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Letter to the Editor

On the Relation between ERG Waves and Retinal Function: Inverted Rod Photoresponses from the Frog Retina

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In rod mass receptor photoresponses recorded across the isolated frog retina, a paradoxical cornea-positive wave may precede the response of normal polarity. We present a model which shows that the light-induced decrease in rod current can give rise to inverted or biphasic ERG signals if the distal part (tip) of the rod outer segment responds more slowly and/or less sensitively than the proximal part (base). The condition is that current entering at the tip is represented with greater weight in the ERG. The model reproduces recorded ERG waveforms well. It further predicts that if there is a light-*in*sensitive conductance in the tip membrane, ERG photoresponses may be non-recordable although current photoresponses are only slightly reduced. The model reveals a type of complexity in the relation between mass potentials and underlying physiological processes which has not previously received attention.

ERG Rod photoreceptors Retina *a*-wave Frog

INTRODUCTION

In a recent article on ERG rod photoresponses recorded across the isolated, aspartate-treated frog retina, Nakamura, Hanawa and Ando (1991) characterize a component of polarity opposite to the photoreceptor response proper. The most remarkable feature of this cornea-positive wave, observed previously by other investigators (Table 1), is that it *precedes* the fast PIII, of which the early parts are usually well described as the extracellular ohmic voltage generated by the rod current response (Baylor, Lamb & Yau, 1979; Donner, 1989; Hood & Birch, 1990). Further, the absolute sensitivity of this leading "hump" appears to be equal to or even higher than that of the normal light response (Donner, Hemilä & Koskelainen, 1987; Nakamura *et al.*, 1991). What should one think of a substantial retinal "signal" which is seemingly both faster and more sensitive to light than that which, according to overwhelming evidence initiates the visual process?

The occurrence of this cornea-positive wave appears unsystematic in two respects. First, the situations (Table 1) have little in common that would clearly implicate a specific link in phototransduction. Second, there is significant variability between individual preparations in the tendency to produce humps (Donner *et al.*, 1987; Nakamura *et al.*, 1991). It should also be noted that no corresponding signal has ever been observed in intracellular membrane voltage recordings of rod photoresponses. All observations (in reasonably physiological perfusing media) are either from the ERG of isolated retinas where synaptic transmission has been blocked by aspartate, or from current responses of isolated rods (Donner *et al.*, 1987, indicating that aspartate is not the common cause). The inverted rod photoresponses observed in zero-sodium (combined with zero-Ca^{2+}, zero-Mg^{2+}) in both membrane voltage and current recordings (Capovilla, Caretta, Cervetto & Torre, 1983; Hodgkin, McNaughton, Nunn & Yau, 1984) are clearly of different origin, as are probably the inverted current responses recorded by Nicol, Schnetkamp, Saimi, Cragoe & Bownds (1987) after treatment with an amiloride derivative.

It therefore seems misguided to look for specific physiological generators such as well-defined components of phototransduction or distinct signals in second-order neurons. Clearly, our earlier evaluation of the phenomenon (Donner *et al.*, 1987) is not commonly known. We write this to bring out new implications which are not evident from our original exposition, and because we think the analysis carries wider interest with regard to the interpretation of mass potentials in general and ERG in particular.

THE MODEL

It is predicted that a cornea-positive wave will appear in the rod ERG photoresponse in situations where the longitudinal inhomogeneity of the rod outer segment becomes pronounced. Briefly, the conditions are:

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TABLE 1. Observations of inverted photoresponses, or initial humps of polarity opposite to a normal photoresponse, in the ERG recorded across the isolated anuran retina with synaptic transmission blocked by aspartate

Conditions favoring	Persistence
*$CH_3SO_3^-$ + low Ca^{2+}	?
†3 mM EDTA + 10 mM EGTA	<20 min
‡Post-dissection trauma	Up to 2 hr
Oxidation	*ca* 15 min
§0.5 mM NEM	Persisted until photoresponses were wholly abolished
¶Low Ca^{2+}	
Low K^+	
Conditions suppressing	
¶Low Na^+	
La^{3+}, Co^{2+}, Cd^{2+}, Ni^{2+}	

*Greenblatt (1983), †Govardovskii and Berman (1985), ‡Donner, Hemilä and Koskelainen (1987), §Donner, Hemilä and Koskelainen (1989), ¶Nakamura, Hanawa and Ando (1991).

