



Published in final edited form as:

*Neuron Glia Biol.* 2004 August ; 1(3): 245–252.

## A dialogue between glia and neurons in the retina: modulation of neuronal excitability

ERIC A. NEWMAN

*Department of Neuroscience, University of Minnesota*

### Abstract

Bidirectional signaling between neurons and glial cells has been demonstrated in brain slices and is believed to mediate glial modulation of synaptic transmission in the CNS. Our laboratory has characterized similar neuron–glia signaling in the mammalian retina. We find that light-evoked neuronal activity elicits  $Ca^{2+}$  increases in Müller cells, which are specialized retinal glial cells. Neuron to glia signaling is likely mediated by the release of ATP from neurons and is potentiated by adenosine. Glia to neuron signaling has also been observed and is mediated by several mechanisms. Stimulation of glial cells can result in either facilitation or depression of synaptic transmission. Release of D-serine from Müller cells might also potentiate NMDA receptor transmission. Müller cells directly inhibit ganglion cells by releasing ATP, which, following hydrolysis to adenosine, activates neuronal  $A_1$  receptors. The existence of bidirectional signaling mechanisms indicates that glial cells participate in information processing in the retina.

### Keywords

Calcium; astrocyte; Müller cell; glial cell; retina; ATP; adenosine; light response; ganglion cell

### INTRODUCTION

In recent years, glial modulation of synaptic transmission and neuronal excitability has been recognized as an important function of glial cells (Volterra *et al.*, 2002; Newman, 2003b). First demonstrated in astrocyte/neuron co-cultures (Parpura *et al.*, 1994; Araque *et al.*, 1998a; Araque *et al.*, 1998b) and later in brain slices (Pasti *et al.*, 1997; Kang *et al.*, 1998), the release of transmitters from glial cells can have a significant influence on neuronal activity. Glial cells have also been shown to modulate neuronal function in the retina (Newman, 2004), which is a useful preparation for studying glia–neuron signaling.

Two types of macroglial cells are present in the retinae of most mammals; astrocytes and Müller cells (Fig. 1) (Newman, 2001a). Astrocytes are confined largely to the nerve fiber layer at the inner border of the retina. They interact primarily with the axons of ganglion cells and do not contact neuronal synapses. In contrast, Müller cells span the entire neural retina from the inner retinal border to the photoreceptor layer. The processes of Müller's cell ramify within the two synaptic layers of the retina, the inner and outer plexiform layers, and surround and sometimes contact neuronal synapses. Throughout most of the retina, Müller cells are the only macroglial cells present and they function as specialized astrocytes.

Glial cells can influence neuronal excitability by several mechanisms (Newman, 2005), including the uptake of glutamate and GABA following release at the synapse, and the

regulation of extracellular  $K^+$  and pH. These processes are considered indirect mechanisms of neuronal modulation (Newman, 2005). Glial cells also modulate neuronal excitability directly, by releasing either transmitters or co-agonists onto neurons (Volterra *et al.*, 2002). Similarly, neurons can modulate glial-cell function by the release of neurotransmitters (Schipke and Kettenmann, 2004). In this review, I focus on work from our laboratory that demonstrates direct communication between neurons and glial cells in the retina through the release of chemical messengers.

### Neuron to glia signaling

Active neurons can evoke increases in  $Ca^{2+}$  in glial cells. In brain slices, electrical stimulation of neurons increases  $Ca^{2+}$  concentrations in neighboring astrocytes (Schipke and Kettenmann, 2004). This neuron to glia signaling is mediated by the release of transmitters from neurons. Release of glutamate (Dani *et al.*, 1992; Porter and McCarthy, 1996; Pasti *et al.*, 1997), GABA (Kang *et al.*, 1998), acetylcholine (Araque *et al.*, 2002) and ATP (Bowser and Khakh, 2004) can evoke  $Ca^{2+}$  increases by activating glial metabotropic receptors. The release of nitric oxide from neurons can also produce glial  $Ca^{2+}$  increases (Matyash *et al.*, 2001).

We have investigated whether similar neuron to glia signaling occurs in the retina. Calcium levels in retinal Müller cells and astrocytes have been monitored with the  $Ca^{2+}$ -indicator dye Fluo-4 when the retina is stimulated with light flashes. Characterizing neuron to glia signaling in the retina has the advantage over similar studies in brain slices in that a natural stimulus, light, rather than electrical stimuli, can be used to excite neurons.

**Müller cell  $Ca^{2+}$  transients**—Previously unpublished experiments from our laboratory demonstrate that transient increases in  $Ca^{2+}$  are generated in Müller cells (Fig. 2). These transients occur at low frequency (~10 transients per Müller cell per 1000 seconds) and do not propagate between cells. Flickering light stimulation increases the occurrence of these  $Ca^{2+}$  transients. Averaging the  $Ca^{2+}$  transients in Müller cells over many trials reveals that  $Ca^{2+}$  rises rapidly at the onset of a flickering stimulus and remains elevated for the duration of the stimulus.  $Ca^{2+}$  transients are not observed in astrocytes.

The light-evoked increase in  $Ca^{2+}$  transients in Müller cells is largely blocked by the purinergic receptor antagonist suramin, indicating that neuronal release of ATP is responsible for the increase in glial  $Ca^{2+}$ . The light-evoked increase in  $Ca^{2+}$  transients is also blocked by tetrodotoxin, which indicates that neuron to glia signaling is mediated by neurons that generate action potentials. In the retina, only amacrine cells and ganglion cells generate action potentials and these two types of inner retinal neurons are likely to signal to glia. Cholinergic starburst amacrine cells, which are believed to co-release ATP, could mediate neuron to glia signaling. Ganglion cells might also contribute to light-evoked increases in  $Ca^{2+}$  in glial cells. Antidromic stimulation of ganglion cell axons evokes a substantial increase in Müller cell  $Ca^{2+}$  transients. This increase is also blocked by suramin, indicating that ganglion cells might release ATP in the retina.

**Adenosine potentiation of Müller cell  $Ca^{2+}$  responses**—Adenosine agonists [either 100  $\mu$ M adenosine or 2  $\mu$ M 1-(6-Amino-9H-purin-9-yl)-1-deoxy-*N*-ethyl-*b*-D-ribofuranuronamide 5'-*N*-Ethylcarboxamidoadenosine (NECA)] potentiate light-evoked Müller cell  $Ca^{2+}$  increases. In the presence of an adenosine agonist, light ON elicits a large increase in  $Ca^{2+}$  in all Müller cells. Adenosine-potentiated increases are ~100-fold larger than the increase observed in the absence of an adenosine agonist. The adenosine-potentiated, light-evoked  $Ca^{2+}$  increase begins in Müller cell processes in the inner plexiform layer and spreads proximally into the endfeet of Müller cells at the inner surface of the retina.

In the presence of adenosine, addition of ATP evokes large increases in Müller cell  $\text{Ca}^{2+}$ . The light-evoked  $\text{Ca}^{2+}$  increase is nearly abolished by suramin and by apyrase, an ectoenzyme that hydrolyzes ATP. By contrast, the light-evoked increase is not reduced substantially by antagonists of glutamate, GABA and acetylcholine receptors. In addition, the metabotropic glutamate agonist trans-ACPD does not evoke Müller cell  $\text{Ca}^{2+}$  increases. Together, these results indicate that neuron to glia signaling in the retina is mediated by the release of ATP from neurons.

