

PHYSIOLOGICAL PROPERTIES AND POSSIBLE FUNCTIONS OF MÜLLER CELLS

ERIC A. NEWMAN

Eye Research Institute of Retina Foundation, Boston, MA 02114 (USA)

INTRODUCTION

Müller cells are the principal glial elements of the vertebrate retina. They are long, radially oriented cells which extend from the outer limiting membrane, near the photoreceptor inner segments, to the inner limiting membrane, at the vitreal border of the retina^{1,2}. Their cell bodies lie in the inner nuclear layer. At the inner limiting membrane, Müller cell processes are terminated by endfeet, which form the inner boundary of the retina. Müller cells are a specialized type of radial glial cell; they are similar, in many respects, to astrocytic glial cells³.

In recent years, there has been increasing interest in the properties and functions of Müller cells. Traditionally, these cells were thought to serve as a structural element in the retina and to support the metabolic functions of retinal neurons. During the past decade, studies have indicated that Müller cells may also be responsible for inactivating a number of neurotransmitters in the retina, including glutamate^{4,5}, GABA^{5,6} and acetylcholine⁵. There have also been suggestions that Müller cells are involved in the visual cycle of synthesis and renewal of visual pigments⁷.

Beginning with the work of Miller and Dowling⁸, physiological studies have suggested additional functions for Müller cells. As will be discussed below, Müller cells are now believed to generate several components of the electroretinogram (ERG), including the b-wave, the d-wave and the slow PIII response. They are also thought to participate in the regulation of extracellular K^+ levels ($[K^+]_o$) in the retina.

MEMBRANE PROPERTIES OF MÜLLER CELLS

Membrane ion selectivity. As in other glial cells, the membrane of Müller cells is almost exclusively permeable to K^+ . Ion substitution experiments^{9,10} have demonstrated that these cells behave as almost perfect K^+ electrodes: Müller cell membrane potential follows the K^+ equilibrium potential as external $[K^+]$ is varied. In at least one preparation⁹, the Müller cell membrane is almost 500 times more permeable to K^+ than to Na^+ .

Non-uniform distribution of K^+ conductance

Over the past decade, a number of experiments have indicated that the K^+ conductance of Müller cells is not distributed uniformly over the cell surface, but rather is concentrated in the cell's end-foot process. Based on their work on the generation of the spreading depression potential, Mori, Müller and Tomita^{11,12} suggested that K^+ currents arising during spreading depression flowed preferentially through Müller cell endfeet. I reached a similar conclusion based on current source-density studies of the generation of the ERG b-wave^{13,14}. These experiments indicated that a large source of b-wave current originated from the endfoot, suggesting that Müller cell endfeet had a higher K^+ conductance than the remainder of the cell. More recently, Tomita and his colleagues have studied retinal potentials generated by Müller cells in response to intraretinal injections of K^+ (ref. 15-17). These experiments, as well, support the notion that Müller cell endfeet have high K^+ conductance.

Input impedance measurements. I have tested the hypothesis that there is a non-uniform distribution of K^+ conductance across the Müller cell surface in a number of ways. The most direct test comes from input impedance measurements on dissociated salamander Müller cells^{9,18}. The input resistance of dissociated cells, measured by passing constant current pulses through the recording electrode, was 7.9 Mohms. When the endfoot process of a cell was cut off by a microdissection procedure, however, the resistance of the remainder of the cell rose to 152 Mohms ($n=24$). The results demonstrate that 95% of the total membrane conductance of Müller cells lies in or near the endfoot process.

K^+ ejection responses. The non-uniform distribution of K^+ conductance across the Müller cell surface was confirmed in a second series of experiments where responses of dissociated cells to focal K^+ ejections were monitored^{9,18,19}. A 125 mM KCl solution was ejected from an extracellular pipette in order to raise $[K^+]_o$ over a restricted region of the cell surface. As shown in Fig. 1, a K^+ ejection directed towards the proximal face of the endfoot (trace a) evoked a much larger cell response than did ejections directed towards other cell regions (traces b-f).