(1) The distal part (tip) reponds more slowly and/or less sensitively to light than the proximal part (base). This includes the possibility that part or all of the Na^+ conductance at the tip is absolutely insensitive to light.* (2) Current entering the rod through channels in the tip part is represented in the transretinal ERG voltage with greater weight than current entering at the base part.

The main features of the model and qualitative consequences can be discussed with reference to the equivalent circuit in Fig. 1, where the fundamental parameters are defined. The equations, which use the "independent activation" kinetics of Baylor, Hodgkin and Lamb (1974) for the time–course of dim-flash current responses, plus Michaelis-type saturation, are given in the Appendix. The parameters which essentially determine response shapes have there been reduced to three: (1) the base/tip sensitivity ratio c, (2) the tip/base time constant ratio k, (3) the tip conductance that is *in*sensitive to light (a "leakage" conductance G_L, not included in our original formulation). As explained in the Appendix, we find it convenient to use scaled parameters, which are denoted by lower-case letters. For example, the scaled leakage conductance is g_L.

SIMULATIONS: EFFECTS OF THE PARAMETERS

Parameters c and k

The simulations in Fig. 2 show how flash responses are affected when the base/tip sensitivity ratio (c, left panels) and the tip/base time constant ratio (k, right panels) are given different values >1. The top panels display dim-flash responses and the bottom panels saturated flash responses. It is seen that a leading cornea-positive ERG wave may arise when the base is either more sensitive or faster than the tip, or both. In either case, closure of base channels will initially dominate over closure of tip channels, forcing more current along the high-resistance tip pathway.

Parameter g_L

A leakage conductance in the distal part will act to decrease the ERG response amplitude and, if large enough, completely reverse the response (Fig. 3, left panels). This is because the light-induced decrease in total current is then associated with an increase in current flowing through the light-insensitive conductance in the tip, generating an ERG signal of opposite polarity.

The leakage that makes the ERG signal vanish in Fig. 3 (middle response in each of the panels on the left) is not very large, 64% of the total "dark" conductance in the tip (the total tip "dark" conductance = $G_L + G_{to}$). The right panels of Fig. 3 show that rod current responses are only moderately affected under these conditions. For example, the zero-amplitude ERG is associated with current responses which have decreased by less than half from the no-leakage situation. Since the functionally relevant signal of the rod, the membrane voltage response, is basically determined by the current response, it is evident that a non-recordable or inverted ERG need by no means imply inactivation of visual function.

SIMULATION OF RECORDED RESPONSE WAVEFORMS

In Fig. 4(A) a series of "hump" responses to three flash intensities [Fig. 1(A) in Nakamura *et al.*, 1991] are juxtaposed with model responses for a corresponding range of intensities, computed with a single set of parameter values ($c = 7.5$, $k = 1.2$, $g_L = 0$). Clearly, the model is able to reproduce the empirically observed waveforms fairly well.

Figure 4(B) displays an ERG response to a very bright flash recorded during perfusion with the sulfhydryl binding reagent n-ethyl-maleimide (NEM) (Fig. 4 in Donner, Hemilä & Koskelainen, 1989). To simulate this waveform, a tip leakage conductance has been introduced. Otherwise the parameters are practically the same as in Fig. 4(A) ($c = 7.5$, $k = 1.4$, $g_L = 0.75$). The success

*The inhomogeneity may also make *current* responses recorded from single rods appear as bi- or even triphasic, if the constriction of the suction pipette divides the outer segment so that one part is within, one part outside the pipette (Donner *et al.*, 1987). Such current responses, which thus depend on a certain recording geometry, will not be considered further in this paper.