### **Glia to neuron signaling**

We have characterized glial modulation of neuronal activity in the retina in a number of studies. Initially, the effect of glial cells on light-evoked spike activity in ganglion cells was characterized. Subsequent studies have determined the mechanisms by which glial cells modulate neuronal activity.

**Glial modulation of light-evoked activity in ganglion cells**—Mechanical stimulation of single astrocytes evokes  $\text{Ca}^{2+}$  increases that spread up to 200  $\mu\text{m}$  from the stimulated cell to neighboring astrocytes and Müller cells (Newman and Zahs, 1997). The effect of these activated glial cells on neurons has been assessed by recording the light-evoked spike activity of a nearby ganglion cell with an extracellular microelectrode (Newman and Zahs, 1998). We found that stimulated glial cells either enhance or depress light-evoked neuronal activity (Fig. 3). In some ganglion cells (9% of all cells recorded) (Fig. 3A) glial-cell stimulation increases cell spiking. In many more ganglion cells, however (47%) (Fig. 3B), glial-cell stimulation decreases cell spiking. Glial modulation of ganglion cell activity is blocked when glial cell  $\text{Ca}^{2+}$  increases are reduced with thapsigargin, suggesting that glia to neuron signaling depends on glial  $\text{Ca}^{2+}$  increases.

**Mechanisms of glia to neuron signaling**—The modulation of neuronal spiking by glial cells described above could be mediated by several mechanisms. Stimulated glial cells might release a transmitter that activates receptors on presynaptic terminals. For example, glial cells release glutamate (Parpura *et al.*, 1994; Jęftinija *et al.*, 1996; Bezzi *et al.*, 1998; Innocenti *et al.*, 2000) and ATP (Cotrina *et al.*, 1998; Newman, 2001b). These gliotransmitters might activate pre-synaptic glutamate, ATP and adenosine metabotropic receptors, either facilitating or depressing synaptic transmission.

Gliotransmitter release could also modulate postsynaptic receptors on ganglion cells. The release of D-serine will facilitate NMDA synaptic transmission by binding to the NMDA co-agonist site (Wolosker *et al.*, 1999; Miller, 2004). Release of glutamate, in contrast, might desensitize glutamatergic receptors, thus, reducing glutamate synaptic transmission.

Gliotransmitters might also directly modulate the excitability of ganglion cells. Release of ATP from glial cells could activate P2X receptors on ganglion cells (Taschenberger *et al.*, 1999), depolarizing the cells. ATP, if converted to adenosine by ectoenzymes, might also activate  $\text{A}_1$  adenosine receptors (Braas *et al.*, 1987) and hyperpolarize ganglion cells.

Our results indicate that retinal glial cells modulate ganglion cell activity by several of these mechanisms, as shown in the following experiments.

**Presynaptic modulation of ganglion-cell activity**—Previously unpublished experiments from our laboratory indicate that glial cells modulate synaptic transmission onto ganglion cells by presynaptic mechanisms. We stimulated the retina with brief light flashes repeated at 5-second intervals. These stimuli evoke excitatory post-synaptic potentials (EPSCs) in ganglion cells. ATP $\gamma$ S ejections, which elicit glial  $\text{Ca}^{2+}$  increases, result in a rise in EPSC amplitude in some ganglion cells (Fig. 4A, middle). It is possible that the ATP $\gamma$ S ejection used

to stimulate glial cells facilitates synaptic transmission, not by stimulating glial cells, but by acting directly on presynaptic terminals. This seems unlikely, however, because activation of glial cells by mechanical stimulation produces a similar facilitation of synaptic transmission (Fig. 4A, bottom).

In some ganglion cells, glial cell stimulation results in a decrease in the amplitude of light-evoked EPSCs. In the example illustrated in Fig. 4B, light flashes evoke a complex synaptic current composed of an initial inhibitory post-synaptic current (IPSC) followed by an EPSC. Glial stimulation reduces the EPSC but has little effect on the IPSC, indicating that glial modulation of synaptic transmission can be selective. Additional experiments to monitor changes in paired pulse facilitation indicate that glial modulation of synaptic transmission reflects a presynaptic mechanism.

Recordings from many ganglion cells reveal that glial modulation of synaptic transmission similar to that illustrated in Fig. 4 occurs relatively rarely. Only a small percentage of ganglion cells show either facilitation or depression of synaptic transmission. We believe that glial modulation of synaptic transmission by presynaptic mechanisms, although present, might play a relatively minor role in glial modulation of ganglion-cell activity.

**Postsynaptic modulation of ganglion cell activity**—Additional experiments demonstrate that robust glial modulation of ganglion-cell activity occurs by direct hyperpolarization of ganglion cells (Newman, 2003a). When glial  $\text{Ca}^{2+}$  increases are evoked by  $\text{ATP}\gamma\text{S}$  ejection, a hyperpolarization, generated by a slow outward current, is elicited in neighboring ganglion cells (Fig. 5A). Of the ganglion cells monitored, 52% show moderate hyperpolarizations ( $<5$  mV), 12% show large hyperpolarizations ( $>5$  mV) and 36% show little or no response.

The hyperpolarizing response is mediated by the stimulated glial cells rather than by a direct neuronal response to the  $\text{ATP}\gamma\text{S}$  ejection (Newman, 2003a). Diverse stimuli, including ejection of ATP, dopamine, thrombin, lysophosphatidic acid and direct mechanical stimulation, evoke glial  $\text{Ca}^{2+}$  increases. Each stimulus also elicits ganglion cell hyperpolarization.

Glial hyperpolarization of ganglion cells can produce substantial changes in neuronal activity. In the experiment illustrated in Fig. 5B, for example, a high rate of spontaneous spiking present in a ganglion cell is blocked completely when neighboring glial cells are stimulated with  $\text{ATP}\gamma\text{S}$ .

Ganglion-cell hyperpolarization is mediated by the release of ATP from glial cells and the activation of adenosine  $\text{A}_1$  receptors on ganglion cells, as demonstrated by several types of experiments (Newman, 2003a). Glial-mediated hyperpolarization of ganglion cells is abolished by DPCPX, an  $\text{A}_1$  receptor antagonist (Fig. 6A). Ejection of adenosine onto ganglion cells mimics the response produced by glial-cell stimulation (Fig. 6B). Inhibition of ecto-ATPases and ectonucleotidases, both of which are needed to convert ATP to adenosine following ATP release from glial cells, substantially reduce the glial-mediated ganglion-cell response (Fig. 6C).

We have used the luciferin–luciferase chemiluminescence assay to image ATP release following glial-cell stimulation. In the presence of  $\text{Cd}^{2+}$ , which blocks transmitter release from neurons, mechanical stimulation of glial cells evokes an ATP release into the inner plexiform (synaptic) layer. This release is most likely from Müller cells. Indeed, selective stimulation of Müller cells and astrocytes demonstrates that stimulation of Müller cells, but not astrocytes, is both necessary and sufficient to elicit ganglion cell hyperpolarization (Newman, 2003a).

Activation of ganglion cell A<sub>1</sub> receptors evokes cell hyperpolarization by opening Ba<sup>2+</sup>-sensitive K<sup>+</sup> channels (Newman, 2003a). The reversal potential of the slow outward current evoked by glial cell stimulation is near the K<sup>+</sup> equilibrium potential. The outward current is blocked by Ba<sup>2+</sup>, indicating that inwardly rectifying K<sup>+</sup> channels mediate the response. Recent experiments in our laboratory demonstrate that the adenosine-evoked current is reduced substantially by tertiapin, which blocks G-protein-coupled inwardly rectifying K<sup>+</sup> channels (GIRKs).

