Chapter 15

Modulation of neuronal activity by glial cells in the retina

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Introduction

Glial cells and neurons conduct an ongoing dialogue in the central nervous system (CNS). The acutely isolated mammalian retina has been studied in order to investigate interactions between glial cells and neurons in intact CNS tissue. This preparation offers several advantages for such studies. First, as in other in situ preparations, normal morphological relations between glial cells and neurons are maintained in the isolated retina. Second, the eyecup, the entire back half of the eye including the retina, pigment epithelium, choroid, and sclera, can be maintained in a healthy state and remain responsive to light for many hours (Newman and Bartosch 1999). Thus, physiologically appropriate 'natural' stimuli can be used to activate neurons in the preparation. Third, retinal glial cells form a coupled network at the inner (vitreal) surface of the retina and are readily accessible for imaging and electrophysiological studies. In addition, a layer of neurons (ganglion cells and displaced amacrine cells) lies directly beneath this glial cell network and is also accessible for physiological studies.

The mammalian retina has been used as a model of CNS tissue in vivo to address several questions related to glial-neuronal interactions: Are Ca^{2+} signals generated in retinal glial cells and, specifically, do intercellular Ca^{2+} waves travel through the glial cell network in the mammalian retina? What mechanism(s) mediate communication between glial cells in the retina? Do Ca^{2+} increases in glial cells result in the modulation of neuronal activity in the retina? Is this modulation excitatory or inhibitory? What mechanism(s) mediate glial cell modulation of neuronal activity?

Glia of the mammalian retina

Only two types of macroglial cells are generally present in the mammalian retina; astrocytes and Müller cells (Newman 2001a; Fig. 15.1). (In a few species, including rabbit, oligodendrocytes are also present.) Astrocytes (Fig. 15.1, o) are restricted primarily to the nerve fibre layer at the inner border of the retina and form a two-dimensional syncytium with their processes lying parallel to the retinal surface.

Müller cells, the principal glial cells of the retina, are radial glial cells that extend from the inner border of the retina to the photoreceptors in the outer retina. Their endfeet (Fig. 15.1, m) form the inner boundary of the retina; their somata (Fig. 15.1, n) are located in the inner
Fig. 15.1 Cells of the mammalian (dog) retina. A Müller cell (n), the principal glial cell of the retina, is shown at the right. Müller cell endfeet (m) form the inner border of the retina. An astrocyte (o), the second type of retinal macroglial cell, lies in the nerve fibre layer. Retinal neurons include rods (f), cones (e), horizontal cells (g), bipolar cells (h), amacrine cells (i), and ganglion cells (layer H). Retinal layers include the pigment epithelium (A), photoreceptor inner and outer segments (B), outer limiting membrane (C), outer nuclear layer (D), outer plexiform layer (E), inner nuclear layer (F), inner plexiform layer (G), ganglion cell layer (H), nerve fibre layer (I) and inner limiting membrane (J). [Drawing by Ramon Y Cajal; used with permission of the Cajal Institute, Madrid.]
nuclear layer. Müller cells are the only macroglial cell present in most retinal layers and, as such, have assumed many of the functions subserved by astrocytes and oligodendrocytes in other areas of the CNS. Like astrocytes, Müller cells possess a high-affinity glutamate transport system (Bouvier et al. 1992; Lehre et al. 1997) and high levels of glutamine synthetase (Riepe and Norenburg 1977; Linser et al. 1984) and are largely responsible for the removal of glutamate from the synaptic cleft. Like oligodendrocytes, Müller cells have high levels of carbonic anhydrase (Linser et al. 1984; Newman 1994) and may participate in pH regulation in the retina (Newman 1996).

Müller cell processes surround neurons in the retina (Müller and Dowling 1970; Rasmussen 1972; Uga and Smelser 1973). Neuronal somata, including those in the ganglion cell layer (Fig. 15.1h), are surrounded by Müller cell lamellae that form basket-shaped profiles around the cell bodies. Neuronal dendrites and synapses within the synaptic layers (the inner plexiform and outer plexiform layers; Fig. 15.1g and e) are surrounded by additional Müller cell processes. In the nerve fibre layer, ganglion cell axons lie adjacent to Müller cells as well as to astrocytes (Holland et al. 1991; Ogden 1983).