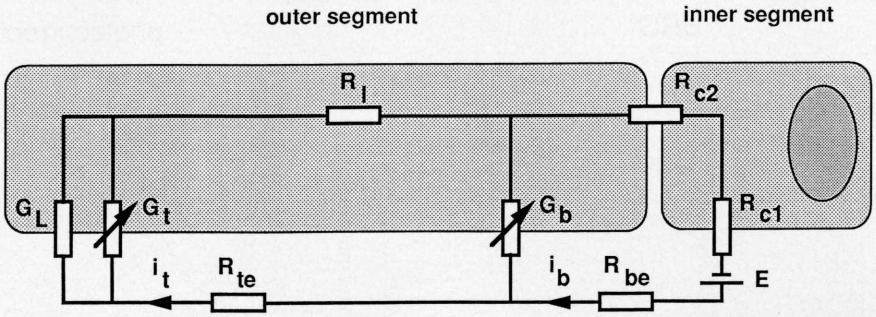

FIGURE 1. Equivalent circuit of the rod layer with the basic parameters of the model indicated. E is the driving force, G_b and G_t are the base and tip portions of the light-sensitive conductance in the outer segment plasma membrane, G_L is the light-insensitive ("leakage") conductance in the tip. R_{be} and R_{te} are the extracellular resistances around the base and the tip, respectively (note that current entering the rod at the tip flows across $R_{be} + R_{te}$). i_b is the total current; i_t is the portion that enters at the tip. R_l is the longitudinal intracellular resistance in the outer segment, R_{cl} the inner segment membrane resistance and R_{c2} the intracellular cilium resistance.