It can be shown that the amplitude of a K^+ response to a focal K^+ ejection is directly proportional to the K^+ conductance of that region of cell membrane experiencing the $[K^+]_o$ increase⁹. Thus, the

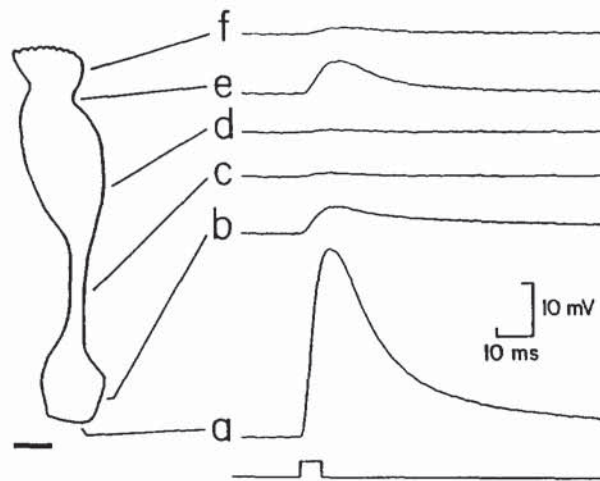


Fig. 1. Responses to focal K^+ ejections recorded from the cell body of a dissociated salamander Müller cell (shown schematically at left). Ejections were directed at: a, proximal face of the endfoot (inner limiting membrane); b, lateral face of the endfoot (nerve fiber layer); c, stalk (inner plexiform layer); d, cell body (inner nuclear layer); e, neck (outer plexiform layer); f, apical end of the cell (rod inner segment layer). Based on experiments such as these, the specific K^+ conductance of the proximal face of the endfoot of Müller cells was estimated to be 63 times larger than that of the cell body. The conductance of the neck region was 6 to 8 times larger than the conductance of neighboring regions. Scale bar for drawing of cell, 10 μ m. From Newman⁹.

K^+ ejection results confirm that the Müller cell endfoot has a much larger K^+ conductance than the remainder of the cell. The results also demonstrate that 1) almost all of the endfoot conductance is localized to that region of the endfoot which faces the vitreous humor in vivo (compare Fig. 1, trace a with trace b), and that 2) the neck of dissociated cells (that region which lies in the outer plexiform layer in situ) has a several-fold larger K^+ conductance than neighboring regions of the cell (compare Fig. 1, trace e with traces c, d and f.) Quantitatively, results from K^+ ejection experiments indicate that, relative to the proximal endfoot, the specific conductance of other regions of the Müller cell are: proximal face of endfoot, 100%; lateral face of endfoot, 18.4%; stalk, 1.8%; cell body, 1.6%; neck, 13.0%; apical end, 2.1%.

Voltage-dependent ion channels

Until recently, Müller cells, like other glial cells, were thought to be electrically passive. During the past two years, however, voltage-dependent ion channels have been described in a number

