposure of human astrocytic cultures to INTERLEUKIN-1 β (IL-1 β) enhances ATP-dependent Ca^{2+} signalling by increasing messenger RNA levels for the **P2Y₂** receptor subunit and by downregulating the expression of **connexin 43** (REF. 45).

There seems to be a tight regulation of the gap-junction- and ATP-mediated signalling pathways. Not only does IL-1 β (REF. 45) modify these two pathways of Ca^{2+} signalling in a coordinated manner, but also other molecules such as ENDOTHELIN⁴⁶, glutamate^{47,48} and ANANDAMIDE⁴⁹ can control gap-junction-mediated communication between astrocytes. It was recently shown that the formation of synaptic connections by striatal neurons and the associated increase in synaptic activity upregulate gap junctions and augment Ca^{2+} waves between astrocytes⁴⁸. This is particularly important if one considers that glia can also regulate the formation of synapses^{50,51}. So a picture is emerging in which astrocytes can regulate synaptogenesis, and the resulting synaptic activity provides feedback regulation of the formation of inter-astrocytic connectivity³².

Astrocytes release chemical transmitters

Given that neuronal activity and transmitters can evoke elevations of astrocytic Ca^{2+} , it is pertinent to ask what is the consequence of such Ca^{2+} elevations and the associated Ca^{2+} waves that propagate between these glial cells. The analysis of superfusates from purified astrocytic cultures has shown that ligands that evoke Ca^{2+} elevations can cause the release of the excitatory amino acids glutamate and aspartate^{17,53,54}. The release of these transmitters, which I shall call chemical transmitters instead of neurotransmitters to emphasize the fact that they are also released by glia, is Ca^{2+} -dependent, as Ca^{2+} elevations are both necessary and sufficient to evoke glutamate release. The results of an experiment⁵⁵ in which the release of glutamate from astrocytes was visualized using an enzymatic assay are shown in FIG. 4. In the presence of the cofactor NAD^+ and the enzyme glutamate dehydrogenase in the bathing solution, glutamate released from astrocytes is converted to α -ketoglutarate and NAD^+ is reduced to NADH. As NADH is fluorescent, it is possible to image the presence of glutamate using fluorescence microscopy. A wave of extracellular NADH that results from the release of glutamate during a radially propagating Ca^{2+} wave⁵⁵ is shown in FIG. 4a.

Although Ca^{2+} -dependent glutamate release has been repeatedly studied with purified astrocytes in cell culture, it is important to ask whether this property is present in astrocytes in normal brain tissue. This is an extremely challenging task, as tissue taken from the brain will also contain synaptic terminals that release the same chemical transmitter. However, acute treatment of hippocampal slices with TETANUS TOXIN^{56–58} selectively blocks the release of glutamate from synapses because astrocytes contain few receptors for the toxin^{59–61}. After this treatment, pharmacological stimuli that mobilize Ca^{2+} in astrocytes elicit glutamate release from the slice¹⁷. This glutamate presumably arises from the astrocytes that were unaffected by the short-term toxin treatment.

In addition to ATP and glutamate, D-serine has been recognized as a molecule that can be released from astrocytes. D-serine, an endogenous ligand for the glycine-binding site of the NMDA (*N*-methyl-D-aspartate) type of glutamate receptor, is synthesized within and released

INTERLEUKIN-1 β
Signalling molecule involved in the inflammatory response that can act as an endogenous pyrogen.

ENDOTHELIN
Molecule with potent vasoconstrictor activity. It is expressed by vascular cells, as well as in brain, kidney and lung.

ANANDAMIDE
An endogenous agonist of cannabinoid receptors.

TETANUS TOXIN
Protein derived from *Clostridium tetani* that can block transmitter release owing to its ability to degrade synaptobrevin. Tetanus toxin is the causative agent of tetanus.

from astrocytes^{62,63}. As selective degradation of D-serine attenuates NMDA-receptor-mediated synaptic transmission⁶⁴, D-serine is probably another important glial-cell-derived signal that regulates certain aspects of synaptic transmission. It is not yet clear whether D-serine release is dependent on Ca²⁺.

Mechanism of glutamate release from astrocytes
A mechanistic understanding of the process that leads to release of the chemical transmitter glutamate from astrocytes has not been achieved. Three prominent mechanisms have been proposed to mediate the release of glutamate from astrocytes — reverse operation of glutamate transporters, an anion-channel-dependent pathway induced by swelling and Ca²⁺-dependent exocytosis⁶⁵ — and significant evidence points towards the involvement of vesicle fusion. On the one hand, glutamate transport inhibitors do not suppress the Ca²⁺-induced release pathway^{17,53} and agonists that induce glutamate release do not cause swelling of astrocytes^{17,66}. On the other, glutamate release from astrocytes is Ca²⁺-dependent, astrocytes express a plethora of proteins normally associated with synaptic transmission^{67–69} and injection of clostridial toxins into astrocytes can block the Ca²⁺-dependent glutamate release^{17,70}. Although these data support the hypothesis that glutamate release is mediated by exocytosis, it is still necessary to determine whether glutamate is stored in and released from vesicles in response to elevated Ca²⁺. Additionally, even if glutamate turns out to be released from vesicles, it does not necessarily follow that ATP or D-serine will be released by a similar mechanism.

Glial glutamate modulates neighbouring neurons
Astrocytes can release glutamate and ATP, and these glial cells are intimately associated with synapses^{5,6}. It is therefore tempting to speculate that glia could locally modulate the synapse through the release of the chemical transmitter glutamate. Indeed, my group has recently suggested that synapses and their associated glia should be considered as tripartite synapses. In our model, the glial cells receive signals from the synapse and integrate this information in the form of Ca²⁺ elevations, which stimulate the release of glutamate and modulate synaptic transmission in a feedback manner⁴. Significant evidence from *in vitro* studies, from brain slice preparations and from acutely isolated tissue now supports this idea.