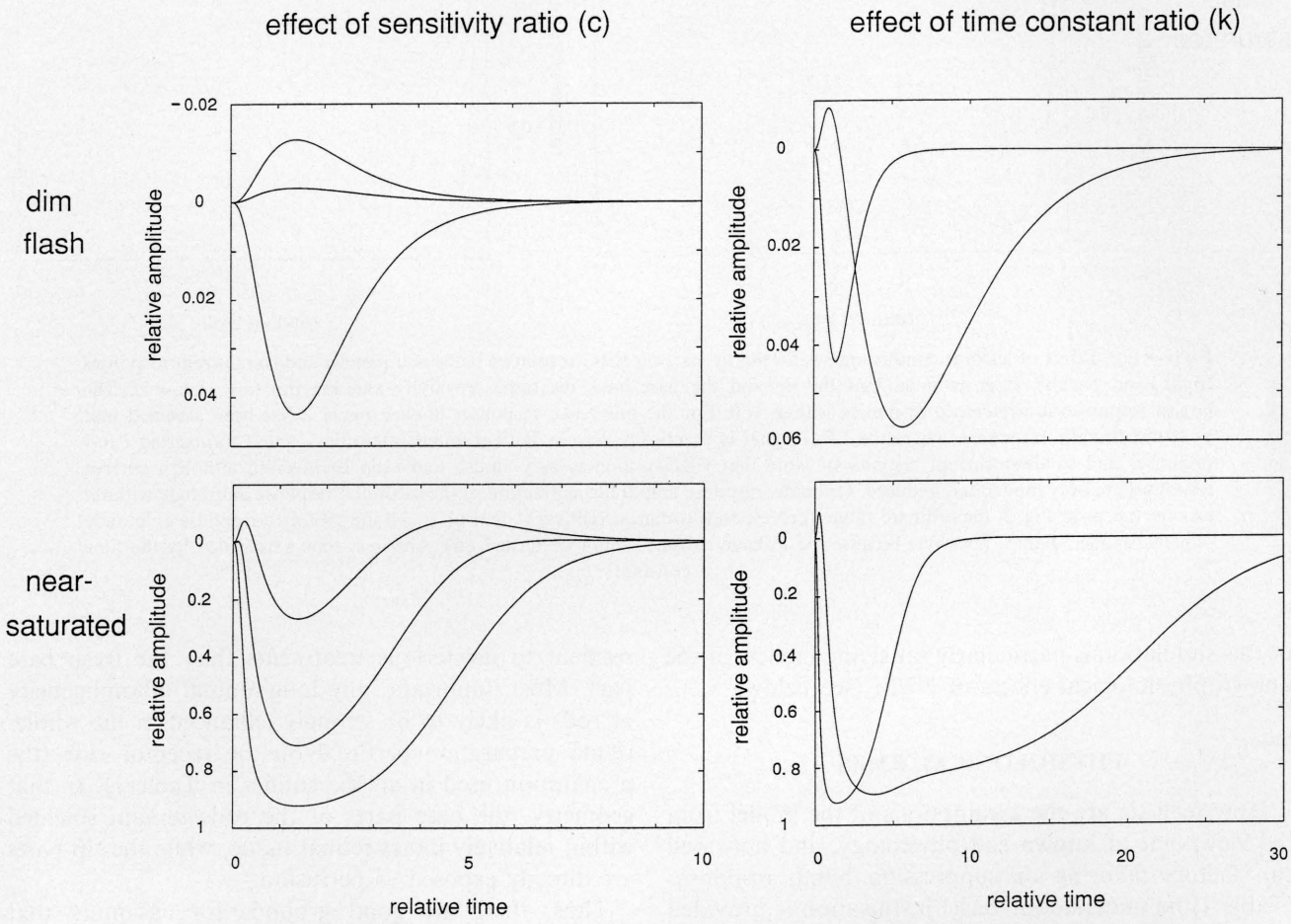

FIGURE 2. Left-hand panels. Effect of the base/tip sensitivity ratio c on model ERG responses. Top panel, dim-flash responses ($cI = 1$), bottom panel, near-saturated responses ($cI = 250$). (Defining the intensity by cI rather than I implies that the excitation of the base is kept constant, but the tip is relatively less excited the larger the c value.) It is assumed that the tip and the base have the same kinetics ($k = 1$) and that there is no leakage ($g_L = 0$). The largest responses in both frames are reference responses with $c = 1$ (no sensitivity difference), the middle responses are obtained when the base is assumed to be 5 times more sensitive than the tip ($c = 5$) and the largely inverted ones for $c = 25$. Note that for $c = 25$ the near-saturated response is triphasic owing to the Michaelis nonlinearity (the corresponding dim-flash response is of simpler waveform). Ordinate, response amplitude as fraction of the saturated response amplitude. Abscissa, time axis scaled by the time constant τ (the largest in the arithmetic sequence) of the independent activation model (Baylor *et al.*, 1974), i.e. in units of t/τ. Right-hand panels. Effect of the tip/base time constant ratio k on model ERG responses. Top panel, dim-flash responses ($I = 1$); bottom panel, near-saturated responses ($I = 250$). It is assumed that the tip and the base have the same sensitivity ($c = 1$) and that there is no leakage ($g_L = 0$). The fast monophasic responses are reference responses with $k = 1$, identical to the largest responses in the left-hand panels, but plotted on a different time scale. The biphasic, extended responses are obtained when the tip is assumed to be 4 times slower than the base ($k = 4$). Ordinate, response amplitude as fraction of the saturated response amplitude. Abscissa, time axis scaled by the base time constant, i.e. in units of kt/τ.

