# Regulation of potassium levels by glial cells in the retina

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*Glial cells in the retina play an important role in clearing light-evoked increases in potassium from extracellular space. Glial cells of the honeybee drone retina remove excess $K^+$ by net uptake of the ion and by $K^+$-mediated current flow through intracellular pathways. In the vertebrate retina, the high membrane conductance of the endfoot process of Muller (glial) cells directs excess retinal $K^+$ into the vitreous humor.*

Maintenance of normal brain function is critically dependent on the regulation of potassium concentration in extracellular space ($[K^+]_o$). Small variations in $[K^+]_o$ lead to changes in the membrane potential of neurons and to alterations in neuronal firing thresholds and the release of neurotransmitters, among other processes. The blood-brain barrier buffers the central nervous system (CNS) from variations in blood $[K^+]$. However, $K^+$ homeostatic mechanisms must also operate within the CNS to regulate $[K^+]_o$ levels.

The task of regulating $[K^+]_o$ within the CNS is formidable $K^+$ released from actively depolarizing neurons can produce large changes in $[K^+]_o$ because the total volume of extracellular space is small. In the honeybee retina, for instance, efflux of $K^+$ from depolarizing photoreceptors would raise $[K^+]_o$ to 80 times its normal level if no $K^+$ regulatory mechanisms operated[1].

Several processes help to limit increases in $[K^+]_o$ produced by neuronal activity[2,3]. These mechanisms include diffusion of $K^+$ through extracellular space, active uptake of $K^+$ through the action of an $Na^+$, $K^+$-ATPase, passive $K^+$ uptake by neurons or glia and $K^+$ spatial buffering by cells permeable to $K^+$.

### $K^+$ spatial buffering

The $K^+$ regulatory mechanism of spatial buffering was first proposed by Orkand, Nicholls and Kuffler[4]. As they suggested, $K^+$ enters $K^+$-permeable cells in regions of tissue where $[K^+]_o$ is raised. In order to maintain net electrical neutrality, an equal amount of $K^+$ exits from these cells, or from cells electrically coupled to them, in regions remote from the initial $[K^+]_o$ increase. A return current through extracellular space completes the current pathway between regions of $K^+$ influx and $K^+$ efflux. The net effect of this current flow is to transfer $K^+$ from regions where $[K^+]_o$ is high, to regions where $[K^+]_o$ is lower.

Glial cells are ideally suited to mediate the process of spatial buffering They are almost exclusively permeable to $K^+$ (Ref. 5 and 6) and form large syncytia of cells coupled together by low resistance gap junctions[5,7].

Recent analyses of $K^+$ dynamics indicate that $K^+$ spatial buffering plays an important role in controlling $[K^+]_o$ in the brain[8,9] Gardner-Medwin[9] suggests that spatial buffering dominates over all other $K^+$ clearance mechanisms when regions of increased $[K^+]_o$ are large (>200 μm) and long lasting (minutes). However, studies of $K^+$ dynamics have been limited by the inherent complexity of brain anatomy and physiology Although $K^+$ spatial buffering is believed to be mediated by glial cells in the brain, it has not been possible to demonstrate this function of glia directly.

Studies of $K^+$ dynamics in the retina have, in contrast, supplied direct evidence for the role that glial cells play in regulating $[K^+]_o$ With its limited number of cell types (from which intracellular $[K^+]$ measurements can be made) and its simple patterns of light-evoked $[K^+]_o$ increases, the retina has proved to be a valuable model system for investigating $K^+$ homeostatic mechanisms Studies utilizing the honeybee drone retina and the amphibian retina have, in particular, provided much of our current understanding of how glial cells regulate $[K^+]_o$

### $K^+$ dynamics in the honeybee retina

The retina of the honeybee drone is composed of photoreceptor (retinula) cells and glial (outer pigment) cells. Each ommatidium (facet of the compound eye) contains six large photoreceptors surrounded by several glial cells which are interconnected by low resistance gap junctions, both within and between ommatidia[1,10,11]

Coles, Tsacopoulos and their colleagues have provided a detailed description of $K^+$ dynamics in this simple system. Using fine-tipped, doubled-barrelled microelectrodes, Coles and Tsacopoulos[12] monitored $[K^+]_o$ in the drone retina as well as intracellular $[K^+]$ within photorecep-