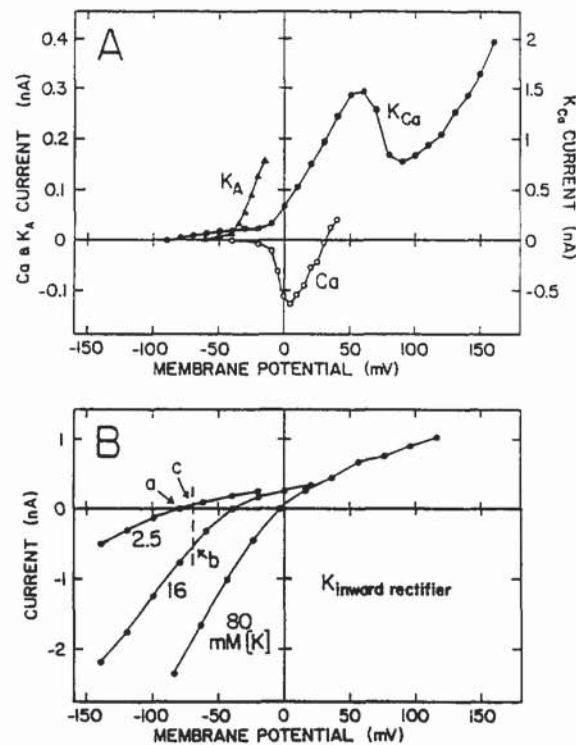


Fig. 2. Current-voltage relations of four voltage-dependent ion currents recorded from dissociated salamander Müller cells. These plots were obtained in whole-cell voltage clamp experiments using single, patch electrodes. (A) Inward Ca^{2+} currents (Ca) and outward Ca^{2+} -activated K^{+} currents (K_{Ca}) were observed when cells were depolarized to past -40 mV. Type-A K^{+} currents (K_{A}) were observed for depolarizations past -60 mV. (B) Inward rectifying K^{+} currents were recorded from a cell bathed sequentially in 2.5, 16 and 80 mM K^{+} perfusate. From Newman²³.

of glial cells in primary cultures²⁰⁻²². Müller cells have similar voltage-dependent channels. For example, if the resting K^{+} conductance of a Müller cell is blocked pharmacologically, the cell can generate action potentials which are based on a voltage-dependent influx of Ca^{2+} current²³.

I have studied the voltage-dependent behavior of Müller cells in a series of voltage clamp studies on dissociated salamander cells. As illustrated in Fig. 2, four voltage-dependent channels were found: a Ca^{2+} channel, a Ca^{2+} -activated K^{+} channel, a type-A K^{+} channel and an inward rectifying K^{+} channel. Of these four voltage-dependent channels, the inward rectifying K^{+} channel is probably the

most important functionally for Müller cells, as it is the only one that is open under normal physiological conditions.

POSSIBLE FUNCTIONS OF MÜLLER CELLS

Generation of the electroretinogram

As suggested by Faber²⁴ and Miller and Dowling⁸, several components of the ERG are believed to be generated by Müller cells. This 'Müller cell' hypothesis of ERG generation predicts that light-evoked changes in $[K^+]_o$ lead to an influx of K^+ into Müller cells and to the generation of a radially directed current flow through the extracellular space of the retina.

The Müller cell hypothesis has received much support since it was originally proposed. 1) Light-evoked responses of Müller cells resemble several components of the ERG, including the b-wave^{8,25}, the M-wave²⁶ and the slow PIII response^{26,27}. 2) Light-evoked changes in $[K^+]_o$, which are thought to generate the radial flow of current through Müller cells, have been measured with ion-selective microelectrodes. A large $[K^+]_o$ increase is seen in the inner plexiform layer while a smaller, transient increase occurs in the outer plexiform layer²⁷⁻³⁰. A $[K^+]_o$ decrease, having the time course of the slow PIII response, occurs at the level of the photoreceptor inner segments^{29,31,32}. 3) Current source-density analysis of the ERG indicates that b-wave^{13,14,24} and slow PIII²⁴ currents flow through Müller cells (see Fig. 3). 4) Computer simulations of ERG currents in the retina demonstrate that, at least in principle, the Müller cell hypothesis can account for the generation of the ERG b-wave and the slow PIII response³³.

High endfoot conductance. The findings that I have presented above on the membrane properties of Müller cells have a direct bearing on the generation of the ERG. According to the Müller cell hypothesis of b-wave generation (summarized in Fig. 3), there is an influx of K^+ into Müller cells within the inner and outer plexiform layers, the sites of light-evoked $[K^+]_o$ increase in the retina. This influx must be balanced by an equal K^+ efflux from other regions of the cell. Because 95% of all cell conductance is localized to the endfoot, most of this current efflux will occur through the endfoot membrane. The return current pathway through extracellular space, from the inner surface of the retina to the two plexiform layers, establishes a transretinal voltage which is recorded as the ERG b-wave.