Early cell-culture studies showed that astrocytes can release glutamate and cause an NMDA-receptor-dependent increase in the Ca²⁺ level of neighbouring neurons^{53,60,71}. These studies were the first to show the potential for chemical-transmitter-mediated astrocyte–neuron signalling^{3,65}. However, it was only in 1997 that a study using a non-cell-culture system showed that this signalling pathway could function in acutely isolated brain slices⁷². A metabotropic glutamate receptor agonist (*trans*-1-amino-1,3-cyclopentanedicarboxylic acid) caused an initial elevation of Ca²⁺ in astrocytes, which was followed by a delayed elevation in neuronal Ca²⁺ levels. This delayed signalling was mediated by glutamate;

antagonists of ionotropic glutamate receptors blocked the neuronal Ca²⁺ elevation, but not the astrocytic Ca²⁺ response. Together with the cell culture experiments mentioned above^{53,60,71}, this study provided the first compelling indication that astrocytes can regulate neurons *in situ* through the release of chemical transmitters.

Glia modulate synaptic transmission

After it was clearly shown that astrocytes can modulate neurons and that physiological astrocytic Ca²⁺ levels are responsible for this modulation¹, electrophysiological and imaging studies have been done to ask whether this modulatory pathway could control the strength of synaptic transmission. When a wave of elevated internal Ca²⁺ was stimulated in an underlying carpet of astrocytes, synaptic transmission between pairs of hippocampal neurons was shown to be reversibly depressed in amplitude⁶¹. This depression of synaptic transmission was due to glutamate released from astrocytes during the Ca²⁺ wave, because antagonists of metabotropic glutamate receptors impaired the ability of astrocytes to modulate synaptic transmission⁶¹.

Using the frog neuromuscular junction, Richard Robitaille⁷³ did extremely difficult experiments that provided a clear demonstration that synaptically associated glia can integrate neuronal inputs and cause feedback modulation of the synapse (in this case, the neuromuscular junction). At the neuromuscular junction, a synaptically associated glial cell termed the perisynaptic Schwann cell (PSC) envelops the presynaptic terminal. PSCs are non-myelinating Schwann cells that regulate the development⁷⁴ and the physiological properties of synaptic connections⁷³.

Stimulation of axons to induce synaptic activity causes a frequency-dependent increase in the internal Ca²⁺ level of PSCs^{75,76}. This Ca²⁺ response requires synaptic activity and can similarly be induced by addition of acetylcholine and its co-transmitter ATP^{75,77}, molecules that are released together from the nerve terminal. Astrocytes and cultured Schwann cells^{17,53,78,79} can release glutamate in response to elevated Ca²⁺, but does the elevation of PSC Ca²⁺ levels cause a similar release of chemical transmitter and modulate synaptic function? To answer this question, Robitaille⁷³ microinjected an activator of GTP-binding proteins directly into a single PSC while focally recording the underlying neuromuscular transmission (FIG. 5). Microinjection of GTPγS, a guanine-nucleotide analogue that activates GTP-binding proteins, caused a considerable depression of transmission from nerve to muscle. Furthermore, the injection of the GTP-binding-protein inhibitor GDPβS into PSCs relieved the activity-dependent depression of synaptic transmission (FIG. 5d, e). This reduction in transmission was once thought to be an intrinsic property of the synapse. As the inactivation of GTP-binding proteins in the PSC reduced this depression, we must conclude that the frog neuromuscular junction acts as a tripartite synapse — elevated synaptic activity increases PSC internal Ca²⁺ levels, which send a feedback signal that depresses the amplitude of synaptic transmission.

