General principles of K^+-dependent transretinal potentials generated by Müller cells.

It is unquestionable that glial Müller cells (MCs) in the vertebrate retina *can* generate transretinal potentials in response to changes of extracellular K^+ concentration ($[K^+]_o$) resulting from light-induced activity of the photoreceptors and postreceptor neurons. The question is *how large* these glial extracellular potentials are? Due to the quantitative nature of this question, it seems beneficial to review the general principles that govern the generation of extracellular voltages by MCs. For convenience we first examine a simple case when $[K^+]_o$ changes happens near one end of the MCs and then a more complicated case when $[K^+]_o$ changes take place somewhere else along the cell inside the retina.

<u>K^+ changes near the end of MCs</u>

The first case is related to the mechanism of generation of the slow PIII (sPIII) subcomponent of the ERG. The light-induced photoreceptor activity is accompanied by a slow decrease of extracellular K^+ concentration ($[K^+]_o$) that hyperpolarizes the distal part of MCs, which in turn causes a cornea-negative sPIII (Oakley & Green, 1976; Tomita, 1976; Steinberg *et al.*, 1980; Bykov *et al.*, 1981). Fig S1.1A presents a simplified equivalent electrical schema that quantifies the relationships between the light-induced $[K^+]_o$ decrease, the MC hyperpolarization, and sPIII. In the schema, R_o is the transretinal resistance of the extracellular space of the retina per area occupied by one MC. R_{m2} is the resistance of the MC membrane in the distal retinal layers (near the photoreceptors) where $[K^+]_o$ changes take place. R_{m1} is the resistance of the MC membrane in the proximal layers where $[K^+]_o$ remains unchanged. For simplicity, the intracellular resistance of the MC cytoplasm is ignored here. The battery E represents the alteration in K^+ equilibrium potential during $[K^+]_o$ changes in the distal retina; it is defined by the modified Nernst equation:

$$E = (RT/F)\ln([K^+]_L/[K^+]_D),$$

where $[K^+]_L$ is the extracellular K^+ concentration during light and $[K^+]_D$ is the extracellular K^+ concentration in the dark. Since $[K^+]_o$ is decreasing here, the battery hyperpolarizes the MC membrane and produces the current:

$$I = E /(R_o+R_{m1}+R_{m2})$$

that flows in the direction marked by arrows near the resistances in the schema. The polarity of the extracellular voltage during illumination ($V_o = I*R_o$) depends on which side of the retina is grounded. With conventional grounding for electroretinography of the photoreceptor (scleral) side (point 2 in Fig. S1.1A), V_o has the cornea-negative polarity of sPIII. The voltage at the proximal extracellular point 1 is higher than at the intracellular point 3, which means that the distal K^+- induced hyperpolarization spreads to proximal parts of the MC ($V_{m1}=I*R_{m1}$). The distal hyperpolarization (between points 2 and 3) is the largest potential in the circuit ($V_{m2}=V_o+V_{m1}$); also

$$V_{m2} = E - I*R_{m2}$$

or

$$V_{m2} = E * (1 - R_{m2} / (R_o+R_{m1}+R_{m2})).$$

The last two equations are the keys to understanding the K^+-dependent potentials generated by MCs. First, the MC apparently cannot be an ideal K^+-selective electrode since the maximal transmembrane response (V_{m2}) is smaller than expected from the $[K^+]_o$ changes (E) by the value of the voltage drop produced by the current flowing across the corresponding part of the MC membrane ($I*R_{m2}$). On the other hand, the MC still can be a fairly good K^+-selective electrode, at least locally: the smaller the R_{m2} relatively to other resistors, the closer the ratio of $R_{m2}/(R_{m1}+R_{m2}+R_o)$ to 0, and V_{m2} to E. And the larger V_{m2}, the larger V_{m1}. As shown in Fig. S1.1B,