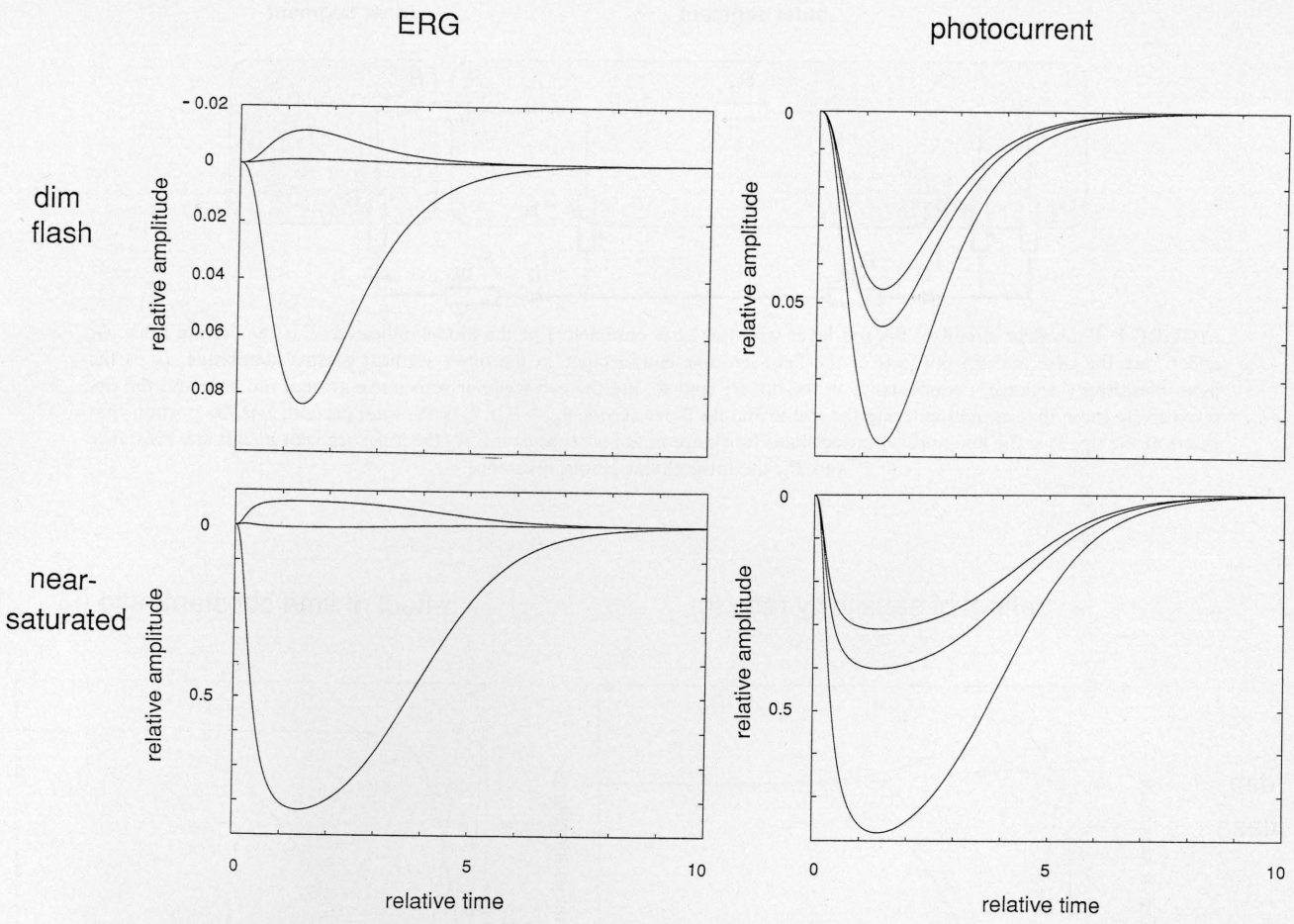

FIGURE 3. Effect of leakage conductance g_L in the tip part on ERG responses (left-hand panels) and rod current responses (right-hand panels). It is assumed that the tip and the base have the same sensitivity and kinetics ($c = 1$, $k = 1$). The largest responses are reference responses with $g_L = 0$. For the other two responses in each panel it has been assumed that $g_L = 0.88$ (middle responses, where the ERG signal is practically zero at both stimulus intensities), and 1.5 (inverted ERG responses and smallest current responses). Note that ERG responses may vanish and even be inverted although current responses are only moderately reduced. Ordinate, response amplitude as fraction of the saturated response amplitude without leakage (i.e. as in Fig. 2, the ordinate value 1 corresponds to the currentless state. For $g_L > 0$ the saturated response amplitude remains smaller than 1, however, because the leakage current cannot be turned off). Abscissa, time axis scaled by the time constant.

of this simulation is particularly satisfying in view of the known physiological effects of NEM (see below).