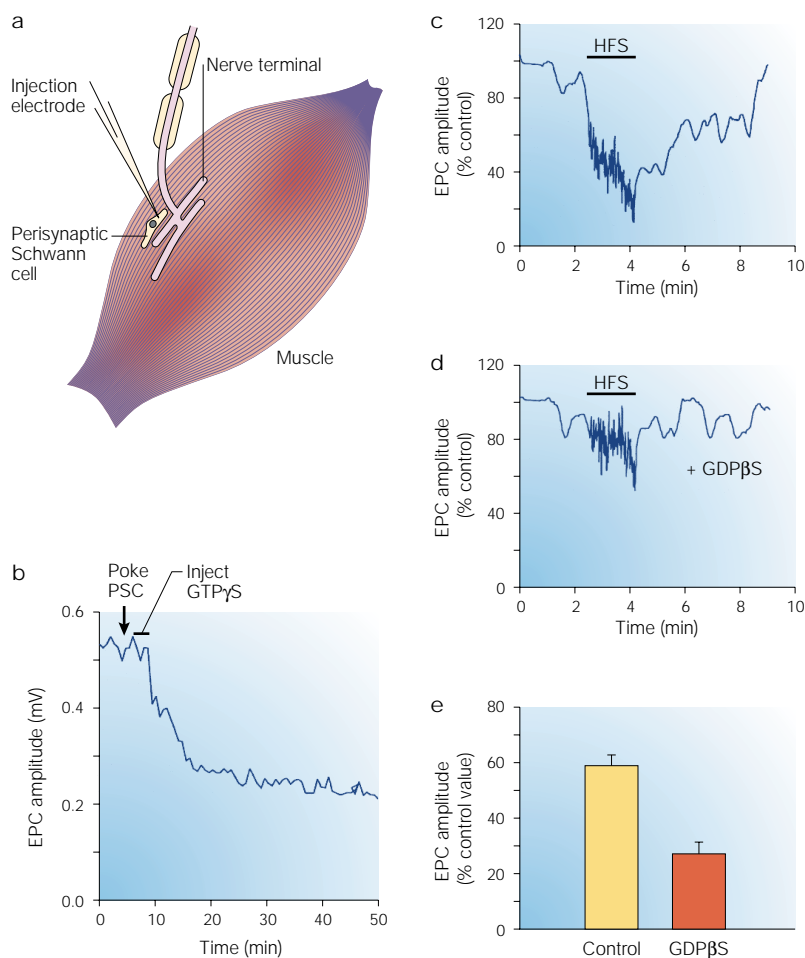


Figure 5 | G-protein-mediated signalling in synaptically associated perisynaptic Schwann cells depresses neuromuscular transmission. **a** | Representation of the tripartite synapse at the neuromuscular junction. **b** | Microinjection of the G-protein activator GTP γ S into perisynaptic Schwann cells (PSCs) that overlie nerve terminals causes a reduction in the amplitude of nerve to muscle transmission. **c** | A plot of the depression of transmission that occurs during high frequency neuronal stimulation (HFS) under control conditions. **d** | Depression of the connection is reduced after injection of the G-protein inhibitor GDP β S into a PSC (compare with **c**). **e** | Summary of experiments similar to those shown in **c** and **d**. (EPC, endplate current.) (Panels **b**–**e** are modified with permission from REE, 73 © (1998) Elsevier Science.)

Glial Ca²⁺ waves can modulate the response of the retina to optical stimulation. Newman and Zahs⁸⁰ elicited a wave of elevated Ca²⁺ between retinal glia while recording ganglion cell activity induced by photoreceptor activation. When the wave of elevated Ca²⁺ invaded the region of the retina where the recordings were made, the output of the ganglion cell was modified; the ganglion cell was either inhibited or excited. Addition of glutamate receptor antagonists, or GABA and glycine receptor antagonists, blocked the inhibitory effect of the glial Ca²⁺ wave. The interpretation of these data is complex. One tentative explanation is that, as elevated Ca²⁺ causes glutamate release from glia^{1,53}, the glial Ca²⁺ wave induces a glutamate-dependent excitation of the GABA interneurons that inhibit ganglion cell activity. But irrespective of the exact mechanism, this study provides a clear example of the ability of glia to modulate neuronal integration within the retina.

Astrocytic transmitter and synaptic potentiation
Astrocytes surround many of the synaptic connections in the central nervous system. In the hippocampus, 57% of the synapses are associated with the process of an astrocyte⁵. The possible roles for this intimate association are not clear. Although it is certain that astrocytes are responsible for transmitter uptake from the synaptic cleft^{81,82}, it is also possible that they release glutamate to modulate synaptic transmission. Maiken Nedergaard's group has obtained compelling evidence to support this possibility in hippocampal slices²⁴. This study focused on the activity-dependent potentiation of inhibitory synaptic transmission from GABA interneurons to pyramidal cells and the surprising observation that a glutamate receptor antagonist blocked the augmentation of this inhibitory connection. It therefore seems that this form of plasticity is not homosynaptic and, as the glutamate terminals of the pyramidal cells are unlikely to be the source of glutamate, it is necessary to consider that a third cell capable of releasing this chemical transmitter is involved in the synaptic modification.

The study by Kang *et al.* indicates that the third cell might be an astrocyte²⁴. The stimulus that elicited the plastic change increased the Ca²⁺ levels in astrocytes that were located adjacent to the stimulated interneuron. Pharmacological studies indicated that this interneuron-induced Ca²⁺ response of the astrocyte is direct, as antagonists of GABA_B receptors prevented the rise in astrocytic Ca²⁺ and suppressed synaptic potentiation, whereas GABA_B receptor agonists mimicked the action of neuronal stimulation. So, activity of the interneurons that is sufficient to cause synaptic plasticity recruits astrocytes through a GABA_B-receptor-dependent pathway and blockade of these receptors prevents the plastic change. Furthermore, to test whether the astrocyte was sufficient to induce the synaptic change, the authors elevated the Ca²⁺ levels of the astrocyte independent of neuronal stimulation by obtaining direct patch-clamp recordings from individual astrocytes. This experimental approach selectively raised Ca²⁺ in astrocytes but not in neurons. Following elevation of astrocytic Ca²⁺, baseline GABA-mediated transmission from interneurons to pyramidal neurons was augmented. Furthermore, this Ca²⁺-dependent astrocyte-induced synaptic augmentation was prevented by addition of a glutamate-receptor antagonist AP-5. The astrocyte can therefore augment inhibitory synaptic transmission through a mechanism that is consistent with the Ca²⁺-dependent release of the chemical transmitter glutamate, which then acts on neuronal NMDA receptors.

The putative circuitry that modulates the augmentation of GABA-mediated transmission is shown in FIG. 6. GABA release not only causes synaptic responses in the postsynaptic neuron, but can also raise the Ca²⁺ level of the synaptically associated astrocyte. As a consequence, a Ca²⁺-dependent glutamate release cascade is initiated, which can lead to the activation of neuronal receptors and cause synaptic modification. The location of these glutamate receptors is not certain, but they are potentially located on both the pre- and postsynaptic terminals⁸³.