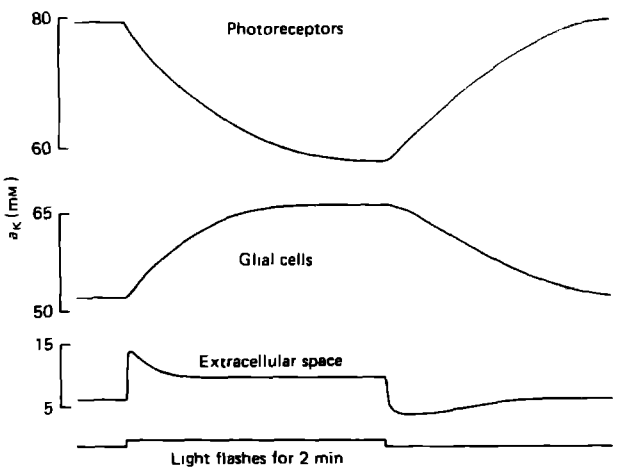


**Fig. 1.** *Summary of $K^+$ level changes that occur in the superfused, cut retina of the honeybee drone during light stimulation Efflux of $K^+$ from the photoreceptors causes a decrease in intracellular $[K^+]$ in these cells (top) Almost all of the $K^+$ released into extracellular space (approximately 99%) enters the glial cells, causing a large increase in $[K^+]$ in the glial syncytium (middle). Both spatial buffering currents and net uptake mechanisms are responsible for the influx of $K^+$ into glial cells Entry of $K^+$ into these cells limits the rise in $[K^+]$ in extracellular space (bottom) to a relatively small increase $K^+$ levels are shown as $K^+$ activities ($a_K$) and represent the mean values measured at each recording site These activities can be converted to concentrations by multiplying by 1 43.* From Coles and Tsacopoulos[12]

tors and glial cells. Their findings are summarized in Fig 1 Drone photoreceptors depolarize in response to light. This depolarization is driven by an influx of $Na^+$ which is accompanied by an approximately equal $K^+$ efflux. This efflux leads to a substantial reduction in $[K^+]$ within the photoreceptor (Fig 1, top)

$K^+$ efflux from the photoreceptors generates a rapid but relatively small rise in $[K^+]_o$ which is maintained for the duration of the light stimulus (Fig 1, bottom) This extracellular increase accounts for only a fraction of the total $K^+$ released by the photoreceptors (approximately 1%) As demonstrated by measurements of intracellular $[K^+]$ in glial cells (Fig 1, middle), nearly all of the remaining $K^+$ released by the photoreceptors enters this intracellular compartment

Influx of $K^+$ into the glial cells could be mediated by passive or active uptake of the ion or by a current flow carried by $K^+$ (spatial buffering). Gardner-Medwin, Coles and Tsacopoulos[1] studied this phenomenon by monitoring $[K^+]_o$ increases and field potentials generated within the drone retina. Their findings, summarized in Fig. 2, demonstrate that light-evoked increases in $[K^+]_o$ establish a current flow which enters glial cells in the region of increased $[K^+]_o$ and exits from regions of the glial syncytium where $[K^+]_o$ is lower (at the cut surface of the retina and in the deep, anoxic retina) The total amount of $K^+$ entering the glial syncytium due to this current flow was estimated from measurements of the extracellular voltage gradient generated by the current The results suggested that a significant fraction of the total $K^+$ influx into the glial syncytium might be attributed to spatial buffering currents

Although the spatial buffering current is carried inwards across the glial membrane by $K^+$, much of the current which flows within the glial syncytium is carried by other ions Thus, $K^+$ should accumulate within glial cells at the site of current influx (This effect is not as pronounced in glial cells of vertebrates, where a larger fraction of intracellular current flow is carried by $K^+$ ) By the same reasoning, internal $K^+$ should be depleted in those regions of the syncytium where spatial buffering current drives $K^+$ outward across glial cell membranes