Astrocytes and Müller cells of the mammalian retina are coupled by an extensive series of gap junctions (Robinson et al. 1993; Zahs and Newman 1997). The homotypic junctions between astrocytes and the heterotypic junctions between astrocytes and Müller cells have different chemical coupling properties. Junctions between astrocytes pass the small tracer molecules biocytin and Neurobiotin as well as the larger tracer, Lucifer yellow. Junctions between astrocytes and Müller cells, by contrast, mediate weaker, asymmetric chemical coupling. The tracer Lucifer yellow does not pass through these junctions at all, while biocytin and Neurobiotin are able to pass from astrocytes to Müller cells but not from Müller cells to astrocytes. In addition, electrical coupling between astrocytes and Müller cells is significantly weaker than that between astrocytes (Ceelen et al. 2001). Electrical coupling between astrocytes and Müller cells is, however, non-rectifying (Ceelen et al. 2001).

Homotypic and heterotypic gap junctions in retinal glial cells are also differentially affected by the junction blocker octanol (Zahs and Newman 1997). Octanol (0.5 mM) blocks chemical coupling between astrocytes and Müller cells but only partially blocks chemical coupling between astrocytes. In the presence of octanol, passage of Lucifer yellow between astrocytes is blocked but passage of the smaller tracer Neurobiotin is not.

**Intercellular calcium waves in retinal glial cells**

Propagation of intercellular Ca$^{2+}$ waves through cultured astrocytes has been characterized in a number of laboratories (Finkbeiner 1993; Verkhovsky et al. 1998). A mechanical or chemical stimulus applied to a single astrocyte evokes an increase in intracellular Ca$^{2+}$ in the stimulated cell that then spreads to neighbouring astrocytes in the culture (see Chapter 9). In initial studies it was not clear, however, whether similar intercellular Ca$^{2+}$ waves could be propagated through glial cell networks in vivo.

Ca$^{2+}$ signalling was monitored within glial cells in the mammalian retina in order to address this question. Astrocytes and Müller cells in the rat retina were labelled with a Ca$^{2+}$ indicator dye (the AM-ester of Calcium Green-1 or Fluo-4) and monitored with confocal fluorescence microscopy. The inner surface of the retina (Fig. 15.1i–j) was imaged so that Ca$^{2+}$ levels in the somata and processes of astrocytes as well as in the endfeet of Müller cells could be monitored.
Fig. 15.2 Propagated Ca\textsuperscript{2+} waves and ATP waves in retinal glial cells. (a) An intercellular Ca\textsuperscript{2+} wave evoked by a mechanical stimulus. The Ca\textsuperscript{2+} wave begins at the stimulated astrocyte (a2) and propagates outwards through neighbouring astrocytes and Müller cells. Pseudocolour images show Ca\textsuperscript{2+} indicator dye intensity in retinal glial cells. [Elapsed time following stimulation in panels 1–6: 0 s; 0.2 s; 1.5 s; 3.5 s; 5.5 s; 9.5 s. Scale bar 50 μm.] (b) Higher magnification view of Ca\textsuperscript{2+} wave propagation in control superfusate solution. The Ca\textsuperscript{2+} wave propagates from the stimulated astrocyte (*) into other astrocytes and Müller cells. (c) Calcium wave propagation in the presence of 100 μM suramin. The Ca\textsuperscript{2+} wave propagates from the stimulated astrocyte (*) into other astrocyte somata (arrows) and processes (arrow heads) but not into Müller cells (the blue regions between the astrocytes). In (b) and (c), the stimulating
Stimulation of a single astrocyte soma with a chemical (ATP, carbachol, phenylephrine), mechanical, or electrical stimulus produced a Ca$^{2+}$ increase in the stimulated cell that traveled into adjacent glial cells as a propagated wave (Newman and Zaho 1997; Fig. 15.2a; Movie 1). The wave travelled nearly synchronously into neighbouring astrocytes and Müller cells to a distance as great as 180 $\mu$m and at a velocity of ~23 $\mu$m/s, similar to the propagation velocity of Ca$^{2+}$ waves observed in cultured astrocytes (Finkbeiner 1993).

The Ca$^{2+}$ increases observed during wave propagation in retinal glial cells are generated by release of Ca$^{2+}$ from internal stores rather than from a Ca$^{2+}$ influx across the plasma membrane (Newman and Zaho 1997). Removal of Ca$^{2+}$ from the external bath solution does not diminish Ca$^{2+}$ wave amplitude. By contrast, addition of thapsigargin (1.5 $\mu$M) or cyclopiazonic acid (30 $\mu$M), which deplete internal stores of Ca$^{2+}$, almost abolish the Ca$^{2+}$ increases seen following astrocyte stimulation. In addition, introduction of heparin, an IP$_3$ receptor blocker, into an astrocyte prevents Ca$^{2+}$ from rising in that cell as a Ca$^{2+}$ wave propagates past the cell (Newman and Zaho 1997). This indicates that mobilization of Ca$^{2+}$ from IP$_3$-sensitive internal stores is responsible for the Ca$^{2+}$ increase observed during wave propagation in retinal glial cells.