As a single astrocyte makes contact with many different synapses, the spread of a Ca^{2+} signal within the astrocyte will affect several synapses that are not directly associated but are indirectly connected through the intermediary astrocyte. So, if it were possible to control the extent of spread of a Ca^{2+} signal within one astrocyte, this would significantly affect the number of synapses that could be regulated. Consistent with this idea, imaging studies in the cerebellum have shown that different regions of Bergmann glia can act as independent functional domains⁶. Microdomains of the arbours of these glia can show localized Ca^{2+} elevations that do not propagate through the rest of the cell. However, stimulation of the PARALLEL FIBRES can evoke a coordinated elevation of Ca^{2+} within the whole glial cell. If astrocytes similarly show localized and global Ca^{2+} signals, then it is feasible that the local Ca^{2+} signal will cause only localized synaptic modification (FIG. 6a), whereas global changes in Ca^{2+} might lead to lateral signalling between distant synapses (FIG. 6b).

Gap-junction-mediated glia–neuron coupling
Much of the preceding discussion has focused on the role of chemical transmitters in mediating astrocyte–neuron signalling. However, there is accumulating evidence for a gap-junction-mediated form of communication between glia and neurons. Early cell-culture studies showed dynamic signalling from astrocytes to neurons and indicated that gap junctions might mediate this form of communication⁸⁴. Two recent reports have provided direct support for the existence of this pathway. Electrophysiological and dye-transfer studies have shown that communication through gap junctions can be detected between cultured astrocytes and neurons⁸⁵. In the LOCUS COERULEUS, noradrenaline neurons are coupled to one another and to neighbouring glial cells by gap junctions⁸⁶. These glia, which are thought to be astrocytes, are weakly connected to the neurons but are likely to provide sufficient electric charge to influence the activity of the neurons. Indeed, experimental stimuli that can depolarize the glial cell induced a secondary increase in the frequency of the neuronal activity. Consequently, gap-junction-dependent communication among astrocytes might serve to coordinate the activity of neuronal populations. Although it is unlikely that glia will regulate neurons through changes in their own membrane potential, inputs that modulate either the gap-junction conductance or other glial conductances will undoubtedly change the electrical load placed on the neurons. As glial cells frequently adopt a more negative membrane potential than neurons (although see REF. 87), a reduction of gap-junction-mediated communication should permit neurons to depolarize and increase the frequency of action-potential discharge as they escape from the negative influence of the glial cells.

In the cerebellum, noradrenergic inputs that are probably derived from the locus coeruleus regulate the Ca^{2+} levels of Bergmann glia¹⁸. In the hippocampus, noradrenaline is known to regulate astrocytic Ca^{2+} levels¹⁹ and electron microscopy has revealed putative noradrenaline terminals in direct association with

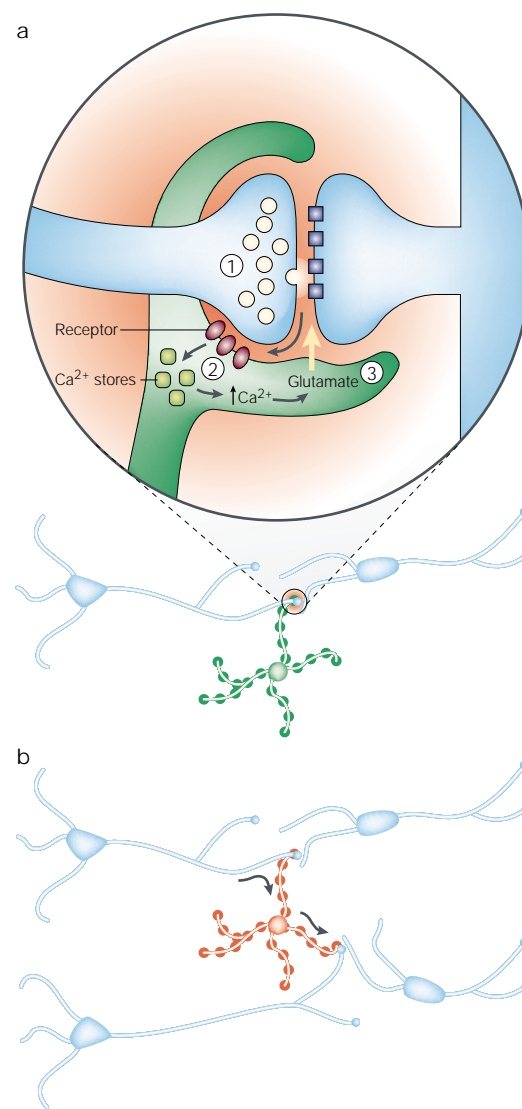


Figure 6 | Intercellular signalling between neurons and astrocytes can have a local modulatory action, as well as participate in the communication between distant synapses. **a** | Representation of a tripartite synapse in which the process of an astrocyte wraps around a synaptic connection. Local signalling between neurons and astrocytes has the potential to modulate the synapse. For example, synaptic activity (1) not only elicits postsynaptic potentials but GABA (γ -aminobutyric acid)²⁴ or glutamate¹⁵ can act on astrocytic receptors and trigger Ins(1,4,5) P_3 -dependent Ca^{2+} release from internal stores to increase astrocytic Ca^{2+} levels (2). Elevated astrocytic Ca^{2+} (3) evokes the local release of the chemical transmitter glutamate, which can modulate the synapse. **b** | Under conditions where neuronal activity causes a global Ca^{2+} elevation in the synaptically associated astrocyte, lateral signalling between synapses could occur through the intermediary astrocyte and its Ca^{2+} -dependent glutamate release pathway.

astrocytic processes^{88,89}. It is tempting to speculate that the regulation of glia–neuron gap-junction-mediated connectivity would control the release of noradrenaline in the hippocampus and cerebellum by controlling neuronal firing frequency at the locus coeruleus. As a consequence, this regulation would also control the

PARALLEL FIBRES

The axons of cerebellar granule cells. Parallel fibres emerge from the molecular layer of the cerebellar cortex towards the periphery, where they extend branches perpendicular to the main axis of the Purkinje neurons and form the so-called *en passant* synapses with this cell type.