Coles and Orkand[11] recently tested this prediction by monitoring internal

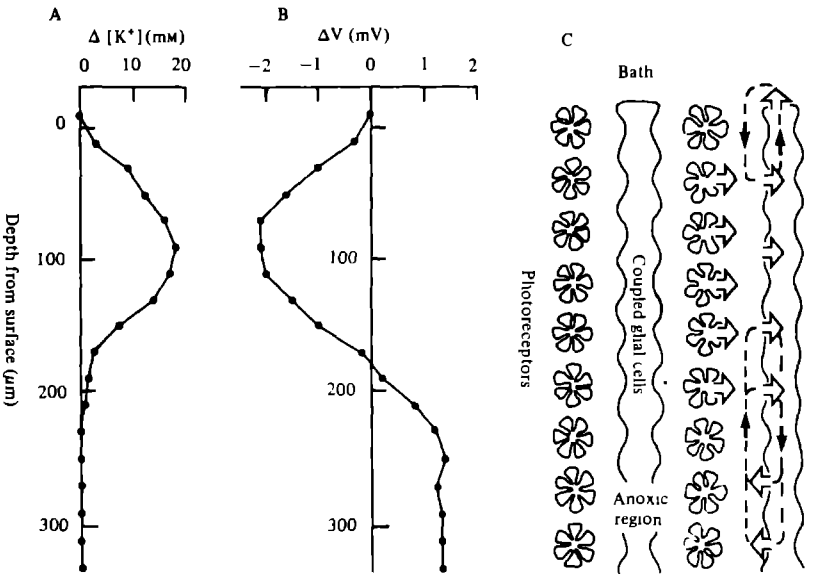


**Fig. 2.** *Spatial buffering currents generated by light-evoked $[K^+]_o$ increases in the cut retina of the drone Measurements made with $K^+$-selective microelectrodes demonstrate that light stimulation generates an increase in $[K^+]_o$ (A) and a negative field potential (B) within the drone retina Both responses reach maximal values at approximately 100 μm below the cut surface of the preparation A schematic representation of the drone eye (C, drawn to the same vertical scale as A and B), illustrates the origin of the field potential $K^+$ released from the photoreceptors (the rosettes in C) enters glial cells in the region of maximal $[K^+]_o$ increase $K^+$ current exits from distant regions of the glial syncytium The two loops of current established by these $K^+$ fluxes (broken lines in C) generate the extracellular field potential measured experimentally (B)* From Gardner-Medwin, Coles and Tsacopoulos[1]

glial cell $[K^+]$ in slices of the drone retina. In contrast to the result expected from the earlier experiments, they found that intracellular $[K^+]$ in glial cells rises, both in regions of efflux of spatial buffering current and in regions of current influx. The increase in intracellular $[K^+]$ at sites of current efflux occurs because net uptake of $K^+$ is greater than current-driven efflux of $K^+$ in these regions. The experimental results indicate that, in at least some circumstances, accumulation of $K^+$ within glial cells in the drone retina is due predominately to net uptake of $K^+$ rather than to spatial buffering This uptake could be driven by either passive or active processes

**The vertebrate retina**

The principal glial cell of the vertebrate retina is the radially oriented Muller cell. This modified astrocyte extends through the full depth of neural retina, from the base of the photoreceptors to the inner (proximal) retinal surface Endfoot expansions at the proximal end of these cells abut the inner limiting membrane and the vitreous humor.

Ion-selective microelectrode measurements have shown that light-evoked increases in $[K^+]_o$ occur in two laminae of the neural retina, the inner plexiform layer and the outer plexiform layer[13,14] (The photoreceptors of the vertebrate retina hyperpolarize in response to light and produce a $[K^+]_o$ decrease )

**Müller cell membrane properties**

Our understanding of the role that Müller cells play in regulating light-evoked $[K^+]_o$ increases in the retina come largely from studies of the membrane properties of these cells Newman[26] measured the membrane permeability of frog Muller cells in a series of ion-substitution experiments He found that the Muller cell membrane potential closely followed the $K^+$ equilibrium potential as $[K^+]_o$ was varied and was insensitive to variations in $[Na^+]_o$ and $[Cl^-]_o$ These results indicate that Muller cells are almost exclusively permeable to $K^+$ and thus are well suited to carry $K^+$ spatial buffering currents

Studies of current flow patterns through Muller cells suggested that the membrane conductance of these cells might be distributed non-uniformly over the cell surface[15,16] Indeed, current source-density analysis of the electroretinogram[17] (ERG, see below) indicated that the endfoot process of Muller cells might contain a large

fraction of total cell conductance

Newman[18,26] measured the regional distribution of Muller cell membrane conductance using enzymatically dissociated Muller cells of the salamander retina. The input resistance of intact dissociated cells was approximately 8 MΩ. However, cell resistance rose to more than 150 MΩ following a microdissection procedure which removed the endfoot process. These measurements demonstrate that approximately 95% of the total membrane conductance of salamander Muller cells is localized in the cell's endfoot process.