**Mechanism of calcium wave propagation**

Propagation of Ca$^{2+}$ waves between glial cells is believed to be mediated by one of two mechanisms (see Chapter 9). Propagation was initially thought to occur by diffusion of the internal messenger IP$_3$ through gap junctions (Sanderson et al. 1994; Sanderson, 1996). Stimulating a single glial cell leads to the production of IP$_3$, triggering the release of Ca$^{2+}$ from internal stores in the stimulated cell as well as in adjacent cells. More recently, experiments in culture have shown that Ca$^{2+}$ waves can be propagated between astrocytes, even when the cells do not contact each other directly (Hassinger et al. 1996; Guthrie et al. 1999). This suggests that wave propagation proceeds by the release of an external messenger. A number of studies indicate that the messenger released by astrocytes is ATP (Cotrina et al. 1998; Guthrie et al. 1999; Wang et al. 2000).

Intercellular Ca$^{2+}$ wave propagation has been studied in the rat retina to determine which of these two mechanisms is responsible for wave propagation in retinal glial cells (Newman 2001b). In control superfusate, mechanical stimulation of an astrocyte soma evokes a propagated Ca$^{2+}$ wave that travels through both astrocytes and Müller cells (Fig. 15.2b, Movie 2). If Ca$^{2+}$ wave propagation between astrocytes and Müller cells is mediated by the diffusion of an intracellular messenger through gap junctions, propagation should be compromised by addition of octanol, which blocks junctions between astrocytes and Müller cells (but not between retinal astrocytes). It was found that wave propagation from astrocytes to Müller
cells was not diminished by 0.5 mM octanol (Table 15.1), suggesting that an extracellular messenger mediates signaling between these glial cells.

In retinal whole-mounts, Ca\(^{2+}\) wave propagation is modified by superfusate flow, further implicating an extracellular messenger (Newman 2001b). With superfusate flow turned off, propagation proceeds symmetrically outwards from the point of stimulation. By contrast, with superfusate flow turned on wave propagation is highly asymmetric; it is skewed towards the direction of flow. Intercellular waves travel an average of 236 \(\mu\)m in the direction of superfusate flow but only 95 \(\mu\)m in the opposite direction. This asymmetric propagation demonstrates that an extracellular messenger, carried by the superfusate, is contributing to propagation of Ca\(^{2+}\) waves in the retina.

The nature of the external messenger was investigated by addition of ATP receptor antagonists (Newman 2001b). In the presence of the P2 receptor antagonists suramin (100 \(\mu\)M) and PPADS (20–50 \(\mu\)M), Ca\(^{2+}\) wave propagation from astrocytes to Müller cells was substantially decreased following stimulation of an astrocyte soma (Table 15.1). In many trials, propagation from astrocytes to Müller cells was completely blocked (Fig. 15.2c, Movie 3), while in others propagation into Müller cells was substantially reduced. In addition, apyrase (80 units/ml), an enzyme that hydrolyses ATP, substantially reduced wave propagation from astrocytes to Müller cells. The delay in wave propagation from astrocytes to Müller cells was also increased by the purinergic antagonists (Table 15.1). By contrast, propagation from the stimulated astrocyte to other astrocytes was reduced to a lesser extent by the purinergic antagonists and by apyrase (Table 15.1).

Calcium wave propagation from one Müller cell to another is also mediated by release of ATP (Newman 2001b). When a mechanical stimulus is applied to Müller cell processes within the inner plexiform layer (Fig. 15.1g), a Ca\(^{2+}\) wave that travels radially into neighbouring