Together, these experiments demonstrate that retinal glial cells can inhibit ganglion cells by releasing ATP. The ATP is converted to adenosine by ectoenzymes, which stimulates ganglion cell A<sub>1</sub> receptors. It is interesting to speculate that released ATP might also directly excite ganglion cells. Ganglion cells express P2X receptors (Taschenberger *et al.*, 1999) and activation of these receptors would depolarize ganglion cells. Thus, retinal glial cells, by releasing ATP, might be able to either excite or inhibit ganglion cells, depending on the type of receptors expressed by the neurons.

**D-serine release from Müller cells**—D-serine released from glial cells is believed to potentiate NMDA receptor responses in the brain (Miller, 2004). D-serine binds to the co-agonist (glycine)-binding site on NMDA receptors (Wolosker *et al.*, 1999) and might be the endogenous co-agonist in the CNS (Mothet *et al.*, 2000; Schell *et al.*, 1995).

Release of D-serine from Müller cells might also potentiate NMDA receptor transmission in the retina (Stevens *et al.*, 2003). Both D-serine and serine racemase, the enzyme that synthesizes D-serine, are localized in Müller cells in the retina. In addition, D-serine potentiates NMDA responses in the retina. D-serine augments the NMDA component of light-evoked ganglion-cell responses and potentiates ganglion-cell responses to NMDA ejection (Fig. 7A). Addition of D-amino acid oxidase, which degrades D-serine, reduces both the NMDA component of light-evoked responses and ganglion-cell responses to NMDA ejection (Fig. 7B). The results demonstrate that endogenous D-serine, which is likely to be released from Müller cells, modulates NMDA synaptic transmission in the retina.

### Glia to glia signaling

The findings outlined above demonstrate bidirectional signaling between neurons and glial cells in the retina. Neuronal activity evokes Ca<sup>2+</sup> increases in Müller cells. Stimulated Müller cells, in turn, release transmitters that modulate synaptic transmission and neuronal excitability. These interactions are likely to take place within a confined area, perhaps within a few microns of the synapse. Release of transmitter from the presynaptic terminal will evoke a Ca<sup>2+</sup> increase within glial processes that contact the synapse. The stimulated processes will then release ATP which would feed back onto either the pre-synaptic or post-synaptic elements of the synapse.

This neuron-glia dialogue need not be restricted to a single synapse, however. Stimulation of a glial process might result in Ca<sup>2+</sup> increases in other regions of the glial cell or in the initiation of a Ca<sup>2+</sup> wave that propagates from the stimulated cell to other glial cells. In this manner, glial cells could influence the excitability of neurons distant from the initial site of glial cell stimulation.

Glial Ca<sup>2+</sup> waves that propagate both within and between cells do occur in brain tissue (Peters *et al.*, 2003) and the retina (Newman and Zahs, 1997). In retinal astrocytes and Müller cells, stimulation of one cell region results in a Ca<sup>2+</sup> increase that propagates into other cells regions (Newman, 2001b). Focal stimulation also elicits intercellular Ca<sup>2+</sup> waves (Fig. 8) (Newman and Zahs, 1997; Newman, 2001b). These waves travel at a velocity of ~23 μm s<sup>-1</sup> and propagate up to ~200 μm from the stimulation site before they die out.

Ca<sup>2+</sup> waves propagate between retinal glial cells by two mechanisms (Newman, 2001b). Astrocyte to astrocyte propagation is mediated principally by diffusion of an intracellular messenger between gap-junction-coupled glial cells (Robinson *et al.*, 1993; Zahs and Newman, 1997). In contrast, propagation from astrocytes to Müller cells and propagation between Müller cells is mediated by glial release of ATP, which functions as an extracellular messenger. ATP release also contributes to wave propagation between retinal astrocytes.

Glial Ca<sup>2+</sup> waves, previously characterized in the retina and brain slices, have been initiated by artificial stimuli such as agonist ejection, mechanical stimulation and electrical stimulation. It remains an open question whether neuronal activity can also initiate intercellular Ca<sup>2+</sup> waves.

Recently, we have obtained evidence that intercellular Ca<sup>2+</sup> waves can be evoked by neuronal activity in the retina. As described above, light stimuli evoke large, transient, Müller cell Ca<sup>2+</sup> increases in the presence of adenosine. In some trials, the initial light-evoked Ca<sup>2+</sup> increase is followed by a delayed Ca<sup>2+</sup> rise in a few Müller cells. This secondary Ca<sup>2+</sup> increase then propagates into neighboring Müller cells as a Ca<sup>2+</sup> wave. Although these Ca<sup>2+</sup> waves propagate into a limited number of glial cells and have, to date, only been observed in the presence of adenosine, their existence indicates that intercellular Ca<sup>2+</sup> waves might occur *in vivo*.

## CONCLUSION

Bidirectional communication between neurons and glial cells has been described in several brain-slice preparations and is believed to be responsible for glial modulation of synaptic transmission in the brain. We have observed similar bidirectional signaling between neurons and Müller cells in the mammalian retina. Light-evoked neuronal activity leads to increases in the generation of Ca<sup>2+</sup> transients in Müller cells. These light-evoked glial responses are potentiated greatly by adenosine. These experiments represent the first demonstration that a natural stimulus can evoke Ca<sup>2+</sup> increases in CNS glia.

Signaling in the reverse direction, from glia to neurons, occurs by a number of mechanisms in the retina. Glial stimulation can either facilitate or depress synaptic transmission onto ganglion cells by presynaptic mechanisms. Glia might also modulate synaptic transmission by releasing D-serine and potentiating postsynaptic NMDA receptors. In addition, Müller cells can inhibit ganglion cells directly by releasing ATP.

Although bidirectional neuron–glia signaling occurs in both the brain and the retina, the principal transmitters that mediate signaling appear to be different in these two CNS regions. In the brain, glutamate is the primary neurotransmitter that is responsible for eliciting Ca<sup>2+</sup> increases in astrocytes. Similarly, glial modulation of neurons in the brain is mediated primarily by astrocytic release of glutamate. By contrast, in the retina ATP appears to be the principal transmitter and is responsible for both neuron to glia signaling and for glia to neuron signaling. It is unclear what accounts for this difference in signaling between the brain and the retina. One possible reason is that glutamate is released tonically from retinal neurons (Miller, 2001) and, thus, might not be well suited to function as the transmitter mediating neuron–glia communication in the retina.

As in the brain, the existence of signaling between neurons and glia in the retina indicates that retinal glial cells might play a role in information processing. Given the relatively slow time course of signaling observed, it is likely that glial cells play a modulatory role in visual processing rather than contributing directly to the generation of rapid, light-evoked signals. Our current understanding of neuron–glia interactions does not permit us to speculate on the precise role of retinal glial cells in the processing of visual information.