PHYSIOLOGICAL BASIS

How realistic are the assumptions of the model from the viewpoint of known rod physiology, and how well can factors favoring or suppressing hump responses (Table 1) be understood? Basic justification is provided by single-rod photocurrent recordings which show that responses originating in the distal part of the outer segment are both slower and less sensitive to light than responses originating in the proximal part (Baylor *et al.*, 1979; Lamb, McNaughton & Yau, 1981; Schnapf, 1983). One family of responses elicited with high spatial resolution from various positions along the toad rod outer segment by Schnapf (1983, her Fig. 2) reveals a base/tip sensitivity ratio as large as 4 and a tip/base time constant ratio of *ca* 1.2. It is also known that the tip recovers more slowly from desensitization by background light (Hemilä & Reuter, 1981; Lamb *et al.*, 1981). The tip is an ageing part of the outer segment which may be generally less resilient to deleterious treatments than the fresh base part. Most important, any longitudinal inhomogeneity of rods is likely to be strongly enhanced in the whole-retina preparation perfused on the receptor side (the preparation used in all the studies in Table 1). In that geometry, the base parts of the rods remain shielded within relatively intact retinal tissue, while the tip parts are directly exposed to perfusion.

Thus, there are good grounds for assuming that treatments affect the tip parts more immediately and more strongly than the base parts. If this is accepted, the treatments in Table 1 can easily be seen to provide conditions for inverted responses and initial humps:

(1) Low Ca^{2+}, EGTA and EDTA are known to slow down responses and depress relative sensitivity (e.g. Lipton, Ostroy & Dowling, 1977; Govardovskii & Berman, 1985).

(2) Isolation of the retina from the pigment epithelium will inevitably entail both mechanical and chemical strain particularly for the rod outer segment tips which are stripped of their pigment epithelium ensheathment. This would explain why inverted responses may appear