### Table 15.1 Effect of agents on Ca\(^{2+}\) wave propagation in the rat eyecup

<table>
<thead>
<tr>
<th>Condition</th>
<th>Astrocyte wave radius ((\mu)m)</th>
<th>Müller cell wave radius ((\mu)m)</th>
<th>Astrocyte → Müller cell delay (s)</th>
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<tbody>
<tr>
<td>Astrocyte stimulation</td>
<td></td>
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</tr>
<tr>
<td>Control</td>
<td>84.7 ± 3.2 (24)</td>
<td>82.2 ± 3.4 (24)</td>
<td>0.85 ± 0.09 (18)</td>
</tr>
<tr>
<td>Octanol (0.5 mM)</td>
<td>100.6 ± 3 (20)*</td>
<td>100.6 ± 3 (20)*</td>
<td>0.99 ± 0.06 (12)</td>
</tr>
<tr>
<td>Suramin (100 (\mu)M)</td>
<td>54.5 ± 6.2 (21)*</td>
<td>12.9 ± 5.2 (21)*</td>
<td>2.38 ± 0.13 (4)*</td>
</tr>
<tr>
<td>PPADS (20 and 50 (\mu)M)</td>
<td>69.0 ± 3.7 (33)*</td>
<td>49.8 ± 6.0 (33)*</td>
<td>2.14 ± 0.14 (22)*</td>
</tr>
<tr>
<td>Apyrase (80 units/ml)</td>
<td>63.3 ± 9.6 (9)*</td>
<td>20.7 ± 6.8 (9)*</td>
<td></td>
</tr>
<tr>
<td>Müller cell stimulation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control (eyecup)†</td>
<td></td>
<td>64.4 ± 2.2 (18)</td>
<td></td>
</tr>
<tr>
<td>Control (slice)</td>
<td></td>
<td>66.0 ± 3.0 (28)</td>
<td></td>
</tr>
<tr>
<td>Suramin (slice, 100 (\mu)M)</td>
<td></td>
<td>32.6 ± 3.5 (22)*</td>
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</tr>
</tbody>
</table>

Calcium waves were evoked by mechanical stimulation of astrocyte somata. Wave radii were measured 10 s after stimulation. In the last column, the time between the onset of a Ca\(^{2+}\) increase in an astrocyte process and the onset an increase in an adjacent Müller cell endfoot is given. Mean ± SEM (n) are given.

* \(p < 0.01\), control vs. test.
† Wave radius measured at the retinal surface. From (Newman 2001b).
Müller cells is evoked at the stimulation site. Propagation of these Müller cell Ca\textsuperscript{2+} waves is substantially reduced by the addition of 100 \textmu M suramin (Table 15.1).

Results from the purinergic antagonist experiments described above indicate that ATP functions as an extracellular messenger during the propagation of Ca\textsuperscript{2+} waves in the retina. This, in turn, suggests that ATP is released from retinal glial cells during the course of Ca\textsuperscript{2+} wave propagation.

We employed the luciferin–luciferase bioluminescence assay to monitor ATP release during wave propagation (Newman 2001b). Following stimulation of an astrocyte soma, a wave of ATP release was observed, as reflected in the generation of bioluminescence, (Fig. 15.2d, Movie 4). ATP release started at the point of stimulation (Fig. 15.2, d2) and propagated radially outwards over time. Comparisons of propagation of ATP release waves with Ca\textsuperscript{2+} waves observed under similar conditions demonstrate that the leading edge of the ATP wave precedes the Ca\textsuperscript{2+} wave by approximately 1 s. Addition of 100 \textmu M suramin substantially reduced the outward propagation of the ATP wave, indicating that the wave is produced by a regenerative mechanism rather than simply reflecting the radial diffusion of ATP released from the stimulated glial cell.

Taken together, these results demonstrate that Ca\textsuperscript{2+} waves are propagated through glial cells in the mammalian retina by two mechanisms. Propagation between astrocytes proceeds primarily by diffusion of iP\textsubscript{3}, an internal messenger, via gap junctions, because it is not greatly reduced by purinergic receptor antagonists or by apyrase. Propagation from astrocytes to Müller cells and from Müller cells to other Müller cells is, by contrast, mediated by the release of ATP, which functions as an extracellular messenger. Propagation between these cells is either completely blocked or is substantially reduced by the purinergic antagonists.

**Glial modulation of neuronal activity**

In cultured astrocytes and in astrocytes of hippocampal slices, increases in intracellular Ca\textsuperscript{2+} result in the release of glutamate which can, in turn, depolarize neighbouring neurons and modulate synaptic signalling between neurons (Hassinger et al. 1995; Araque et al. 1998a,b; Bezzi et al. 1998; Sanzgiri et al. 1999; Innocenti et al. 2000). Experiments have been conducted in the mammalian retina to determine whether increases in glial Ca\textsuperscript{2+} observed during Ca\textsuperscript{2+} wave propagation result in the modulation of neuronal activity in this intact CNS tissue (Newman and Zahs 1998). Experiments were conducted on the rat eyecup (Newman and Bartosch 1999), which remains responsive to light for many hours following isolation.