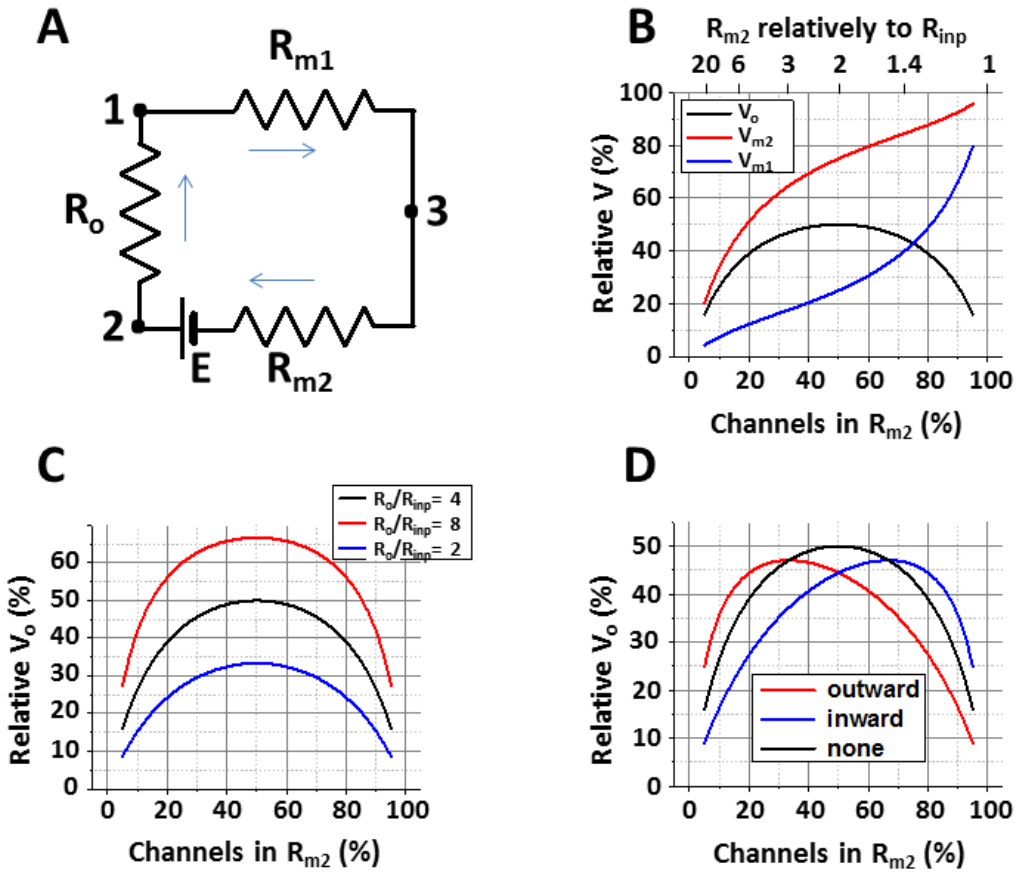

Fig.S1.1. Quantification of sPIII generation by MCs in response to light-evoked photoreceptor-induced changes of $[K^+]_o$ in the distal retina. *A:* the equivalent electrical schema; the definitions of the circuit elements are in the text. *B:* dependence of the extracellular transretinal potential (V_o, as a percentage of E) and transmembrane hyperpolarization in proximal and distal parts of the MC (V_{m1} and V_{m2}, respectively, as a percentage of E) on the distribution of K^+ conductance between the distal and proximal parts of the MC. The distribution of K^+ conductance is expressed as the percentage of the channels that are in R_{m2} (i.e., moving to the right represents lower values of R_{m2} and larger values of R_{m1}). The relative value of R_{m2} is presented on the top for reference. The input resistance of the MC (R_{inp}) remains constant and the ratio R_o/R_{inp} is set at 4. *C*: effect of the distribution of K^+ conductance on amplitude of the transretinal potential (V_o) with different ratios of R_o/R_{inp}. *D*: effect of the distribution of K^+ conductance on amplitude of the transretinal potential (V_o) in the presence of inward and outward rectification. For inward rectification the transmembrane resistors have half the values for inward current and twice the values for outward current compared to the non-rectified channels that were used for calculations in parts B and C; for outward rectification the resistance values were reversed.

both V_{m2} and V_{m1} increase when K^+ conductance is concentrated in the area of K^+ changes (i.e. R_{m2} decreases). Accordingly, it is possible to determine the distribution of K^+ conductance along

isolated MCs by puffing K^+ onto different parts of the cell and recording intracellular responses. Using that method, it was determined that in amphibian MCs the majority (up to 95%) of the cell K^+ conductance was concentrated in its endfoot (an enlargement of the proximal end of the MC that faces the ILM), while in mammalian retinae other parts of the cell can have a similar or even larger K^+ conductance than the endfoot (Newman, 1984, 1985, 1987).