## Acknowledgements

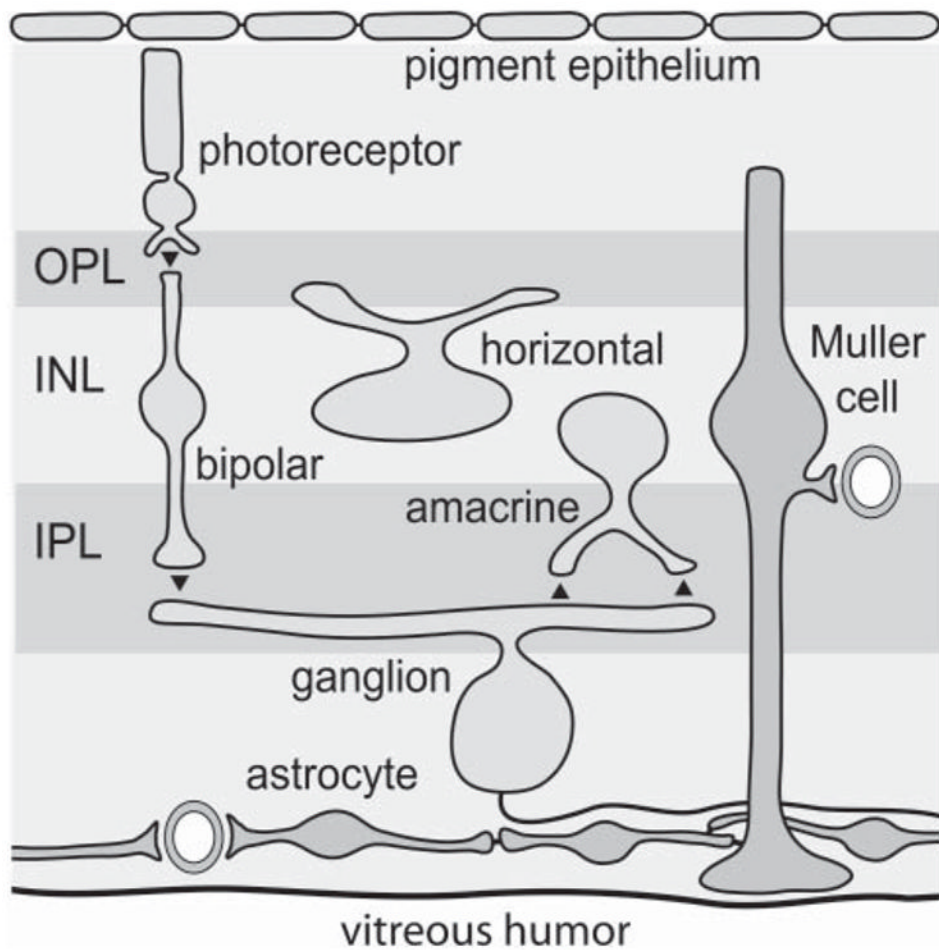
I thank J.I. Gepner and M. Metea for helpful discussions. This research is supported by NIH grant EY004077.

## References

- Araque A, Martin ED, Perea G, Arellano JI, Buno W. Synaptically released acetylcholine evokes  $Ca^{2+}$  elevations in astrocytes in hippocampal slices. *Journal of Neuroscience* 2002;22:2443–2450. [PubMed: 11923408]
- Araque A, Parpura V, Sanzgiri RP, Haydon PG. Glutamate-dependent astrocyte modulation of synaptic transmission between cultured hippocampal neurons. *European Journal of Neuroscience* 1998a; 10:2129–2142. [PubMed: 9753099]
- Araque A, Sanzgiri RP, Parpura V, Haydon PG. Calcium elevation in astrocytes causes an NMDA receptor-dependent increase in the frequency of miniature synaptic currents in cultured hippocampal neurons. *Journal of Neuroscience* 1998b;18:6822–6829. [PubMed: 9712653]
- Bezzi P, Carmignoto G, Pasti L, Vesce S, Rossi D, Lodi, et al. Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* 1998;391:281–285. [PubMed: 9440691]
- Bowser DN, Khakh BS. ATP Excites Interneurons and Astrocytes to Increase Synaptic Inhibition in Neuronal Networks. *Journal of Neuroscience* 2004;24:8606–8620. [PubMed: 15456834]
- Braas KM, Zarbin MA, Snyder SH. Endogenous adenosine and adenosine receptors localized to ganglion cells of the retina. *Proceedings of the National Academy of Sciences of the USA* 1987;84:3906–3910. [PubMed: 3473489]
- Cotrina ML, Lin JHC, Alves-Rodrigues A, Liu S, Li J, Azmi-Ghadimi H, et al. Connexins regulate calcium signaling by controlling ATP release. *Proceedings of the National Academy of Sciences of the USA* 1998;95:15735–15740. [PubMed: 9861039]
- Dani JW, Chernjavsky A, Smith SJ. Neuronal activity triggers calcium waves in hippocampal astrocyte networks. *Neuron* 1992;8:429–440. [PubMed: 1347996]
- Innocenti B, Parpura V, Haydon PG. Imaging extracellular waves of glutamate during calcium signaling in cultured astrocytes. *Journal of Neuroscience* 2000;20:1800–1808. [PubMed: 10684881]
- Jeftinija SD, Jeftinija KV, Stefanovic G, Liu F. Neuroligand-evoked calcium-dependent release of excitatory amino acids from cultured astrocytes. *Journal of Neurochemistry* 1996;66:676–684. [PubMed: 8592139]
- Kang J, Goldman SA, Nedergaard M. Astrocyte-mediated potentiation of inhibitory synaptic transmission. *Nature Neuroscience* 1998;1:683–692.
- Matyash V, Filippov V, Mohrhagen K, Kettenmann H. Nitric oxide signals parallel fiber activity to Bergmann glial cells in the mouse cerebellar slice. *Molecular and Cell Neuroscience* 2001;18:664–670.
- Miller, RF. The physiology and morphology of the vertebrate retina. In: Ryan, SJ., editor. *Retina*. Mosby; 2001. p. 138-170.
- Miller RF. D-Serine as a glial modulator of nerve cells. *Glia* 2004;47:275–283. [PubMed: 15252817]
- Mothet J-P, Parent AT, Wolosker H, Brady RO Jr, Linden DJ, Ferris CD, et al. D-serine is an endogenous ligand for the glycine site of the N-methyl-D-aspartate receptor. *Proceedings of the National Academy of Sciences of the USA* 2000;97:4926–4931. [PubMed: 10781100]
- Newman, EA. Glia of the retina. In: Ryan, SJ., editor. *Retina*. Mosby; 2001a. p. 89-103.
- Newman EA. Propagation of intercellular calcium waves in retinal astrocytes and Müller cells. *Journal of Neuroscience* 2001b;21:2215–2223. [PubMed: 11264297]
- Newman EA. Glial cell inhibition of neurons by release of ATP. *Journal of Neuroscience* 2003a;23:1659–1666. [PubMed: 12629170]
- Newman EA. New Roles for astrocytes: Regulation of synaptic transmission. *Trends in Neurosciences* 2003b;26:536–542. [PubMed: 14522146]
- Newman EA. Glial modulation of synaptic transmission in the retina. *Glia* 2004;47:268–274. [PubMed: 15252816]
- Newman, EA. Glia and synaptic transmission. In: Kettenmann, H.; Ransom, BR., editors. *Neuroglia*. Oxford University Press; 2005.