LOCUS COERULEUS

Nucleus of the brainstem. The main provider of noradrenaline to the brain.

Ca²⁺-dependent release of glutamate from astrocytes. So, the electrical and chemical connections from astrocytes to neurons are likely to modulate neuronal activity and synaptic transmission significantly.

Concluding remarks

The studies that I have reviewed here prompt us to look at the nervous system from a different perspective. Although it is clear that neurons are essential to nervous system function, it is time to acknowledge that glial cells are dynamic signalling components with the potential to modulate neuronal action on a slow timescale. However, it is difficult to imagine brain function if it were based on astrocytic signalling on a timescale that is slower than the action potential by six orders of magnitude. So, what active functions do astrocytes have? Consider the brain to be like a theatrical production. Although the actors (neurons) take centre stage, the play is nothing without the stagehands, the director and so on (the glial cells). The more technically sophisticated the performance, the greater the number of backstage people needed. Perhaps it is no coincidence that the ratio of glia to neurons increases through phylogeny. For example, the nematode *Caenorhabditis elegans* has about one glial cell for every five neurons⁹⁰, whereas the ratio in the human brain is thought to be at least ten glia per neuron.

We have come a long way in our appreciation of potential dynamic roles for glial cells but we are only beginning to scratch the surface in our understanding of their functions. A challenge in the field is to develop the necessary tools to allow the selective manipulation of astrocytes within the nervous system. For neurons, this has been relatively simple, as they can be stimulated electrically. The electrically unexcitable glia, however, are less tractable. We therefore need to develop selective stimulation approaches to activate glial cells within the nervous system. This will undoubtedly require a combination of optical approaches to control focally Ca²⁺ levels within astrocytes, as well as new imaging methods to detect neuronal and glia activity *in vivo*. It will also be essential to harness the power of molecular genetics to perturb the intrinsic glial signalling pathways by regulated expression of molecules within astrocytes, and to determine the consequences for behaviour, learning, memory and synaptic transmission. This combination of methods will be essential to advance from our current knowledge that glia are involved in signalling to identifying the roles of glia–neuron connections in higher brain function.

 Links

DATABASE LINKS [PLC](#) | [P2Y₁](#) | [IL-1β](#) | [P2Y₂](#) | [connexin 43](#)
 FURTHER INFORMATION [Haydon lab](#)