The extracellular potential (V_o), in contrast to intracellular ones, does not increase when K^+ conductance concentrates in the area of $[K^+]_o$ changes. Instead, V_o (Fig.S1.1B, black line) is at its maximum when this area holds exactly half of the conductance and consequently $R_{m1} = R_{m2}$. In real MCs with definite input resistance (R_{inp}), making the distribution of K^+ conductance more asymmetric leads to an increase of the sum $R_{m1}+R_{m2}$. When the input resistance is measured, the transmembrane resistors are connected in parallel, so the cell with $R_{m1} = R_{m2} = 20$ MΩ and the cell with $R_{m1} = 100$ MΩ and $R_{m2} = 11.1$ MΩ both have $R_{inp} = 10$ MΩ, since $R_{inp} = 1/(1/R_{m1} + 1/R_{m2})$. But for the current generated by the K^+ battery, the transmembrane resistors are connected in series, so $R_{m1}+R_{m2}$ will be 40 MΩ in the first case and 111.11 MΩ in the second. Thus, the largest current can be generated when the sum $R_{m1}+R_{m2}$ is smallest, which is when $R_{m1}=R_{m2}$. In that situation, $(R_{m1}+R_{m2})/R_{inp} = 4$, the minimum value of this ratio. And this is the most favorable distribution of K^+ conductance for generating the largest possible extracellular potential.

Obviously, the extracellular V_o depends on the value of the extracellular resistor R_o, or more accurately on its value relative to R_{inp} (Fig. S1.1C). Theoretically, in favorable conditions, i.e. when R_o is significantly greater than R_{inp} and $R_{m1} \approx R_{m2}$, the glial potential V_o could exceed 75% of the voltage of the K^+ battery E. The former ($R_o > R_{inp}$) is expected (which is, by the way, a very important condition for an effective K^+ spatial buffering). For instance, as stated in the main paper, the best fit between experimental and theoretical data was found with $R_o = 160$ mΩ, and $R_{inp} \sim 20$ mΩ. But the effective extracellular resistance for the glial current is much smaller than the total transretinal R_o because only a small fraction of this current runs from one end of the MC

to another and most of the current loops are shorter and flow through only a part of that distance. More importantly, the area of the MC membrane exposed to $[K^+]_o$ changes is much smaller than the rest of the cell, and, accordingly, R_{m2} is not equal to, but significantly larger than R_{m1}. As a result, the maximal amplitude of sPIII experimentally recorded in isolated mouse retina is only about 25% of E calculated from simultaneously recorded light-induced $[K^+]_o$ changes (see the main paper). And this is with a relatively uniform distribution of K^+ conductance along MCs, as demonstrated in mouse. Much smaller sPIII could be expected if the K^+ conductance was highly unevenly distributed similar to what was demonstrated for isolated amphibian MCs.

To complete this part, the rectifying properties of the channels that comprise the K^+ conductance of the MCs are taken into consideration. MCs possess K^+ channels of different types, but the voltage-gated inwardly rectifying K^+ channels, Kir, dominate (Newman, 1993; Newman & Reichenbach, 1996). It should be remembered that in physiological conditions when light-induced changes of $[K^+]_o$ rarely exceed 1-2 mM there is not a large difference in the conductance of the K^+ channels for inward and outward currents. Still this is an interesting theoretical case to explore.

When MCs respond to changes of $[K^+]_o$ they generate a current which is both inward and outward (Fig S1.1A) in different parts of the cell. Inward rectification can be interpreted as a lower channel resistance for current directed into the cell and a higher channel resistance for current directed out of the cell. To simulate inward rectification with a factor of 4, the value of R_{m1} (outward current) was doubled and the value of R_{m2} (inward current) was halved (Fig. S1.1D, blue line). Not surprisingly, under these conditions V_o was not largest when 50% of the channels were in R_{m1} but occurred when more channels were in R_{m2}. The mirror image of this graph was obtained when outward rectification with a factor of 4 was assumed. If instead of a $[K^+]_o$ decrease an increase was simulated, the current would be reversed and the graph for inward rectification would be the same as the one for outward rectification with a decrease of $[K^+]_o$. To summarize,

rectification (regardless of direction) affects the extracellular voltage generated by MCs, increasing it in some range of relative R_{m2} and decreasing in another range, but the effect is modest compared to effects of changes in relative values of R_o, R_{m1} and R_{m2}.