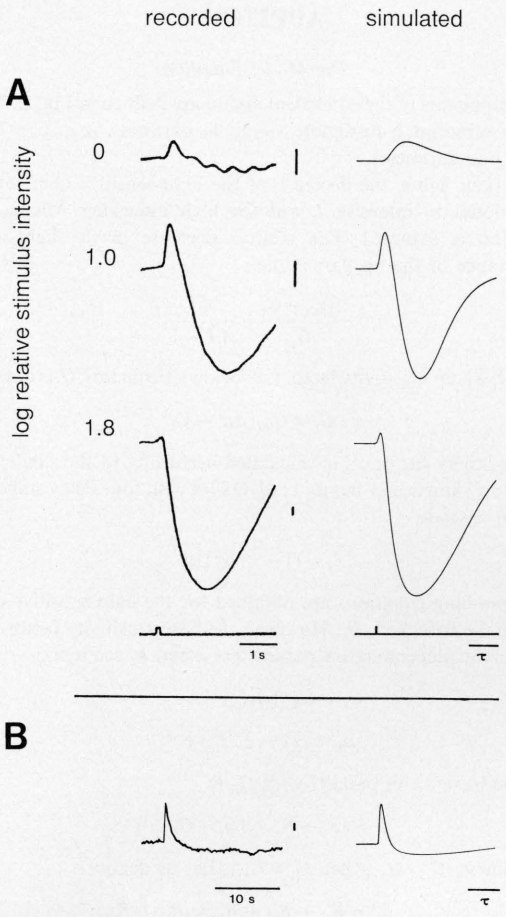

FIGURE 4. Simulation of recorded ERG responses by the model. (A) ERG flash responses from Nakamura *et al.* [1991, Fig. 1(A)] to log stimulus intensities denoted −7, −6 and −5.2 in that paper. The parameters for all three model responses are $c = 7.5$, $k = 1.2$ and $g_1 = 0$, and the log intensity differences between the responses are the same as for the experimental ones (starting from the intensity parameter 0.45 for the smallest response). (B) Experimental ERG response in NEM perfusion (Donner *et al.*, 1989) and a simulated response with $c = 7.5$, $k = 1.4$, $g_1 = 0.75$ and log relative stimulus intensity 2.6 [by the scale used in (A)]. The amplitude scale bar to the right of each recorded response marks 1 μV; note that the smaller responses have been relatively enlarged. The simulated responses have been scaled in the same way. The time scale bars for the recorded responses mark 1 sec (A) and 10 sec (B); for the simulated responses they mark the tip time constant.

spontaneously in normal Ringer. The disappearance of humps after longer times in perfusion could result, e.g. from permanent closure of tip channels.

(3) Oxidation has been shown to injure light-sensitive conductance (Wormington & Cone, 1978). In the isolated retina, oxidation transiently depresses sensitivity and slows down rod photoresponses (Donner *et al.*, 1987).

(4) In both ERG and single-rod current recordings, prolonged perfusion with NEM depresses light sensitivity and slows down photoresponses. Further, a current component appears that cannot be turned off by light (Donner *et al.*, 1989). By patch-clamp recording, we have shown directly that NEM indeed induces a light-*in*sensitive conductance in the rod outer segment plasma membrane (Donner, Hemilä, Kalamkarov, Koskelainen, Pogozheva & Rebrik, 1990). Hence, the simulation in Fig. 4(B) has a firm experimental basis.

(5) Divalent cations, found to *abolish* humps, have been shown to strongly reduce the conductance of the light-sensitive channel (Co^{2+}, as well as higher concentrations of Ca^{2+}: MacLeish, Schwartz & Tachibana, 1984). La^{3+} blocks the Na^+ channel more or less completely (Cervetto & McNaughton, 1986). Obviously, total blockage of the tip conductance would eliminate the conditions for humps or inverted responses.

(6) Perfusion with low Na^+ was likewise found to abolish humps (Nakamura *et al.*, 1991), which can be given the following explanation. In the rod, there is probably a substantial longitudinal Na^+ concentration gradient, because sodium enters all along the outer segment but is extruded only by Na^+/K^+ pumping in the inner segment (see Schnapf, 1983). Given that $[Na^+]_i$ is higher in the tip, a decrease in $[Na^+]_0$ (which will decrease the inward driving force for Na^+, thus the dark current) would affect tip responses more strongly than base responses, reducing or abolishing humps.

In Table 1, there remains one treatment conducive to humps where it is hardly fruitful to make specific guesses about mechanisms. Lowered K^+ will strongly affect all retinal neurons, not least the Müller cells, and it is possible to envisage several possible secondary changes that might be involved.

CONCLUSION

We think that a thorough evaluation of the cornea-positive hump recorded across the isolated frog retina is worthwhile mainly because of the general lessons that may be learnt about mass potentials and their relation to physiology. Granit (1933) first showed that an ERG wave does not correspond to a physiological process, but is the result of algebraic summation of voltage components. It is by now also commonly appreciated that mass potentials do not faithfully reflect underlying currents but are modified by changes in extracellular resistances (resulting from, e.g. cell swelling or loss of cells). The present model points to an additional level of interpretational complexity: a light-induced *de*crease in total current can cause a paradoxical *in*crease (hence also more complicated patterns) in the extracellular ohmic voltage generated by that current. Conversely, it is worth noting that the disappearance of a recordable ERG signal need not signify disappearance of the corresponding function, but may have several other causes: (1) decreased extracellular resistivity; (2) shortened radial paths for the underlying currents; (3) mechanisms of the type described here, whereby a decrease in total current is counterbalanced by an increase in a component which is reflected with greater weight in the ERG.