- Parpura, V. & Haydon, P. G. Physiological astrocytic calcium levels stimulate glutamate release to modulate adjacent neurons. *Proc. Natl Acad. Sci. USA* **97**, 8629–8634 (2000).
By using quantitative imaging and photorelease of Ca²⁺, this study showed that physiological levels of Ca²⁺ evoke sufficient release of glutamate from astrocytes to modulate neurons.
- LoTurco, J. J. Neural circuits in the 21st century: synaptic networks of neurons and glia. *Proc. Natl Acad. Sci. USA* **97**, 8196–8197 (2000).
- Smith, S. J. Neural signalling. Neuromodulatory astrocytes. *Curr. Biol.* **4**, 807–810 (1994).
- Araque, A., Parpura, V., Sanzgiri, R. P. & Haydon, P. G. Tripartite synapses: glia, the unacknowledged partner. *Trends Neurosci.* **22**, 208–215 (1999).
- Ventura, R. & Harris, K. M. Three-dimensional relationships between hippocampal synapses and astrocytes. *J. Neurosci.* **19**, 6897–6906 (1999).
- Grosche, J. *et al.* Microdomains for neuron–glia interaction: parallel fiber signaling to Bergmann glial cells. *Nature Neurosci.* **2**, 139–143 (1999).
Subregions or microdomains of a single glial cell can show Ca²⁺ signals independent of the rest of the cell. This indicates that distinct regions of a glial cell could integrate information.
- Orkand, R. K., Nicholls, J. G. & Kuffler, S. W. Effect of nerve impulses on the membrane potential of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* **29**, 788–806 (1966).
- Bergles, D. E., Roberts, J. D., Somogyi, P. & Jahr, C. E. Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. *Nature* **405**, 187–191 (2000).
- Grynkiewicz, G., Poenie, M. & Tsien, R. Y. A new generation of Ca²⁺ indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260**, 3440–3450 (1985).
- Cornell Bell, A. H., Finkbeiner, S. M., Cooper, M. S. & Smith, S. J. Glutamate induces calcium waves in cultured astrocytes: long-range glial signaling. *Science* **247**, 470–473 (1990).
Original cell-culture study showing transmitter-induced Ca²⁺ signalling in astrocytes and the potential for long-range signal transmission between these cells. The results of this study opened the field to the possibility that astrocytes could form a pathway for information transfer.
- Cornell Bell, A. H. & Finkbeiner, S. M. Ca²⁺ waves in astrocytes. *Cell Calcium* **12**, 185–204 (1991).
- Smith, S. J. Do astrocytes process neural information? *Prog. Brain Res.* **94**, 119–136 (1992).
- Finkbeiner, S. Calcium waves in astrocytes — filling in the gaps. *Neuron* **8**, 1101–1108 (1992).
- Stevens, B. & Fields, R. D. Response of Schwann cells to action potentials in development. *Science* **287**, 2267–2271 (2000).
- Porter, J. T. & McCarthy, K. D. Hippocampal astrocytes *in situ* respond to glutamate released from synaptic terminals. *J. Neurosci.* **16**, 5073–5081 (1996).
By using acutely isolated hippocampal brain slices, this study showed that neural activity could regulate astrocytic Ca²⁺ levels as a result of a synaptic transmitter acting on astrocytic receptors, indicating that glutamate-mediated neuron–glia signalling could function in the intact nervous system.
- Dani, J. W., Chernjavsky, A. & Smith, S. J. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* **8**, 429–440 (1992).
- Bezzi, P. *et al.* Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* **391**, 281–285 (1998).
- Kulik, A., Haentzsch, A., Luckermann, M., Reichelt, W. & Ballanyi, K. Neuron–glia signaling via α1 adrenoceptor-mediated Ca²⁺ release in Bergmann glial cells *in situ*. *J. Neurosci.* **19**, 8401–8488 (1999).
- Duffy, S. & MacVicar, B. A. Adrenergic calcium signaling in astrocyte networks within the hippocampal slice. *J. Neurosci.* **15**, 5535–5550 (1995).
- Shelton, M. K. & McCarthy, K. D. Hippocampal astrocytes exhibit Ca²⁺-elevating muscarinic cholinergic and histaminergic receptors *in situ*. *J. Neurochem.* **74**, 555–563 (2000).
- Wang, Z., Haydon, P. G. & Yeung, E. S. Direct observation of calcium-independent intercellular ATP signaling in astrocytes. *Anal. Chem.* **72**, 2001–2007 (2000).
- Guthrie, P. B. *et al.* ATP released from astrocytes mediates glial calcium waves. *J. Neurosci.* **19**, 520–528 (1999).
- Cotrina, M. L. *et al.* Connexins regulate calcium signaling by controlling ATP release. *Proc. Natl Acad. Sci. USA* **95**, 15735–15740 (1998).
- Kang, J., Jiang, L., Goldman, S. A. & Nedergaard, M. Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nature Neurosci.* **1**, 683–692 (1998).
This study provided the first evidence that astrocytes are a necessary intermediary in some forms of synaptic potentiation.
- Verkhratsky, A., Orkand, R. K. & Kettenmann, H. Glial calcium: homeostasis and signaling function. *Physiol. Rev.* **78**, 99–141 (1998).
- Verkhratsky, A. & Kettenmann, H. Calcium signalling in glial cells. *Trends Neurosci.* **19**, 346–352 (1996).
- Porter, J. T. & McCarthy, K. D. Astrocytic neurotransmitter receptors *in situ* and *in vivo*. *Prog. Neurobiol.* **51**, 439–455 (1997).
- Hassinger, T. D., Guthrie, P. B., Atkinson, P. B., Bennett, M. V. & Kater, S. B. An extracellular signaling component in propagation of astrocytic calcium waves. *Proc. Natl Acad. Sci. USA* **93**, 13268–13273 (1996).
The presence of an extracellular signal, which was later shown to be ATP, mediating astrocyte–astrocyte signalling was clearly shown in this study, which provided evidence that Ca²⁺ waves could 'jump' over cell-free regions.
- Charles, A. C., Merrill, J. E., Dirksen, E. R. & Sanderson, M. J. Intercellular signalling in glial cells: calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron* **6**, 983–992 (1991).
- Newman, E. A. & Zahs, K. R. Calcium waves in retinal glial cells. *Science* **275**, 844–847 (1997).
- Harris-White, M. E., Zanotti, S. A., Frautschy, S. A. & Charles, A. C. Spiral intercellular calcium waves in hippocampal slice cultures. *J. Neurophysiol.* **79**, 1045–1052 (1998).
- Sneyd, J., Wetton, B. T., Charles, A. C. & Sanderson, M. J. Intercellular calcium waves mediated by diffusion of inositol trisphosphate: a two-dimensional model. *Am. J. Physiol.* **268**, C1537–C1545 (1995).
- Sanderson, M. J., Charles, A. C., Boitano, S. & Dirksen, E. R. Mechanisms and function of intercellular calcium signaling. *Mol. Cell Endocrinol.* **98**, 173–187 (1994).
- Charles, A. C. *et al.* Intercellular calcium signaling via gap junctions in glioma cells. *J. Cell Biol.* **118**, 195–201 (1992).
- Sneyd, J., Charles, A. C. & Sanderson, M. J. A model for the propagation of intercellular calcium waves. *Am. J. Physiol.* **266**, C293–C302 (1994).
- Leybaert, L., Paemeleire, K., Strahonja, A. & Sanderson, M. J. Inositol-trisphosphate-dependent intercellular calcium signaling in and between astrocytes and endothelial cells. *Glia* **24**, 398–407 (1998).
- Giaume, C. & Venance, L. Intercellular calcium signaling and gap junctional communication in astrocytes. *Glia* **24**, 50–64 (1998).