<u>K^+ changes somewhere in the middle of MCs</u>

In this part a hypothetical mechanism of the MC's contribution to the b-wave will be explored. Similar to the previous example with sPIII, MCs are expected to react to increases of $[K^+]_o$ evoked by the retinal neurons, but it is more complicated, since these K^+ changes do not happen near an end of the MC, but somewhere else along the cell. For the simplest case of a single localized K^+ increase, one more transmembrane element has to be added to the minimal equivalent electrical schema (Fig. S1.2A), and the current generated by the K^+-dependent battery E is now branching (the directions of the flow are marked in the schema by arrows near the resistances). In the ideally symmetrical circuit (i.e. $R_{m1}=R_{m3}$ and $R_{o1}=R_{o2}$) the current is divided equally between two directions of flow:

$$I_1 = I_2 = E / (2*(R_{m2} + 1/(1/(R_{m1}+R_{o1}) + 1/(R_{m3}+R_{o2}))))$$

and the depolarizations in the distal ($V_{m3}=I_2*R_{m3}$) and proximal ($V_{m1}=I_1*R_{m1}$) parts of the MC will be equal. The distal and proximal components of the extracellular potential (V_{o2} and V_{o1}, respectively) will also be equal in absolute values, but with opposite signs, and the resulting transretinal potential (V_o) will be zero. In real life, the circuit is never symmetrical, and the unevenly branched currents run through unequal extracellular and transmembrane resistors. The value and the polarity of the resulting transretinal voltage, V_o, depends on three factors: 1) the value of R_{m2} relative to other resistors that determine the amplitude of the MC depolarization in the area of the $[K^+]_o$ increase, V_{m2}, 2) the ratio of resistances for the proximal and distal currents ($R_{m1}+R_{o1}$ vs $R_{m3}+R_{o2}$) that determines how the K^+ current branches, and 3) the ratios of

extracellular and transmembrane resistors (R_{o1}/R_{m1} and R_{o2}/R_{m3}) that determine the fractions of V_{m2} which are manifested extracellularly as V_{o1} and V_{o2}.

These relations are illustrated in Fig. S1.2B, where intra- and extracellular voltages are plotted against the fraction of K^+ conductance in the proximal part of the MC, i.e. in R_{m1}. This part includes the MC endfoot, which is recognized as critically important for MC electrophysiology, but $[K^+]_o$ hardly changes in this area. Other assumptions for Fig. S1.2B are that $R_{o1} = R_{o2} = R_{inp}$ and $R_{m2} = R_{m3}$. If the total number of K^+ channels is constant, the accumulation of the K^+ channels in the proximal part of the MC means that R_{m1} decreases but R_{m2} and R_{m3} increase. The larger R_{m2}, the smaller the depolarizations in all parts of the MC, as in the previously described case of sPIII generation. Additionally, the redistribution of K^+ conductance between the proximal part of the MC and the rest of the cell leads to changes in branching of the current. When only a small fraction of the K^+ conductance is localized in the proximal part of the MC, R_{m1} is large and R_{m3} is small. So, the proximal current (I_1) is smaller than the distal (I_2), the cornea-positive voltage in the proximal part of the retina (V_{o1}) is smaller than the cornea-negative voltage in the distal part of the retina (V_{o2}), and the total transretinal potential is negative. When the K^+ conductance is shifted to the proximal part of the MC, R_{m1} decreases, R_{m3} increases, and more current shifts to the proximal direction. At some point (when 33.3% of the conductance is in the proximal part, i.e. $R_{m1}=R_{m2}=R_{m3}$), $I_1=I_2$ and $V_o=0$. After this point the total transretinal potential begins to be cornea-positive, like the b-wave of the ERG, but its amplitude, as well as other voltages, decreases when too much K^+ conductance is localized in the proximal end. Concentration of 95% of the total conductance in the proximal part seems extreme, but that is what was suggested for just the endfoot of amphibian MCs based on measurements on isolated cells, as indicated earlier.