- Newman EA, Zahs KR. Calcium waves in retinal glial cells. *Science* 1997;275:844–847. [PubMed: 9012354]
- Newman EA, Zahs KR. Modulation of neuronal activity by glial cells in the retina. *Journal of Neuroscience* 1998;18:4022–4028. [PubMed: 9592083]
- Parpura V, Basarsky TA, Liu F, Jefčinija K, Jefčinija S, Haydon PG. Glutamate-mediated astrocyte-neuron signalling. *Nature* 1994;369:744–747. [PubMed: 7911978]
- 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*. *Journal of Neuroscience* 1997;17:7817–7830. [PubMed: 9315902]
- Peters O, Schipke CG, Hashimoto Y, Kettenmann H. Different mechanisms promote astrocyte  $\text{Ca}^{2+}$  waves and spreading depression in the mouse neocortex. *Journal of Neuroscience* 2003;23:9888–9896. [PubMed: 14586018]
- Porter JT, McCarthy KD. Hippocampal astrocytes *in situ* respond to glutamate released from synaptic terminals. *Journal of Neuroscience* 1996;16:5073–5081. [PubMed: 8756437]
- Robinson SR, Hampson ECGM, Munro MN, Vaney DI. Unidirectional coupling of gap junctions between neuroglia. *Science* 1993;262:1072–1074. [PubMed: 8093125]
- Schell MJ, Molliver ME, Snyder SH. D-serine, an endogenous synaptic modulator: Localization to astrocytes and glutamate-stimulated release. *Proceedings of the National Academy of Sciences of the USA* 1995;92:3948–3952. [PubMed: 7732010]
- Schipke CG, Kettenmann H. Astrocyte responses to neuronal activity. *Glia* 2004;47:226–232. [PubMed: 15252811]
- Stevens ER, Esquerra M, Kim P, Newman EA, Snyder SH, Zahs KR, et al. D-serine and serine racemase are present in the vertebrate retina and contribute to the functional expression of NMDA receptors. *Proceedings of the National Academy of Sciences of the USA* 2003;100:6789–6794. [PubMed: 12750462]
- Taschenberger H, Jüttner R, Grantyn R.  $\text{Ca}^{2+}$ -permeable P2X receptor channels in cultured rat retinal ganglion cells. *Journal of Neuroscience* 1999;19:3353–3366. [PubMed: 10212295]
- Volterra, A.; Magistretti, PJ.; Haydon, PG. *Glia in synaptic transmission*. Oxford University Press; 2002. The Tripartite Synapse.
- Wolosker H, Blackshaw S, Snyder SH. Serine racemase: a glial enzyme synthesizing D-serine to regulate glutamate-N-methyl-D-aspartate neurotransmission. *Proceedings of the National Academy of Sciences of the USA* 1999;96:13409–13414. [PubMed: 10557334]
- Zahs KR, Newman EA. Asymmetric gap junctional coupling between glial cells in the rat retina. *Glia* 1997;20:10–22. [PubMed: 9145301]