38. Enkvist, M. O. & McCarthy, K. D. Activation of protein kinase C blocks astroglial gap junction communication and inhibits the spread of calcium waves. *J. Neurochem.* **59**, 519–526 (1992).
39. Charles, A. Inter-cellular calcium waves in glia. *Glia* **24**, 39–49 (1998).
40. Fam, S. R., Gallagher, C. J. & Salter, M. W. P2Y₁ purinoreceptor-mediated Ca²⁺ signaling and Ca²⁺ wave propagation in dorsal spinal cord astrocytes. *J. Neurosci.* **20**, 2800–2808 (2000).
41. Newman, E. A. Propagation of intercellular calcium waves in retinal astrocytes and Müller cells. *J. Neurosci.* (in the press).
42. Cotrina, M. L. *et al.* Connexins regulate calcium signaling by controlling ATP release. *Proc. Natl Acad. Sci. USA* **95**, 15735–15740 (1998).
43. Scemes, E., Suardicani, S. O. & Spray, D. C. Intercellular communication in spinal cord astrocytes: fine tuning between gap junctions and P2 nucleotide receptors in calcium wave propagation. *J. Neurosci.* **20**, 1435–1445 (2000).
44. Scemes, E., Dermietzel, R. & Spray, D. C. Calcium waves between astrocytes from Cx43 knockout mice. *Glia* **24**, 65–73 (1998).
45. John, G. R. *et al.* IL-1 β differentially regulates calcium wave propagation between primary human fetal astrocytes via pathways involving P2 receptors and gap junction channels. *Proc. Natl Acad. Sci. USA* **96**, 11613–11618 (1999).
46. Blomstrand, F., Giaume, C., Hansson, E. & Ronnback, L. Distinct pharmacological properties of ET-1 and ET-3 on astroglial gap junctions and Ca²⁺ signaling. *Am. J. Physiol.* **277**, C616–C627 (1999).
47. Enkvist, M. O. & McCarthy, K. D. Astroglial gap junction communication is increased by treatment with either glutamate or high K⁺ concentration. *J. Neurochem.* **62**, 489–495 (1994).
48. Rouach, N., Glowinski, J. & Giaume, C. Activity-dependent neuronal control of gap-junctional communication in astrocytes. *J. Cell Biol.* **149**, 1513–1526 (2000).
49. Venance, L., Plomelli, D., Glowinski, J. & Giaume, C. Inhibition by anandamide of gap junctions and intercellular calcium signalling in striatal astrocytes. *Nature* **376**, 590–594 (1995).
50. Ullian, E. M., Sapperstein, S. K., Christopherson, K. S. & Barres, B. A. Control of synapse number by glia. *Science* **291**, 657–661 (2001).
51. Pfrieger, F. W. & Barres, B. A. Synaptic efficacy enhanced by glial cells *in vitro*. *Science* **277**, 1684–1687 (1997).
52. Haydon, P. G. Neuroglial networks: neurons and glia talk to each other. *Curr. Biol.* **10**, R712–R714 (2000).
53. Parpura, V. *et al.* Glutamate-mediated astrocyte–neuron signalling. *Nature* **369**, 744–747 (1994).
- Original study showing that Ca²⁺ elevations in astrocytes evoke the release of glutamate, which signals to neighbouring neurons.**
54. Jęftinija, S. D., Jęftinija, K. V., Stefanovic, G. & Liu, F. Neuroigand-evoked calcium-dependent release of excitatory amino acids from cultured astrocytes. *J. Neurochem.* **66**, 676–684 (1996).
55. Innocenti, B., Parpura, V. & Haydon, P. G. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. *J. Neurosci.* **20**, 1800–1808 (2000).
56. Jahn, R., Hanson, P. I., Otto, H. & Ahnert Hilger, G. Botulinum and tetanus neurotoxins: emerging tools for the study of membrane fusion. *Cold Spring Harb. Symp. Quant. Biol.* **60**, 329–335 (1995).
57. Link, E. *et al.* Tetanus and botulinum neurotoxins. Tools to understand exocytosis in neurons. *Adv. Second Messenger Phosphoprotein Res.* **29**, 47–58 (1994).
58. Niemann, H., Blasi, J. & Jahn, R. Clostridial neurotoxins: new tools for dissecting exocytosis. *Trends Pharmacol. Sci.* **4**, 179–185 (1994).
59. Ahnert Hilger, G. & Bigalke, H. Molecular aspects of tetanus and botulinum neurotoxin poisoning. *Prog. Neurobiol.* **46**, 83–96 (1995).
60. Charles, A. C. Glia–neuron intercellular calcium signaling. *Dev. Neurosci.* **16**, 196–206 (1994).
61. Araque, A., Parpura, V., Sanzgiri, R. P. & Haydon, P. G. Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons. *Eur. J. Neurosci.* **10**, 2129–2142 (1998).
- First study to show that a chemical transmitter released from astrocytes can modulate synaptic transmission.**
62. Wolosker, H. *et al.* Purification of serine racemase: biosynthesis of the neuromodulator D-serine. *Proc. Natl Acad. Sci. USA* **96**, 721–725 (1999).
63. Schell, M. J., Molliver, M. E. & Snyder, S. H. D-serine, an endogenous synaptic modulator: localization to astrocytes and glutamate-stimulated release. *Proc. Natl Acad. Sci. USA* **92**, 3948–3952 (1995).
64. Molthet, J. P. *et al.* D-Serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proc. Natl Acad. Sci. USA* **97**, 4926–4931 (2000).
65. Attwell, D. Glia and neurons in dialogue. *Nature* **369**, 707–708 (1994).
66. Parpura, V., Liu, F., Brethorst, S., Jęftinija, K., Jęftinija, S. & Haydon, P. G. α -Latrotoxin stimulates glutamate release from cortical astrocytes in cell culture. *FEBS Lett.* **360**, 266–270 (1995).
67. Parpura, V., Fang, Y., Basarsky, T., Jahn, R. & Haydon, P. G. Expression of synaptobrevin II, cellubrevin and syntaxin but not SNAP-25 in cultured astrocytes. *FEBS Lett.* **377**, 489–492 (1995).