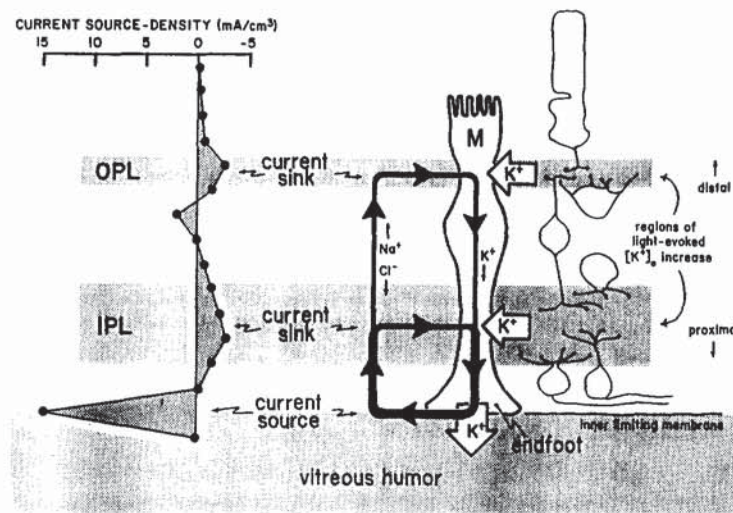


Fig. 3. Generation of the ERG b-wave by Müller cells. The results of a current source-density analysis of the b-wave (shown at left) demonstrated that the ERG response is generated by two current sinks in the inner and outer plexiform layers and by a current source at the vitreal surface of the retina (data from a frog eyecup¹⁴). The current sinks arise as K^+ current enters the cell in regions of light-evoked increase in $[K^+]_o$ (indicated by open arrows at right of fig). Almost all of this current exits from the endfoot of the Müller cell. This efflux is recorded as a current source. The current flow through extracellular space established by these K^+ fluxes (solid lines) generates the b-wave. This pattern of current flow also leads to the transfer of excess K^+ from the plexiform layers to the vitreous humor. M, Müller cell; OPL, outer plexiform layer; IPL, inner plexiform layer. From Newman³⁰.

In this scheme, the high conductance of the endfoot membrane is directly responsible for determining the pathway along which ERG currents travel within the retina. This pathway, in turn, determines the magnitude of the ERG potential. Computer simulations show, for instance, that the Müller cell produces a b-wave response which is five times larger than would be generated if the endfoot membrane had the same specific K^+ conductance as the remainder of the cell³³.

Membrane conductance in the outer plexiform layer. Other specializations of the Müller cell membrane may also influence the generation of the ERG. As described above, that region of the Müller cell which lies within the outer plexiform layer has a significantly larger K^+ conductance than do neighboring regions of the cell. (K^+

ejection experiments, Fig. 1, suggest that in salamanders, the specific conductance of the cell in the outer plexiform layer is 7.5 times the cell conductance in the inner plexiform layer.) This implies that a light-evoked $[K^+]_o$ increase in the outer plexiform layer will give rise to a larger influx of K^+ into Müller cells, and thus, to a larger transretinal potential, than will a similar $[K^+]_o$ increase in the inner plexiform layer.

This phenomenon may help to resolve one of the more important questions concerning the Müller cell hypothesis of b-wave generation (see ref. 30). During b-wave generation, light-evoked $[K^+]_o$ increases are much smaller in the outer plexiform layer than they are in the inner plexiform layer³⁰. Yet, K^+ current influx into Müller cells in the outer plexiform layer is roughly equivalent to the current influx in the inner plexiform layer¹⁴. This apparent contradiction may be explained by regional differences in the membrane conductance of Müller cells; because the membrane conductance is larger in the outer plexiform layer, a relatively small increase in $[K^+]_o$ in this region could give rise to a large current influx.