The retina was stimulated by a repetitively flashing light, and the resulting spike activity of single neurons in the ganglion cell layer (Fig. 15.1h) recorded with an extracellular microelectrode. Displaced amacrine cells as well as ganglion cells may have been recorded, as both are found in the ganglion cell layer (Perry and Walker 1980). Light-evoked activity was recorded for a 30 s control period. A glial Ca\textsuperscript{2+} wave was then elicited by a mechanical stimulus, and propagation of the wave past the recorded neuron was followed with confocal imaging.

The light-evoked spike activity of a majority of the neurons tested was modulated when a glial Ca\textsuperscript{2+} wave propagated past the neuron (Table 15.2). In five of the 53 neurons analysed in detail, spike activity increased with the arrival of a Ca\textsuperscript{2+} wave (Fig. 15.3a, Movie 5). In 25 other cells, an inhibition of activity was observed (Fig. 15.3b).
Fig. 15.3 Glial cell modulation of neuronal activity. (a) Excitatory modulation in an ON-type neuron recorded in the ganglion cell layer. For each of the trials (1–3), a running average of spike frequency is shown in the upper trace and Ca\(^{2+}\) concentration within the glial cells adjacent to the neuron is shown in the lower trace. Vertical arrows mark initiation of the glial Ca\(^{2+}\) wave. In trials 1 and 3, the Ca\(^{2+}\) wave reaches the neuron and an increase in spike frequency is observed. In trial 2, the Ca\(^{2+}\) wave fails to reach the neuron and the spike frequency does not change. (b) Inhibitory modulation in an ON–OFF-type neuron. The display is the same as in a. A decrease in spike frequency is observed when a Ca\(^{2+}\) wave reaches the neuron (trials 1 and 3). No change in spike frequency is seen when the Ca\(^{2+}\) wave fails to reach the neuron (trial 2). (c) The magnitude of inhibitory modulation in retinal neurons is correlated with the amplitude of the Ca\(^{2+}\) increase in glial cells adjacent to the neuron. Each data point represents a single trial in neurons displaying inhibitory modulation. The points at the left (ΔF/F = 0) represent trials in which the Ca\(^{2+}\) wave failed to reach the neuron. [From Newman and Zahs 1998.]
Modulation of neuronal activity was mediated by the arrival of the Ca\(^{2+}\) wave in the glial cells adjacent to the neuron, rather than by the mechanical stimulus used to evoke the wave (Newman and Zahs 1998). In trials in which the wave propagated beyond the neuron (Fig. 15.3, a1, a3, b1, and b3), modulation of neuronal activity was observed. In trials in which a Ca\(^{2+}\) wave was evoked by the mechanical stimulus, but died out before reaching the neuron (Fig. 15.3, a2 and b2), no modulation of activity was seen.

The magnitude of the modulation was substantial in those neurons studied. The change in average spike frequency ranged from 25% to 35% when a large glial Ca\(^{2+}\) wave reached the neuron (Table 15.2). No modulation was observed in the same neurons when a glial Ca\(^{2+}\) wave failed to reach the cell. The magnitude of glial modulation of neuronal activity was, in addition, correlated with the size of the Ca\(^{2+}\) increase in the glial cells adjacent to the neuron (Newman and Zahs 1998). This correlation was analysed for all neurons that displayed significant inhibitory modulation. Greater inhibition of spike activity was observed in those trials in which larger glial Ca\(^{2+}\) increases occurred (Fig. 15.3c).

**Mechanism of glial modulation of neuronal activity**

These results indicate that Ca\(^{2+}\) increases within glial cells result in the modulation of the electrical activity of neighbouring retinal neurons. Pharmacological experiments were conducted to determine the mechanism of this modulation (Newman and Zahs 1998). Efforts so far have been focused on characterizing inhibitory modulation of neuronal activity.