68. Hepp, R. *et al.* Cultured glial cells express the SNAP-25 analogue SNAP-23. *Glia* **27**, 181–187 (1999).
69. Maienschein, V., Marxen, M., Volkhardt, W. & Zimmermann, H. A plethora of presynaptic proteins associated with ATP-storing organelles in cultured astrocytes. *Glia* **26**, 233–244 (1999).
70. Araque, A., Li, N., Doyle, R. T. & Haydon, P. G. SNARE protein-dependent glutamate release from astrocytes. *J. Neurosci.* **20**, 666–673 (2000).
71. Hassinger, T. D. *et al.* Evidence for glutamate-mediated activation of hippocampal neurons by glial calcium waves. *J. Neurobiol.* **28**, 159–170 (1995).
72. Pasti, L., Volterra, A., Pozzan, T. & Carmignoto, G. Intracellular calcium oscillations in astrocytes: a highly plastic, bidirectional form of communication between neurons and astrocytes *in situ*. *J. Neurosci.* **17**, 7817–7830 (1997).
- First study in a brain slice preparation supporting the cell-culture evidence that astrocytes can signal to neurons by releasing glutamate.**
73. Robitaille, R. Modulation of synaptic efficacy and synaptic depression by glial cells at the frog neuromuscular junction. *Neuron* **21**, 847–855 (1998).
- This elegant study on the neuromuscular junction shows that synaptically associated glial cells are required for the modulation of synaptic transmission between nerve and muscle.**
74. Son, Y. J., Trachtenberg, J. T. & Thompson, W. J. Schwann cells induce and guide sprouting and reinnervation of neuromuscular junctions. *Trends Neurosci.* **19**, 280–285 (1996).
75. Jahromi, B. S., Robitaille, R. & Charlton, M. P. Transmitter release increases intracellular calcium in perisynaptic Schwann cells *in situ*. *Neuron* **8**, 1069–1077 (1992).
76. Reist, N. E. & Smith, S. J. Neurally evoked calcium transients in terminal Schwann cells at the neuromuscular junction. *Proc. Natl Acad. Sci. USA* **89**, 7625–7629 (1992).
77. Robitaille, R. Purinergic receptors and their activation by endogenous purines at perisynaptic glial cells of the frog neuromuscular junction. *J. Neurosci.* **15**, 7121–7131 (1995).
78. Parpura, V., Liu, F., Jęftinija, K. V., Haydon, P. G. & Jęftinija, S. D. Neuroigand-evoked calcium-dependent release of excitatory amino acids from Schwann cells. *J. Neurosci.* **15**, 5831–5839 (1995).
79. Jęftinija, S. D. & Jęftinija, K. V. ATP stimulates release of excitatory amino acids from cultured Schwann cells. *Neuroscience* **82**, 927–934 (1998).
80. Newman, E. A. & Zahs, K. R. Modulation of neuronal activity by glial cells in the retina. *J. Neurosci.* **18**, 4022–4028 (1998).
- This study showed that Ca²⁺ elevations within glial cells of the retina can modulate information transfer from the photoreceptor to the retinal ganglion cell. This modulatory action provides compelling evidence that glial Ca²⁺ signals are intimately involved in the regulation of information processing in the nervous system.**
81. Bergles, D. E. & Jahr, C. E. Glial contribution to glutamate uptake at Schaffer collateral-commissural synapses in the hippocampus. *J. Neurosci.* **18**, 7709–7716 (1998).
82. Bergles, D. E. & Jahr, C. E. Synaptic activation of glutamate transporters in hippocampal astrocytes. *Neuron* **19**, 1297–1308 (1997).
83. Araque, A., Sanzgiri, R. P., Parpura, V. & Haydon, P. G. Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *J. Neurosci.* **18**, 6822–6829 (1998).
84. Nedergaard, M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science* **263**, 1768–1771 (1994).
85. Froes, M. M. *et al.* Gap-junctional coupling between neurons and astrocytes in primary central nervous system cultures. *Proc. Natl Acad. Sci. USA* **96**, 7541–7546 (1999).
86. Alvarez-Maubecin, V., Garcia-Hernandez, F., Williams, J. T. & Van Bockstaele, E. J. Functional coupling between neurons and glia. *J. Neurosci.* **20**, 4091–4098 (2000).
87. McKhann, G. M., D'Ambrosio, R. & Janigro, D. Heterogeneity of astrocyte resting membrane potentials and intercellular coupling revealed by whole-cell and gramicidin-perforated patch recordings from cultured neocortical and hippocampal slice astrocytes. *J. Neurosci.* **17**, 6850–6863 (1997).
88. Milner, T. A., Kurucz, O. S., Veznedaroglu, E. & Pierce, J. P. Septohippocampal neurons in the rat septal complex have substantial glial coverage and receive direct contacts from noradrenergic terminals. *Brain Res.* **670**, 121–136 (1995).
89. Paspalas, C. D. & Papadopoulos, G. C. Ultrastructural relationships between noradrenergic nerve fibers and non-neuronal elements in the rat cerebral cortex. *Glia* **17**, 133–146 (1996).
90. Sulston, J. E., Schierenberg, E., White, J. G. & Thomson, J. N. The embryonic cell lineage of the nematode *Caenorhabditis elegans*. *Dev. Biol.* **100**, 64–119 (1983).

Acknowledgements

P.G.H. wishes to thank R. Doyle, H. Chen and J.-Y. Sul for comments on an earlier version of this manuscript, and V. Parpura, S. Shen, M. McCloskey and D. Sakaguchi for their stimulating discussions at various stages of these studies. Work done by P.G.H. is supported by grants from the National Institutes of Health.