Inward rectifying K^+ channels. The presence of voltage-dependent ion channels in Müller cells will also influence ERG generation. Under normal physiological conditions, when Müller cells are not depolarized by more than 10 or 20 mV, influx of K^+ into Müller cells will be carried largely by inward rectifying K^+ channels. Because these channels rectify, the magnitude of the current entering a cell for a given increase in $[K^+]_o$ will be larger than the value suggested by the membrane conductance at rest. Based on the current-voltage relation of the inward rectifying channel (Fig. 2, B), the conductance of a region of membrane exposed to 16 mM $[K^+]_o$ will be 4.2 times greater than the membrane exposed to resting (2.5 mM) K^+ (ref. 23).

Regulation of extracellular K^+ levels

K^+ spatial buffering. In 1966, Orkand, Nichols and Kuffler³⁴ suggested that astrocytes might aid in the regulation of $[K^+]_o$ in the brain through the process of K^+ spatial buffering. According to their hypothesis, K^+ enters glial 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. The net effect of these current fluxes is to transfer K^+ from regions where $[K^+]_o$ is high to regions where $[K^+]_o$ is low.

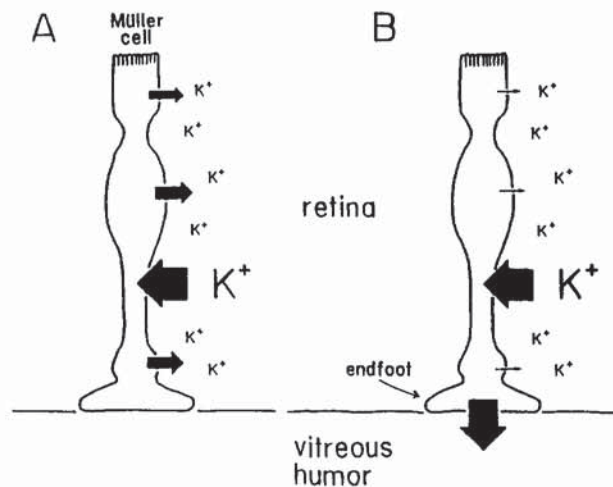


Fig. 4. Diagrams illustrating the process of K^+ siphoning by Müller cells in the retina. (A) If K^+ conductance was distributed uniformly over the cell surface, an increase in $[K^+]_i$ in the inner plexiform layer would cause a K^+ influx into Müller cells in this region (large arrow) and a K^+ efflux from all other regions of the cell (small arrows). (B) Because most of the conductance of the Müller cell is localized to the endfoot process, almost all K^+ efflux will occur from this region. Excess K^+ from the inner plexiform layer will be transferred to the vitreous humor rather than to other retinal layers, as in A. From Newman¹⁹.

Müller cells may play a similar regulatory role in the retina. If K^+ conductance were distributed uniformly over the cell surface, an influx of K^+ into Müller cells in regions of light-evoked $[K^+]_o$ increase would be balanced by an efflux of K^+ in other retinal layers. This is illustrated in Fig. 4A, which shows the distribution of K^+ fluxes that would be present following a $[K^+]_o$ increase in the inner plexiform layer. In this case, the original $[K^+]_o$ increase is dissipated, but at the expense of raising $[K^+]_o$ in other regions of the retina.

Because the K^+ conductance of Müller cells is localized in the endfoot, the actual distribution of K^+ fluxes will be quite different. As shown in Fig. 4B, almost all of the K^+ influx into Müller cells in the inner plexiform layer will be balanced by an efflux from the endfoot membrane. The K^+ exiting from the endfoot will diffuse directly into the vitreous, which will function as a large K^+ sink. This modified form of spatial buffering, termed ' K^+ siphoning',²⁵ has a distinct advantage over the traditionally envi-