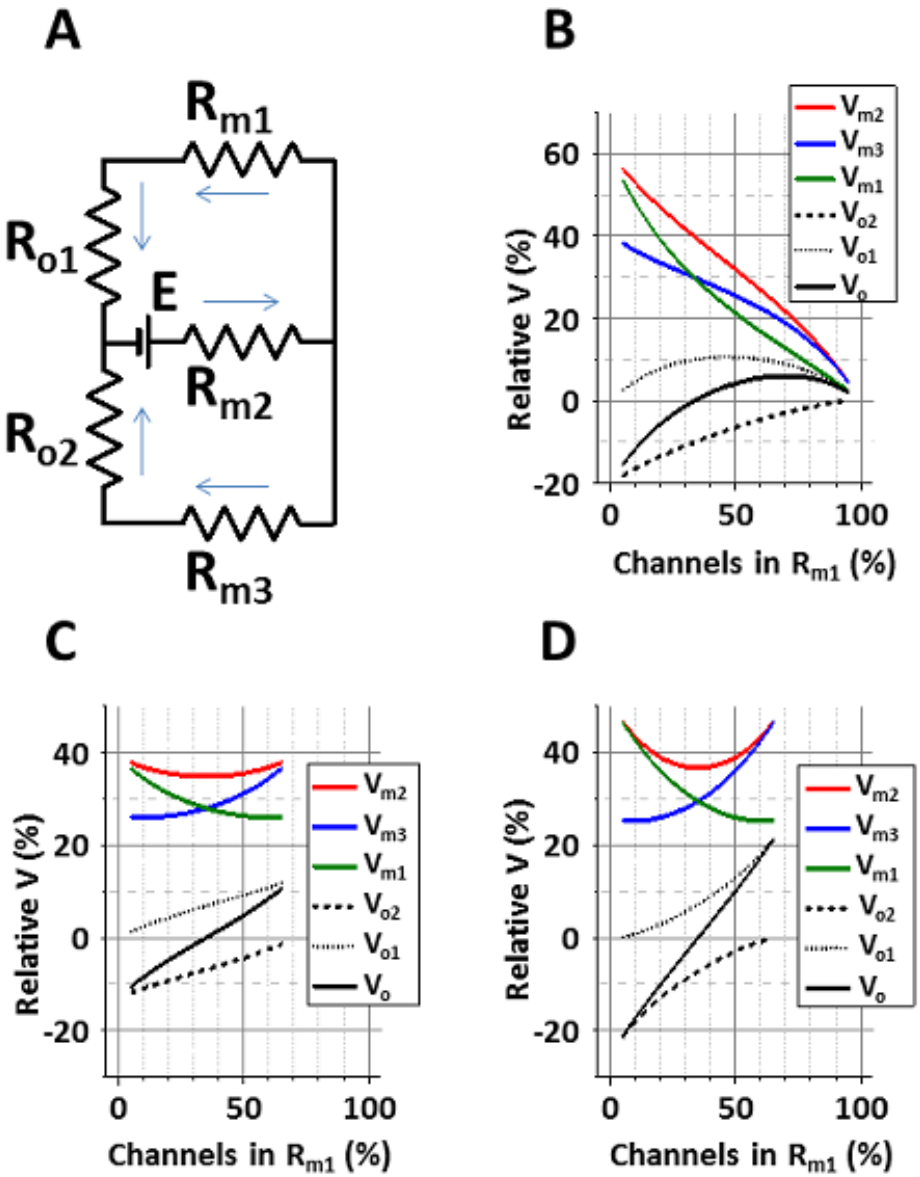

Fig.S1.2. Quantification of MC responses to an increase of $[K^+]_o$ in the inner layers of the retina. *A:* the equivalent electrical schema; the definitions of the circuit elements are in the text. The polarity of the battery is reversed relative to Fig. S1.1 because the increase in $[K^+]_o$ makes the cell relatively more positive. *B:* effects of the concentration of K^+ conductance in the proximal part of MC on the extracellular potentials proximally and distally to the area of $[K^+]_o$ changes (V_{o1} and V_{o2}, respectively), total transretinal potential (V_o), and transmembrane depolarizations in proximal, middle and distal parts of the MC (V_{m1} V_{m2}, and V_{m3}, respectively). The distribution of K^+ conductance is expressed in the percentage of channels that make R_{m1}; the remainder of the channels is divided equally between R_{m3} and R_{m2}. The input resistance of the MC is constant and $R_{inp}=R_{o1}=R_{o2}$. *C*: similar to *B*, but R_{m2} is fixed (contains 30% of the K^+ channels) and a decrease in R_{m1} leads to an increase only in R_{m3}; also, $R_{o1}=R_{o2}=R_{inp}*0.7$. *D*: similar to *C*, but R_{o1} and R_{o2} also change proportionally to the fraction of K^+ channels in the proximal part of MC: $R_{o1} = 0$ and $R_{o2}= R_o$ with no channels in R_{m1} ($R_{m1}=0$), but $R_{o1} = R_o$ and $R_{o2} = 0$ with 70% of the channels in R_{m1}. R_o (i.e. $R_{o1}+ R_{o2}$) is constant and equal to $R_{inp}*1.4$.