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APPENDIX

The Model Equations

The components of the equivalent circuit are defined in Fig. 1. We shall use the subscript o to denote values in darkness, (e.g. $G_t = G_{to}$ and $G_b = G_{bo}$ in darkness).

For weak lights, the decrease of the light-sensitive conductance is proportional to intensity I, but for high intensities Michaelis-type saturation is assumed. The relative decrease in the light-sensitive conductance of the tip part is then

$$\frac{G_{to} - G_t}{G_{to}} = \frac{A_t I}{A_t I + 1}$$

where A_t is a tip sensitivity factor (see below). From this, G_t is obtained as

$$G_t = G_{to}/(A_t I + 1).$$

The sensitivity factor A_t is calculated according to the "independent activation" kinetics of Baylor *et al.* (1974) with four delay stages in the reaction cascade

$$A_t = (1 - e^{-t}/\tau)^3 e^{-t/\tau}.$$

Corresponding equations are obtained for the light-sensitive conductance in the base part, G_b. However, the base sensitivity factor A_b may be different (depending on parameters c and k, see text):

$$G_b = G_{bo}/(A_b I + 1)$$

$$A_b = c(1 - e^{-kt/\tau})^3 e^{-kt/\tau}.$$

The conductance in parallel with G_b is

$$G = [R_i + R_{te} + (G_t + G_L)^{-1}]^{-1}.$$

In darkness, $G = G_o$ (when $G_t = G_{to}$). Let us denote

$$R' = R_{c1} + R_{c2} + R_{be} \approx R_{c1} + R_{c2}$$

$$X = R_{be}/(R_{be} + R_{te}).$$

The extracellular currents are then

$$i_t = \frac{EG}{1 + R'(G + G_b)}$$

$$i_b = \frac{E(G + G_b)}{1 + R'(G + G_b)}.$$

The transretinal voltage is $U = R_{be} i_b + R_{te} i_t$; in the dark this becomes $U_o = R_{be} i_{bo} + R_{te} i_{to}$. The mass receptor photoresponse is $\Delta U = U - U_o$. Substituting the expressions for i_t and i_b in light and darkness gives the relative ERG photoresponse

$$\frac{\Delta U}{U_o} = \frac{G + XG_b}{G_o + XG_{bo}} \cdot \frac{1 + R'(G_o + G_{bo})}{1 + R'(G + G_b)} - 1.$$

The relative current photoresponse is

$$\frac{\Delta i_b}{i_{bo}} = \frac{i_b - i_{bo}}{i_{bo}} = \frac{1 + R'(G_{bo} + G_o)}{1 + R'(G_b + G_o)} - 1.$$

Scaling is used to reduce the number of parameters. The dimensionless time is t/τ and the dimensionless conductances are $g = R'G$. Thus in addition to the parameters c, k, and X defined above we have four scaled conductances.

$$g_{to} = R'G_{to}, \quad g_{bo} = R'G_{bo}, \quad g_L = R'G_L, \quad g_i = R'/(R_i + R_{te}).$$

The total light-sensitive conductance is of the same order of magnitude as the inner segment membrane conductance, so we set $g_{to} + g_{bo} = 1$. Setting, further, tip and base conductances equal in darkness then gives $g_{to} = g_{bo} = 0.5$. The ratio g_i is determined by the resistive properties of the rod, here $1/g_i$ is always assumed to be 0.3 (as in Donner *et al.*, 1987). In all the simulations presented here we have chosen $X = 0.1$. Thus the three parameters used for fitting are c, k and g_L. Since $g_{to} = 0.5$, a leakage conductance of, e.g. $g_L = 0.5$ means that $G_L = G_{to}$.