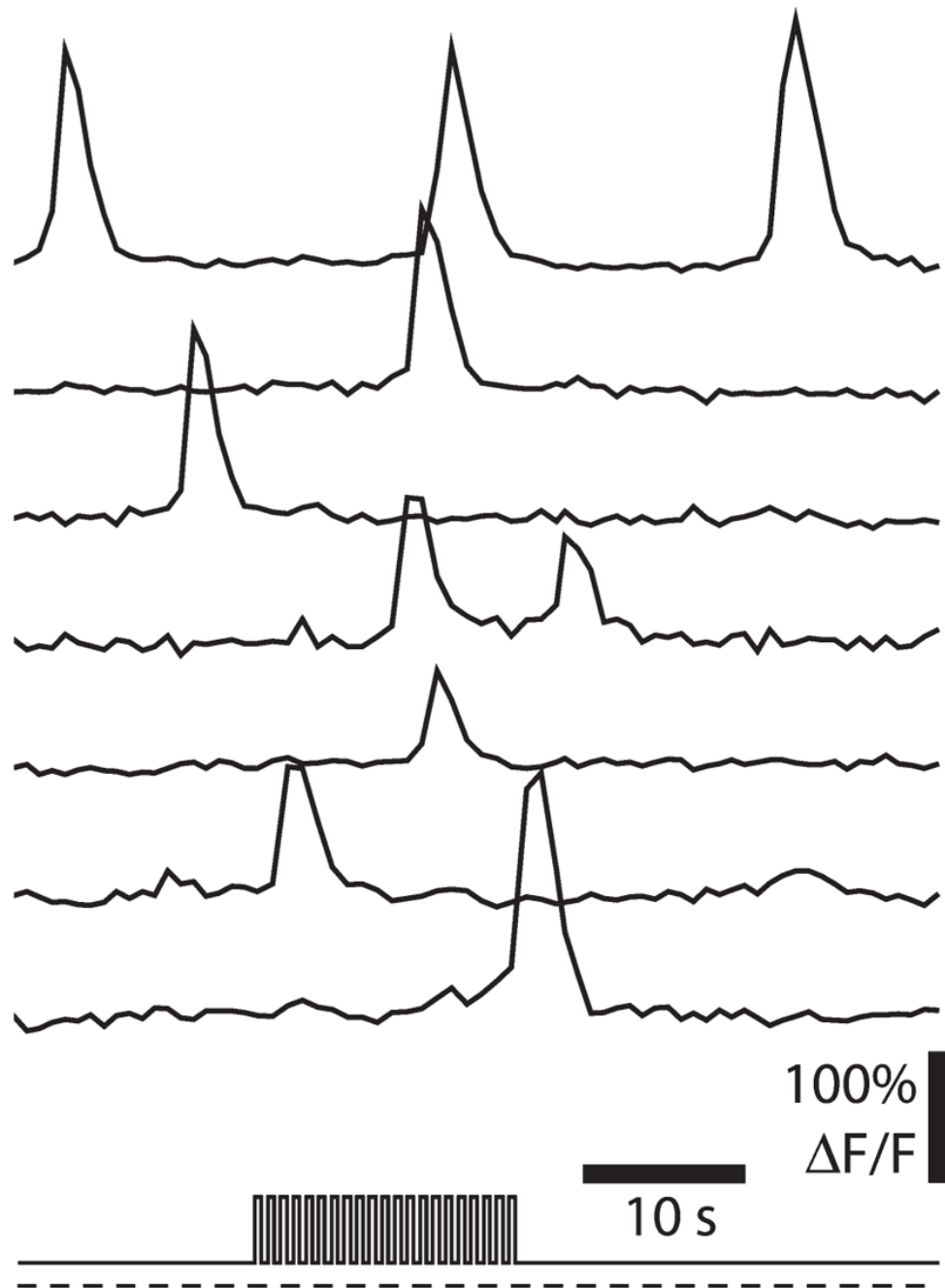




**Fig. 1. Glial cells of the mammalian retina**

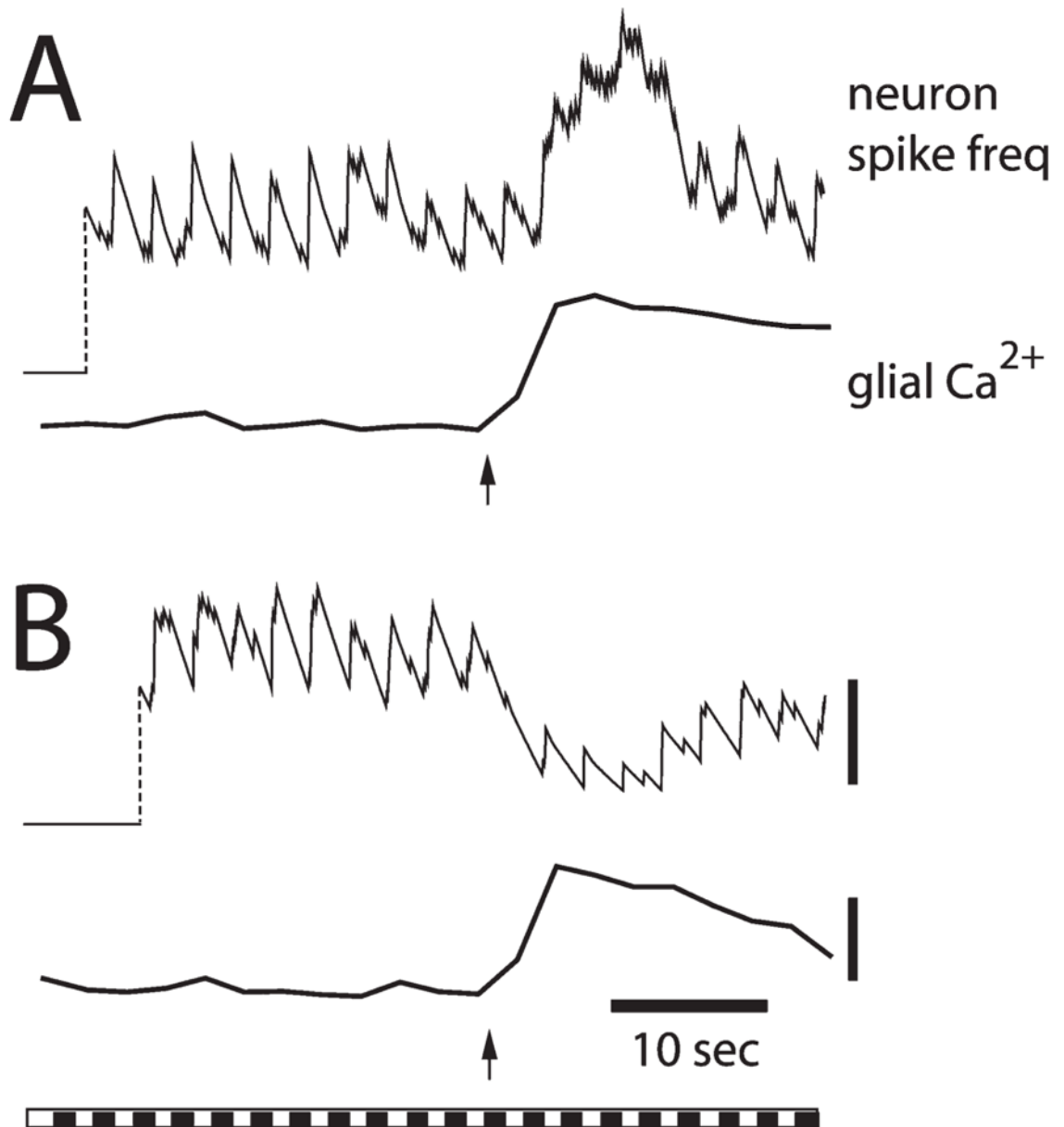
Astrocytes, ubiquitous CNS glial cells, are confined primarily to the nerve-fiber layer adjacent to the inner (vitreal) surface of the retina. Müller cells, which are specialized radial glial cells found only in the retina, extend from the vitreal surface to the photoreceptor layer. Müller cells, but not astrocytes, span the two synaptic layers of the retina, the inner plexiform layer (IPL) and the outer plexiform layer (OPL). Their somata lie in the inner nuclear layer (INL).

Astrocytes are coupled to each other and to Müller cells by gap junctions. The major classes of retinal neurons are also illustrated. Synapses are indicated by filled triangles.



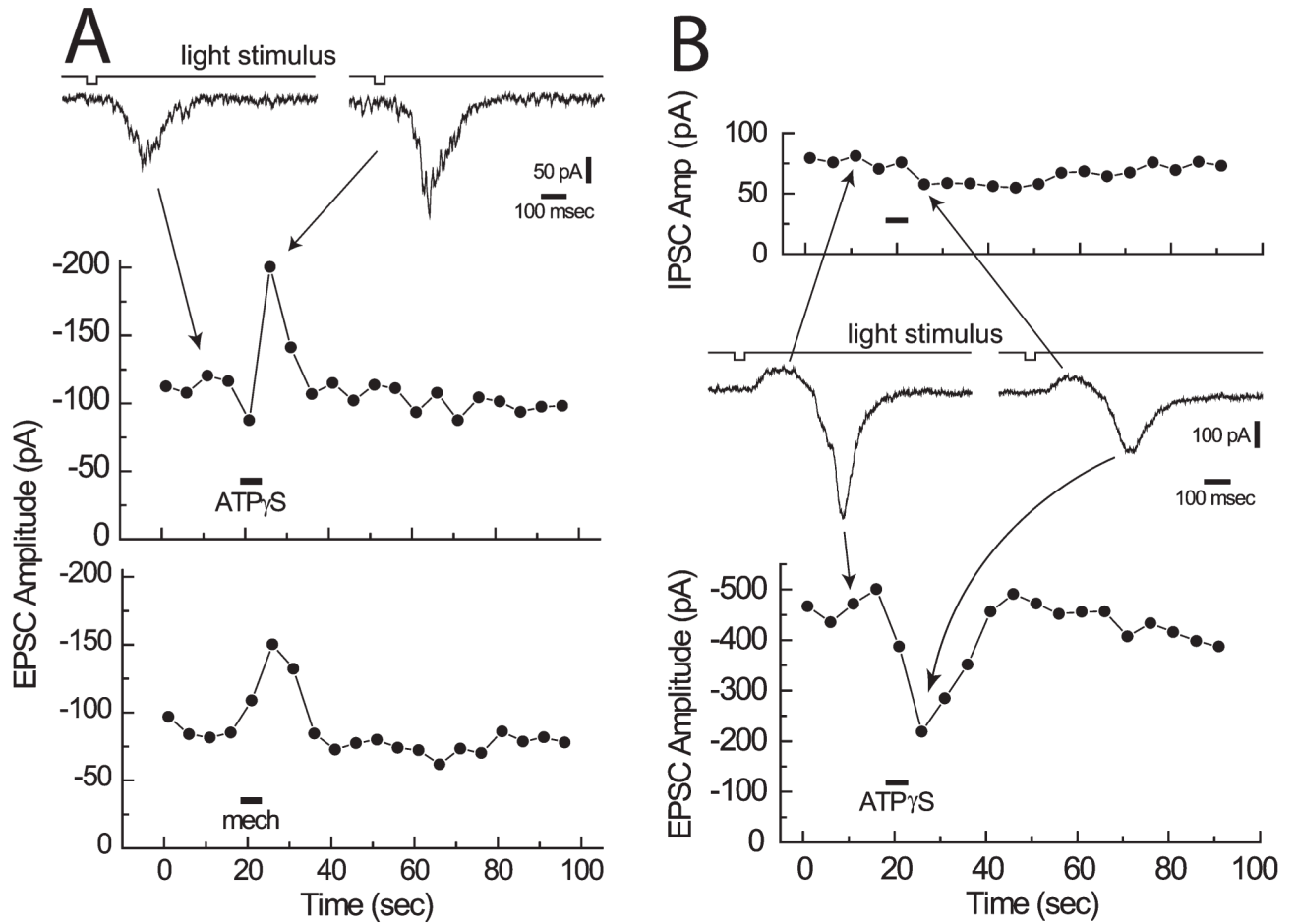
**Fig. 2. Calcium transients in retinal glial cells**

Calcium fluorescence is measured simultaneously in seven Müller cells at the vitreal surface of the retina. The light-stimulus protocol, a 16-second episode of flickering light superimposed on a dim background light, is illustrated at the bottom. Calcium transients are generated in each of the Müller cells. On average,  $\text{Ca}^{2+}$  transients occur at a higher rate during the flickering stimulus than during the dim background illumination. Unpublished results.