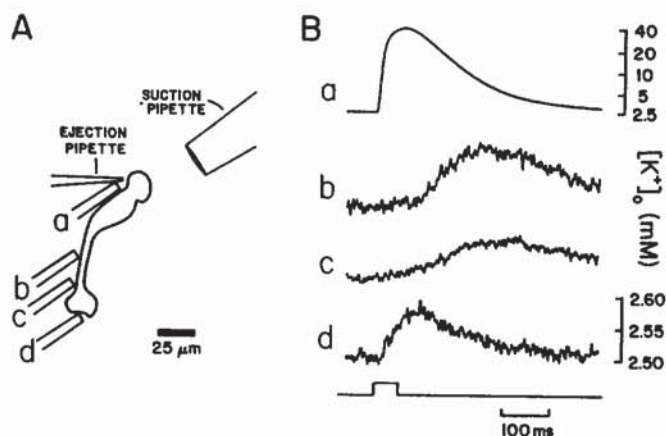


Fig. 5. Experimental demonstration of K^+ siphoning. (A) The apical end of a dissociated salamander Müller cell was exposed to increased $[K^+]_o$ by ejecting an 85 mM KCl solution from an ejection pipette. Efflux of K^+ from other regions of the cell was monitored with a K^+ -selective microelectrode (a through d). (B) Responses recorded from the K^+ -selective electrode. K^+ efflux from the cell was only detected at site d, the proximal face of the endfoot. Traces b, c and d are expanded vertically relative to trace a. From Newman, Frambach and Odette³⁵.

sioned process of spatial buffering: excess K^+ , instead of being transferred into neighboring regions of neural tissue, is deposited in the vitreous, where it will not influence retinal activity.

Demonstration of K^+ siphoning. The process of K^+ siphoning has been demonstrated experimentally using dissociated salamander Müller cells³⁵. As illustrated in Fig. 5, the apical (photoreceptor) end of Müller cells were exposed to increased $[K^+]_o$ by pressure ejecting an 85 mM KCl solution from an extracellular pipette. Efflux of K^+ from other regions of the cells was monitored with an ion-selective microelectrode positioned near the cell surface (Fig. 5A, b through d). Efflux of K^+ was only detected at the proximal face of the endfoot (Fig. 5B, trace d). The results demonstrate that when K^+ enters a Müller cell in a region of $[K^+]_o$ increase, efflux of K^+ from the cell occurs principally from the high conductance membrane of the endfoot.

Membrane specializations and K^+ siphoning. As observed above, Müller cell membrane conductance is larger in the outer plexiform layer than it is in neighboring laminae. This membrane specialization may have evolved in order to make K^+ siphoning by Müller cells more effective. A larger K^+ conductance in the region of a $[K^+]_o$ increase, such as in the outer plexiform layer, will lead to a

greater influx of K^+ into the cell, and thus to better regulation of $[K^+]_o$. At the same time, the K^+ conductance of the cell membrane in other retinal layers remains low, ensuring that most excess K^+ will be siphoned into the vitreous humor instead of into retinal tissue.

The voltage- and K^+ -dependent properties of the inward rectifying K^+ channel will have a similar effect on K^+ siphoning. The conductance of the membrane will increase in those regions of the Müller cell experiencing $[K^+]_o$ increases. This will lead to a greater influx of K^+ . At the same time, the conductance of the membrane will actually decrease in those regions where $[K^+]_o$ does not rise²³, thus reducing the amount of K^+ that will flow into other retinal layers.

Relation between K^+ siphoning and ERG generation

The process of K^+ siphoning and the generation of the ERG are closely related phenomena. As is illustrated in Fig. 3, the currents that arise due to the influx of K^+ into Müller cells in regions of increased $[K^+]_o$ are responsible for the siphoning of K^+ out through the endfoot and into the vitreous humor. These same currents, flowing radially through extracellular space, are responsible for generating components of the ERG. In this respect, the b-wave, and other ERG components of Müller cell origin, may be thought of as epiphenomena, arising as a consequence of the process of K^+ siphoning by Müller cells³⁶.