The effect of redirecting K^+ current and changing the polarity of the extracellular potential due to redistribution of K^+ conductance along the MC is more clearly demonstrated in Fig. S1.2C. For these calculations R_{m2} is assumed to be constant (30% of total conductance). In such a condition V_{m2} is about the same regardless of how the remainder of the transmembrane conductance (70%) is divided between the distal and proximal parts. But the transretinal potential is strongly affected when R_{m1} decreases and R_{m3} increases, and its sign changes from cornea-negative to cornea-positive. This theoretical setting is a bit similar to a situation where the source of the $[K^+]_o$ increase shifts from the MC endfoot toward its distal end. One unrealistic assumption here is that the extracellular resistances distally and proximally to the source of $[K^+]_o$ changes remain constant. In a more reasonable case presented in Fig. S1.2D a shift of the K^+ increase toward the distal part of the MC is simulated by not only a relative increase in proximal K^+ conductance, but also an increase in proximal extracellular resistance and a decrease of distal extracellular resistance, with their sum (R_{o1}+R_{o2}) remaining constant. These assumptions have effects on several things, but the most important for extracellular potentials is that the maximal ratios R_{o2}/R_{m3} (at 5% of the K^+ channels in R_{m1}) and R_{o1}/R_{m1} (at 65%) here are about 2 times larger than in Fig 2C. Accordingly the range of V_o changes is approximately doubled.

The calculations described above illustrated how three factors – 1) the location of the $[K^+]_o$ increase, 2) the distribution of K^+ conductance along the MC and 3) the extracellular resistances of the retinal layers - determine not only the value but also the polarity of the transretinal potential generated by MCs. In the vertebrate retina, there are actually two light-evoked $[K^+]_o$ increases, one in more distal and another in more proximal layers, as discussed in the main paper. The *proximal* $[K^+]_o$ is large in amplitude and covers a broad area of the MC membrane in both the IPL and INL, but it is badly situated for generation of a cornea-positive extracellular potential that can contribute to the b-wave of the ERG. Its location suggests that it would evoke rather a cornea-negative transretinal potential if the distribution of K^+ conductance along the MC were more or

less uniform, such as was shown for MCs isolated from mammalian retinae. On the other hand, the location of *distal* $[K^+]_o$ is favorable for generation of a cornea-positive transretinal potential, but these K^+ changes are small and restricted to narrow OPL layer.

As we can see, the theoretical reasons make it hard to believe that MCs can have a *major* contribution to generation of the b-wave of the ERG. The detailed and quantitative evaluation of the ability of MCs to generate the extracellular potential is presented in the main paper.

BYKOV, K., DMITRIEV, A. & SKACHKOV, S. (1981). Relationship between photoinduced changes in the intercellular concentration of potassium ions and transretinal potential generation by the Muller cells of the retina. *Biofizika* **26,** 104.

NEWMAN, E. & REICHENBACH, A. (1996). The Müller cell: a functional element of the retina. *Trends in neurosciences* **19,** 307-312.

NEWMAN, E.A. (1984). Regional specialization of retinal glial cell membrane. *Nature* **309,** 155.

NEWMAN, E.A. (1985). Membrane physiology of retinal glial (Muller) cells. *Journal of Neuroscience* **5,** 2225-2239.

NEWMAN, E.A. (1987). Distribution of potassium conductance in mammalian Muller (glial) cells: a comparative study. *The Journal of neuroscience* **7,** 2423-2432.

NEWMAN, E.A. (1993). Inward-rectifying potassium channels in retinal glial (Muller) cells. *Journal of Neuroscience* **13,** 3333-3345.

OAKLEY, B. & GREEN, D.G. (1976). Correlation of light-induced changes in retinal extracellular potassium concentration with c-wave of the electroretinogram. *Journal of neurophysiology* **39,** 1117-1133.

STEINBERG, R., OAKLEY 2ND, B. & NIEMEYER, G. (1980). Light-evoked changes in [K+] 0 in retina of intact cat eye. *Journal of neurophysiology* **44,** 897-921.

TOMITA, T. (1976). Electrophysiological studies of retinal cell function (Proctor Medal Lecture). *Invest Ophthalmol* **15,** 171-187.