Regional differences in membrane conductance were also assessed in the dissociated cell preparation by monitoring passive cell depolarizations generated in response to increases in $[K^+]_o$ over localized regions of the cell surface[18,26] $[K^+]_o$ increases at the proximal face of the endfoot process produced depolarizations 24 to 50 times greater than did increases in other cell regions. These results suggest that the high conductance region of the cell is largely restricted to that portion of the endfoot membrane which, *in situ*, directly apposes the vitreous humor

This remarkably non-uniform conductance distribution influences the flow of spatial buffering currents through Muller cells As we observed for glial cells of the drone retina, $K^+$ enters Müller cells in regions where neuronal activity generates increases in $[K^+]_o$ This current influx is balanced by an equal current efflux from other regions of the cell. Because of the low membrane resistance of the endfoot, almost all of this current is shunted out through the endfoot process

Newman, Frambach and Odette[19] obtained experimental confirmation of this specialized form of $K^+$ spatial buffering. They exposed the distal (photoreceptor) end of dissociated salamander Müller cells to increased $[K^+]_o$ and monitored efflux of $K^+$ from other regions of the cell using ion-selective microelectrodes. Results are shown in Fig. 3 $K^+$ efflux was only detected at the proximal face of the endfoot, indicating that most of the $K^+$ current entering the cell at the site of $[K^+]_o$ increase exited from the endfoot region This finding represents a direct demonstration of the mechanism of spatial buffering

Analysis of light-evoked current flow within the retina[16,17] confirms that this asymmetric pattern of $K^+$ spatial buffering occurs *in situ* The results, summarized in Fig 4, demonstrate that an influx of current into Müller cells occurs at the level of the inner plexiform and outer plexiform layers, where the largest light-evoked $[K^+]_o$ increases are found[13,14]. This influx is balanced by a current efflux at the proximal surface of the retina, the location of the Müller cell endfoot Thus, in the intact retina, as in isolated cells, most $K^+$ spatial buffering current is directed out through the high-conductance endfoot processes.

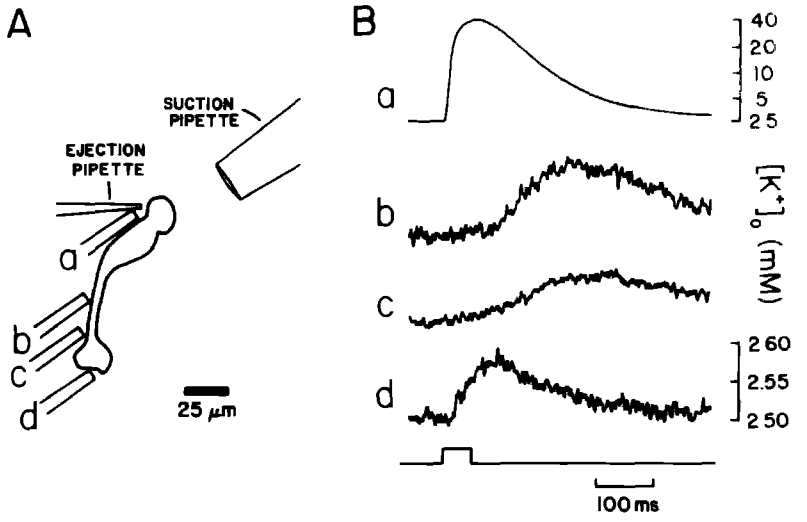


**Fig. 3.** *Experimental demonstration of $K^+$ spatial buffering (A) The distal (photoreceptor) end of an isolated salamander Müller cell is exposed to an increase in $[K^+]_o$ by ejecting an 85 mM KCl solution from an extracellular pipette. Efflux of $K^+$ from the cell is measured at sites (a) through (d) with a $K^+$-selective micropipette (B) A large $[K^+]_o$ increase is recorded at the site of $K^+$ ejection (a) At sites (b) and (c) small, slower increases are measured (note that the vertical gain is much larger in (b) through (d) than in (a)) These increases are due to bulk flow and diffusion of $K^+$ through extracellular space, from the ejection site. Only at site (d), the proximal face of the endfoot, is efflux of $K^+$ from the cell detected The short latency of the endfoot response at site (d) (as short as 4 ms in some cases) demonstrates that the efflux is driven by spatial buffering current rather than by diffusion* From Newman, Frambach and Odette[20]

This directed flow of $K^+$ current, termed $K^+$ siphoning[19], makes spatial buffering a far more powerful process than it would otherwise be. In the absence of an endfoot membrane specialization, spatial buffering by Müller cells would redistribute localized increases in $[K^+]_o$ evenly throughout the retina. However, because of the high conductance of the endfoot, almost all $K^+$ current entering Müller cells in regions of increased $[K^+]_o$ is shunted out through the endfoot process, directly into the vitreous humor. The vitreous, in turn, functions as a large $K^+$ sink, storing excess $K^+$ until retinal $[K^+]_o$ levels return to normal

Newman, Frambach and Odette[19] have assessed the importance of Muller cell spatial buffering using a computer model of $K^+$ dynamics in the retina. Results of the simulation indicate that Müller cell $K^+$ siphoning removes a 1 mM increase in $[K^+]_o$ from the inner plexiform layer of the retina with a one-half clearance time 2 1 s. In contrast, $K^+$ diffusion through extracellular space is less effective, having a one-half clearance time of 7.7 s.