Inhibitory modulation was reduced by the addition of receptor antagonists to GABA and glycine, the two principal inhibitory neurotransmitters of amacrine cells (Table 15.3). In the
Table 15.3 Effect of neurotransmitter antagonists on inhibitory modulation of neuronal activity

<table>
<thead>
<tr>
<th>Condition</th>
<th>Control (%)</th>
<th>Antagonist (%)</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inhibitory neurotransmitter antagonists</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bicuculline (5 µM)</td>
<td>−31 ± 5 (15)</td>
<td>−13 ± 3 (17)*</td>
<td>−26 ± 3 (16)*</td>
</tr>
<tr>
<td>Strychnine (1 µM)</td>
<td>−28 ± 5 (20)</td>
<td>−8 ± 3 (17)*</td>
<td>−25 ± 4 (19)*</td>
</tr>
<tr>
<td>Bicuculline + Strychnine</td>
<td>−28 ± 4 (28)</td>
<td>0 ± 1 (23)*</td>
<td>−20 ± 2 (32)*</td>
</tr>
<tr>
<td><strong>Glutamate antagonists</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NBQX (10 µM)</td>
<td>−25 ± 5 (22)</td>
<td>−6 ± 3 (22)*</td>
<td>−15 ± 2 (23)*</td>
</tr>
<tr>
<td>D-AP7 (200 µM)</td>
<td>−24 ± 5 (21)</td>
<td>−12 ± 4 (18)†</td>
<td>−22 ± 5 (14)</td>
</tr>
<tr>
<td>NBQX + D-AP7</td>
<td>−18 ± 2 (19)</td>
<td>−2 ± 2 (19)*</td>
<td>−12 ± 3 (18)*</td>
</tr>
</tbody>
</table>

Shown are the average modulation values for trials when a Ca\(^{2+}\) wave reached the neuron. Only neurons with significant inhibitory modulation were tested. Antagonists were applied for 15 min prior to testing. Recovery followed a 60 min washout.

* p < 0.01, † p < 0.05, antagonist vs. control, recovery vs. antagonist. From (Newman and Zahs 1998).

Presence of either bicuculline (a GABA antagonist) or strychnine (a glycine antagonist), inhibitory neuronal modulation was reduced substantially. Addition of both antagonists together completely abolished the inhibitory modulation. The effects of the antagonists were partially reversible (Table 15.3).

Similarly, antagonists to glutamate receptors reduced the inhibitory modulation (Table 15.3). Addition of NBQX (an AMPA/kainate antagonist) or D-AP7 (an NMDA antagonist) reduced the modulation, while addition of both antagonists together nearly abolished the inhibitory modulation. These pharmacological results demonstrate that excitatory glutamate transmission as well as inhibitory GABA and glycine transmission are required for glial cell inhibition of ganglion cell activity.

Summary and perspectives

The mechanical stimulus used to evoke glial cell Ca\(^{2+}\) waves in the experiments in the rat retina is decidedly non-physiological. It is not known whether Ca\(^{2+}\) waves or smaller, localized Ca\(^{2+}\) responses occur in glial cells in vivo. In other preparations, including hippocampal slices (Dani et al. 1992; Porter and McCarthy 1996; Pasti et al. 1997), cerebellar slices (Grosche et al. 1999), and the neuromuscular junction (Jahromi et al. 1992); see Chapters 10–12 and 17), neuronal activity leads to Ca\(^{2+}\) increases in neighbouring glial cells. These Ca\(^{2+}\) responses are mediated by the release of transmitters from neurons. A similar mechanism could operate in the retina. It is not known, however, whether neuronal activity in the retina results in Ca\(^{2+}\) increases in retinal astrocytes or Müller cells.

If Ca\(^{2+}\) increases are generated in retinal glial cells in response to neuronal activity, the increases may be smaller in amplitude than those evoked by mechanical stimulation. It remains to be demonstrated whether smaller glial Ca\(^{2+}\) increases can modulate the activity of retinal neurons.
The pharmacological experiments described above demonstrate that both glutamate and GABA/glycine chemical transmitter pathways are involved in glial cell inhibition of neuronal activity. The cells involved in this inhibitory pathway have not, however, been identified. Do astrocytes, Müller cells, or both mediate inhibition? Are inhibitory interneurons involved? The cells and transmitter systems involved in glial cell excitation of neuronal activity must also be determined.

The function of glial modulation of neuronal activity is not known. At the neuromuscular junction, high rates of presynaptic fibre activity evoke Ca\(^{2+}\) increases in perisynaptic Schwann cells (Jahromi et al. 1992) and lead to a decrease in the efficacy of synaptic transmission (Robitaille 1998; see Chapter 17). This glial cell negative feedback loop is responsible for activity-dependent depression at the neuromuscular junction and may function to limit the activation of muscle fibres. A similar glial feedback loop may operate in the retina and may provide either a positive or negative input onto neurons, serving to reinforce or subdue neuronal activity. Glial Ca\(^{2+}\) increases and glial modulation of neuronal activity have relatively slow time courses. Glial contributions to information processing in the retina would thus be limited to the modulation of neuronal responses over a timescale of seconds.

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**References**