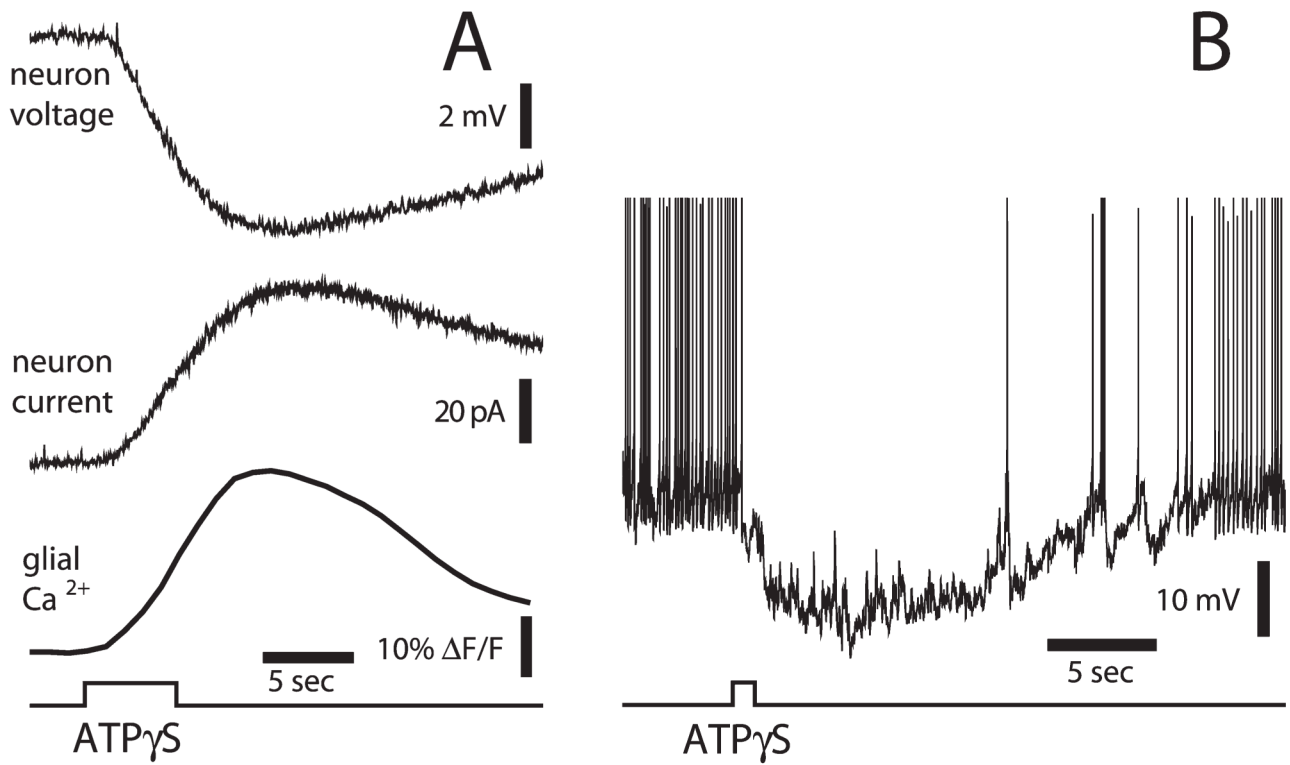
**Fig. 3. Glial modulation of light-evoked spiking in rat ganglion cells**

Facilitation of neuron spiking (A) and depression of spiking (B) are illustrated in recordings from two ganglion cells. A frequency plot of spike activity (top trace) and Ca<sup>2+</sup> levels in glial cells adjacent to the neuron (bottom trace) are shown for each trial. Arrows indicate initiation of the glial Ca<sup>2+</sup> wave. The bar at the bottom shows the repetitive light stimulus that evokes neuronal spiking. Calibration bars; 3 spikes second<sup>-1</sup>; 20%  $\Delta F/F$ . Modified from Newman and Zahs (1998).



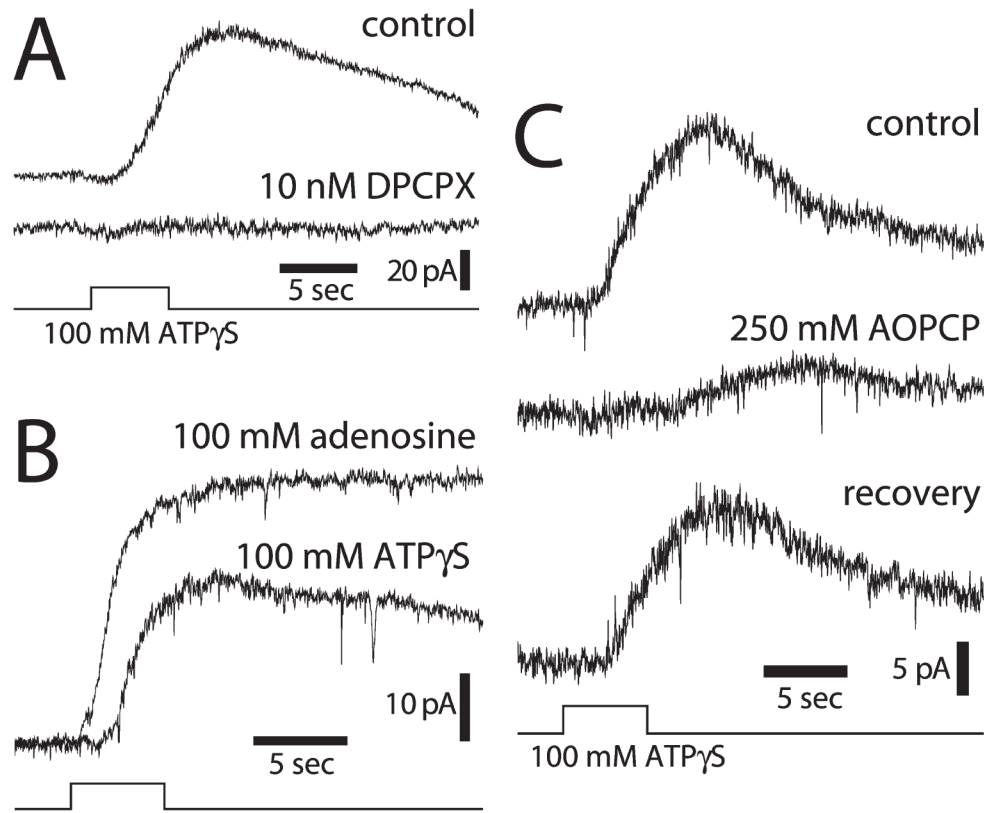
**Fig. 4. Glial facilitation and depression of synaptic transmission**

Light flashes repeated at 5-second intervals evoke EPSCs in ganglion cells. (A) Facilitation of synaptic transmission. Sample ganglion cell EPSCs are shown at the top. EPSC amplitudes are plotted as a function of time in the middle and bottom. Stimulation of glial cells with either ATP $\gamma$ S (middle) or a mechanical stimulus (bottom) results in a transient increase in EPSC amplitude. The two trials are from the same ganglion cell. (B) Depression of synaptic transmission. Sample ganglion cell synaptic currents (an IPSC followed by an EPSC) are shown in the middle. The amplitudes of IPSCs (top) and EPSCs (bottom) are shown. Stimulation of glial cells with ATP $\gamma$ S results in a transient decrease in EPSC amplitude but little change in IPSC amplitude. Unpublished results.