CONCLUSION

During the past few years we have learned that the physiological properties of Müller cells are far more complex than originally thought. The K^+ conductance of these cells is distributed in a highly non-uniform manner over the cell surface. A large fraction of the total cell conductance is localized to that portion of the endfoot which faces the vitreous humor. Along the remainder of the cell, the membrane conductance is larger in the outer plexiform layer than in neighboring laminae. The cell also possesses four types of voltage-dependent ion channels, including an inward rectifying K^+ channel. These membrane specializations play an important role in the generation of ERG components and in the regulation of $[K^+]_o$ in the retina.

ACKNOWLEDGEMENTS

Research in my laboratory is supported by National Institutes of Health grant EY 04077.

REFERENCES

1. Ramon y Cajal S (1972) The Structure of the Retina. Charles C Thomas, Springfield, Illinois
2. Pedler C (1963) Exp Eye Res 2:296-303
3. Newman EA (1986) In: Fedoroff S, Vernadakis A (eds) Astrocytes. Academic Press
4. Ehinger B (1977) Exp Eye Res 25:221-234
5. Sarthy PV, Lam DMK (1978) J Cell Biol 78:675-684
6. Sarthy PV (1982) J Neurosci Meth 5:77-82
7. Bridges CDB, Alvarez RA, Fong S-L, Gonzalez-Fernandez F, Lam DMK, Liou GI (1984) Vision Res 24:1581-1594
8. Miller RF, Dowling JE (1970) J Neurophysiol 33:323-341
9. Newman EA (1985) J Neurosci 5:2225-2239
10. Connor JD, Detwiler PB, Sarthy PV (1985) J Physiol 362:79-92
11. Mori S, Miller WH, Tomita T (1976) Proc Natl Acad Sci USA 73:1351-1354
12. Mori S, Miller WH, Tomita T (1976) Jap J Physiol 26:203-217
13. Newman EA (1979) Vision Res 19:227-234
14. Newman EA (1980) J Neurophysiol 43:1355-1366
15. Fujimoto M, Tomita T (1981) Brain Res 204:51-64
16. Yanagida T, Tomita T (1982) Brain Res 237:479-483
17. Yanagida T, Tomita T (1984) Jpn J Ophthalmol 28:215-221
18. Newman EA (1984) Nature 309:155-157
19. Newman EA (1986) N Y Acad Sci, in press
20. Chiu SY, Schrager P, Ritchie JM (1984) Nature 311:156-157
21. MacVicar BA (1984) Science 226:1345-1347
22. Bevan S, Raff M (1985) Nature 315:229-232
23. Newman EA (1985) Nature 317:809-811
24. Faber DS (1969) Buffalo: State University of New York (Ph.D. Thesis)

25. Zimmerman RP, Corfman TP (1984) *Neurosci* 12:77-84
26. Karwoski CJ, Proenza LM (1977) *J Neurophysiol* 40:244-259
27. Dick E, Miller RF (1985) *J Gen Physiol* 85:885-909
28. Kline RP, Ripps H, Dowling JE (1978) *Proc Natl Acad Sci USA* 75:5727-5731
29. Karwoski CJ, Proenza LM (1978) *Brain Res* 142:515-530
30. Karwoski CJ, Newman EA, Shimazaki H, Proenza LM (1985) *J Gen Physiol* 86:189-213
31. Oakley B II, Green DG (1976) *J Neurophysiol* 39:1117-1133
32. Steinberg RH, Oakley B II, Niemeyer G (1980) *J Neurophysiol* 44:897-921
33. Newman EA, Odette LL (1984) *J Neurophysiol* 51:164-182
34. Orkand RK, Nicholls JG, Kuffler SW (1966) *J Neurophysiol* 29:788-806
35. Newman EA, Frambach DA, Odette LL (1984) *Science* 225:1174-1175
36. Newman EA (1985) *Trends in Neurosci* 8:156-159