Although the major light-evoked increases in $[K^+]_o$ are restricted to the plexiform layers of the retina, increases also occur in the fiber layer during the conduction of action potentials Hildebrand and Waxman[20] have recently demonstrated that fine processes of Muller cells in the nerve fiber layer of the rat are closely apposed to specialized patches of axon membrane which may function as node-like 'hot spots' of current generation. They suggest that these Müller cell processes help to clear excess $K^+$ from the extracellular space surrounding axons.

As noted above, spatial buffering currents flowing through extracellular space can generate intraretinal and transretinal field potentials. Faber[21] and Miller and Dowling[22] suggested that Müller cell currents arising from $[K^+]_o$ increases at the onset of light lead to the generation of the ERG b-wave (see Fig 4) In this respect, the b-wave and other field potentials of glial origin[23,24], may be viewed as epiphenomena, arising as a consequence of $K^+$ spatial buffering by glia.

Muller cells may also contribute to

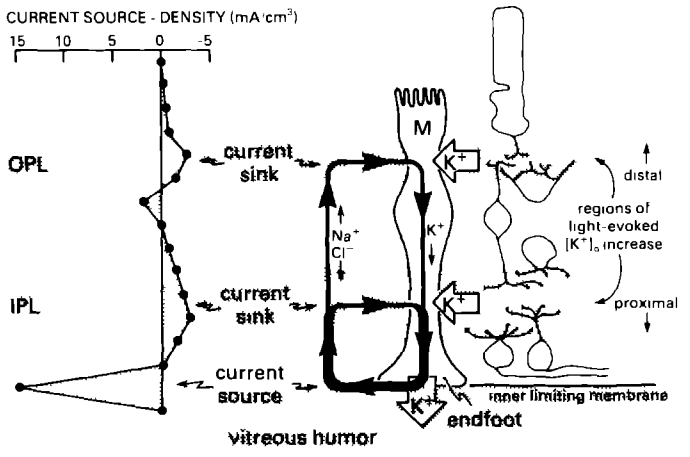


**Fig. 4.** *Spatial buffering currents in the vertebrate retina Results of a current source-density analysis of the b-wave of the ERG (shown at left) demonstrate that the b-wave is generated by two current sinks in the inner and outer plexiform layers and by a current source at the vitreal surface of the retina (data from the frog eyecup preparation) Light-evoked increases in $[K^+]_o$ in the two plexiform layers (shaded regions) cause a $K^+$ influx into Muller cells (open arrows) which are recorded as current sinks Almost all of this $K^+$ current exits from the high conductance endfoot process of the Muller cell The current flow established by these fluxes (solid lines) generates the b-wave The current is carried largely by $K^+$ within the Muller cell and by $Na^+$ and $Cl^-$ in extracellular space The net effect of this directed flow of spatial buffering current is to transfer $K^+$ from the plexiform layers to the vitreous Muller cells and the extracellular space each occupy approximately 5 to 10% of the total volume of the retina M, Muller cell, OPL, outer plexiform layer, IPL, inner plexiform layer* Current source-density data from Newman[18]

$K^+$ homeostasis in the retina by net uptake of $K^+$, although the question remains open[26] Astrocytes[25] and oligodendrocytes[6] in primary culture show net $K^+$ uptake when exposed to increases in $[K^+]_o$. Substantial increases in intracellular $[K^+]$ in Muller cells have been measured during episodes of spreading depression[15]. However, the $[K^+]_o$ increases that occur during such episodes are much larger than those which occur normally

## Summary

Experiments in both invertebrate and vertebrate retinas demonstrate that glial cells are instrumental in clearing activity-dependent $[K^+]$ increases from extracellular space Spatial buffering and, in at least some systems, uptake mechanisms play a role in this process Similar glial cell mechanisms are likely to participate in $K^+$ homeostasis in the brain.

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