**Fig. 5. Stimulation of glial cells inhibits ganglion cells**

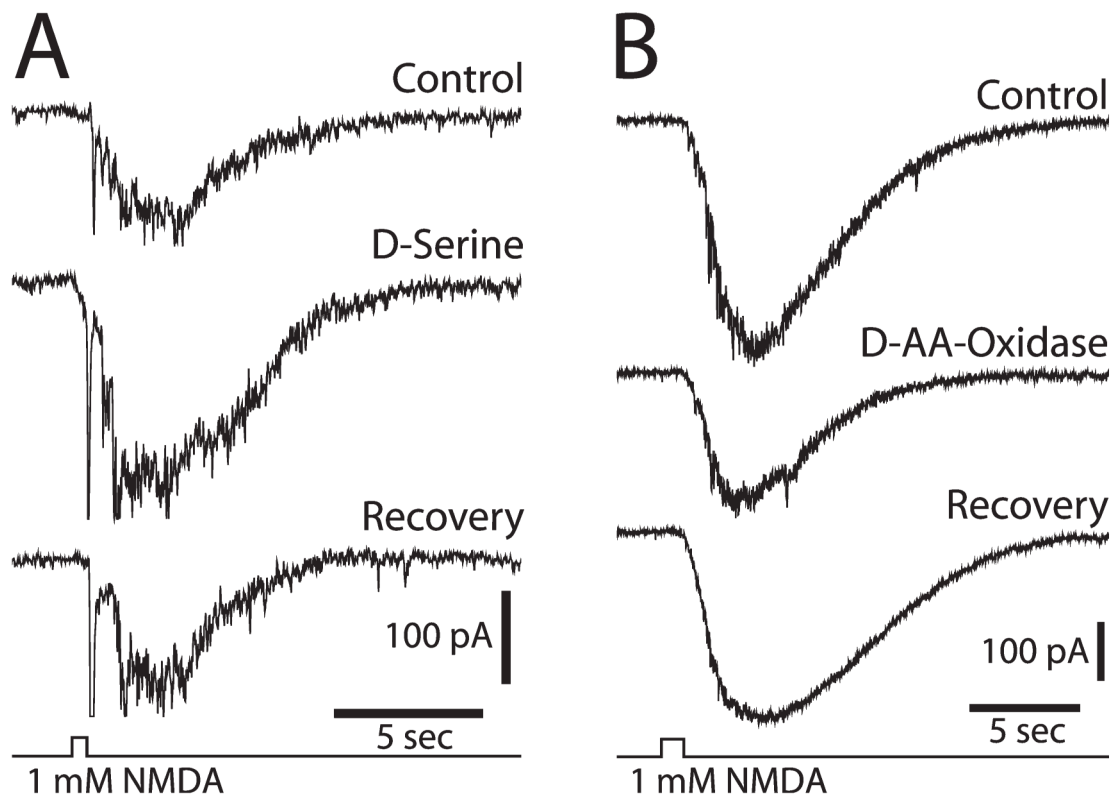
(A) Ejection of ATP $\gamma$ S onto the retinal surface evokes a Ca<sup>2+</sup> increase in glial cells and a hyperpolarization (current-clamp recording) and an outward current (voltage-clamp recording) in a neighboring ganglion cell (Newman, 2003a). (B) ATP $\gamma$ S stimulation of glial cells hyperpolarizes a ganglion cell and blocks spontaneous action potentials in the cell. Unpublished results.



**Fig. 6. Glial inhibition of ganglion cells is mediated by glial release of ATP and activation of neuronal adenosine receptors**

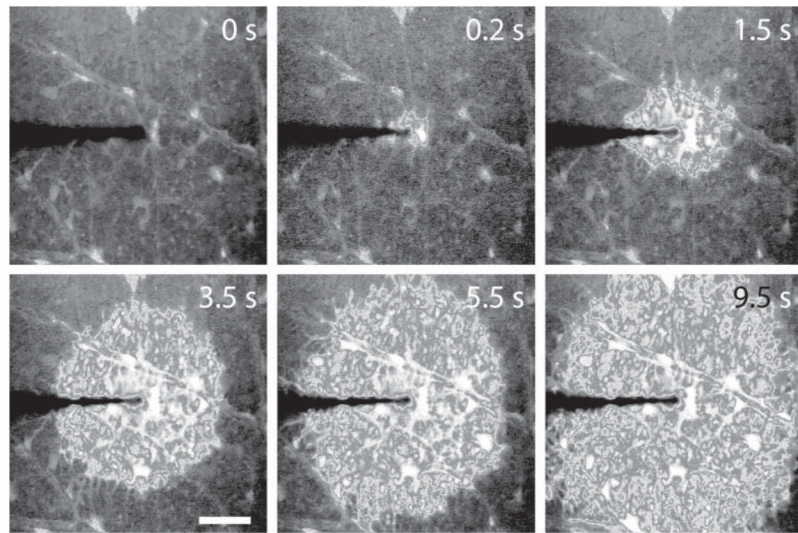
(A) Stimulation of glial cells with ATP $\gamma$ S evokes an inhibitory outward current in a ganglion cell (control), which is abolished by addition of DPCPX, an A<sub>1</sub> adenosine receptor antagonist. (B) Adenosine ejection evokes a larger, shorter-latency current in a ganglion cell than ATP $\gamma$ S ejection at the same retinal location. (C) Addition of AOPCP, an ectonucleotidase inhibitor that blocks the conversion of AMP to adenosine, reduces and slows the time-course of outward ganglion cell current evoked by ATP $\gamma$ S ejection. The effect is largely reversible (Newman, 2003a).





**Fig. 7. Modulation of NMDA receptor transmission by D-serine in the retina**

(A) Addition of D-serine, an NMDA receptor co-agonist, potentiates the inward current recorded from a ganglion cell in response to NMDA ejection. (B) Addition of D-amino-acid-oxidase, an enzyme that degrades D-serine, reduces the inward current evoked by NMDA ejection (Stevens *et al.*, 2003).



**Fig. 8. Intercellular  $\text{Ca}^{2+}$  wave in retinal glial cells**

Mechanical stimulation of an astrocyte evokes an intercellular  $\text{Ca}^{2+}$  wave that propagates through both astrocytes and Müller cells. Calcium fluorescence images at the vitreal surface of the retina are shown. Numbers indicate elapsed time following stimulation. Scale bar, 50  $\mu\text{M}$ . Unpublished results.