Role of Synaptic Feedback and Intrinsic Voltage-Gated Currents in Shaping Cone Light Responses

Garrett T. Kenyon[1,3], Bryan J. Travis[2,3], David W. Marshak[4]

[1]P-21 Biophysics, MS D454, [2]EES-6 Geological Processes, Simulation, and Modeling, MS T003, [3]Los Alamos National Laboratory, Los Alamos, NM, 87545; [4]Department of Neurobiology and Anatomy, University of Texas Health Science Center, Houston, 6431 Fannin, Houston Texas, 77030

Abstract

A model of the outer primate retina was used to investigate how cone light responses could be shaped by both synaptic feedback from horizontal cells and intrinsic voltage-gated conductances. Published data was used to estimate the photoconductance, convergence/divergence ratios, synaptic transfer functions, passive membrane properties and voltage-gated conductances, the latter using a Levenberg-Marquardt search algorithm. When the cone resting potential was sufficiently depolarized, synaptic feedback from horizontal cells could account for the flash response measured experimentally. Voltage-gated conductances were not significantly activated during the flash response but rather maintained the cone membrane potential inside a narrow physiological range.

Introduction

Mammalian photoreceptors represent a potentially useful system for examining how the input/output properties of biological neurons are influenced by two factors: 1) synaptic feedback and 2) intrinsic voltage-gated conductances. Here, we use the light-induced photocurrent as a measure of the input to the cone photoreceptor and the resulting membrane voltage as a measure of photoreceptor output. Voltage responses recorded from the inner segments of primate cones following brief light flashes are more transient than the underlying photocurrents. Photocurrents elicited by a 10 msec light flash show an average time-to-peak in dark adapted cones of 51 msec [1] for non-saturating flash intensities. In contrast, the time-to-peak of the voltage response generated under the same conditions decreases from 35 msec for dim flashes to 10 msec for brighter but still non-saturating intensities [2]. The pronounced difference in the time course of the voltage response compared to the photocurrent demonstrates that there is a non-linear input/output transformation right at the very first synapse of the visual system.

In order to better understand the physiological factors that determine the input/output characteristics of cone photoreceptors when embedded within the circuitry of the outer retina, a computer model was used to investigate the relative contributions of synaptic interactions and voltage-gated conductances. To isolate the contribution due to synaptic interactions, an anatomically realistic model of the outer retina was developed in which cones and horizontal cells were initially implemented as passive elements that contained no explicit voltage-gated conductances. With physiologically reasonable assumptions, the passive model was able to reproduce the measured responses of primate cones to brief light flashes. Voltage-gated conductances that have been described in both cones and horizontal cells were then added to the model to investigate how intrinsic membrane properties might also contribute to signal processing in the outer retina. Our results suggest that for primate cones, synaptic feedback is the primary factor in shaping light responses, whereas voltage-gated conductances act primarily to keep the cone membrane potential within a relatively narrow operating range.

Methods

Simulations were conducted using the LCM3D simulator [3]. The model consisted of an 8×8 array of cones and a 4×4 array of H1 horizontal cells connected with convergence/divergence ratios inferred from anatomical studies [4]. Each cone made excitatory synapses (V_{eq} = –10 mV) onto 4 H1 cells, and each H1 cell made inhibitory synapses (V_{eq} = –55 mV) onto all sixteen cones within its dendritic field. The assumption that horizontal cell inhibition is mediated by conventional GABA receptors is supported by evidence that mammalian cones respond to GABA [5], although alternative inhibitory mechanisms have been described in goldfish [6]. Synaptic release was modeled using Fermi functions. For cones, the transfer function had a gain of 1.75 mV (g_{max} = 2 nS, $V_{½}$ = –30 mV), consistent with the kinetics of a non-inactivating calcium current in the primate cone [7] and a fourth power dependence on calcium influx [8]. The transfer function governing horizontal cell output had a gain of 15 mV (g_{max} = 20 pS, $V_{½}$ = –10 mV), based on a calcium-independent release mechanism [9]. The synaptic transfer function of the model cones was thus much more non-linear than that of the horizontal cells (fig. 1). The photoconductance of the cone (V_{eq} = –10 mV, g_{max} = 58 pS) was adapted from a realistic photocurrent model [1]. Passive membrane properties of the model cone (R_{in} = 1 GΩ, τ = 10 msec) were based on measured values in lower vertebrate photoreceptors after taking into account damage to the cell membranes resulting from electrode penetration [10, 11]. The resting potential of the model cone (in the absence of a photocurrent) was set to –40 mV. This relatively depolarized resting potential, which was necessary to account for the plateau phase of the cone's flash response, might result from cGMP-gated cation channels [12, 13]. The resting potentials of the H1 cells were set to –60 mV, based on cat type-B horizontal cells [14]. H1 input impedance (R_{in} = 1 GΩ) was based on data from cultured postnatal rabbit horizontal cells [15]. The passive H1 membrane time constant was set to 20 msec. The H1 time constant was not well constrained by available experimental data but its precise value did not affect our main conclusions.

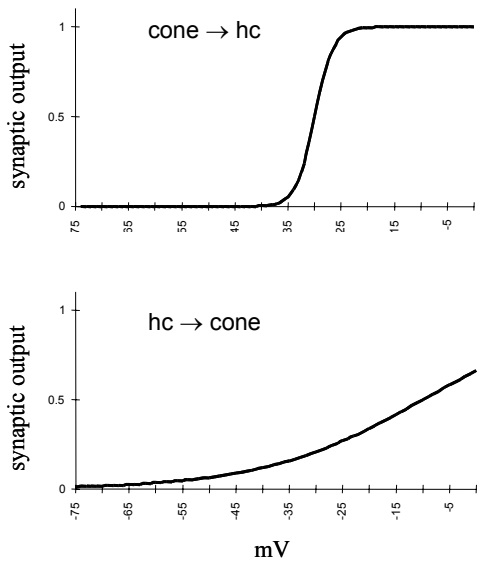

Fig. 1. Synaptic transfer functions in retinal model. Cone output to horizontal cells (top) depends non-linearly on membrane potential [8] while horizontal cell feedback to cones (bottom) is approximately linear [9].

For the active membrane model, voltage-gated conductances were implemented as previously described [16]. Hodgkin-Huxley activation and inactivation parameters were fit to voltage-clamp data recorded from primate cones [7] and to similar data from cat [14] and cultured rabbit [15] horizontal cells using a Levenberg-Marquardt search algorithm [17]. The calcium-dependent chloride current present in the primate cone was based on a previously developed model of a mammalian calcium-dependent potassium current [16], except that the dependence of the chloride current on intracellular Ca^{2+} was strongly non-linear. Space does not permit a full description of each of the voltage-gated currents included in the active active model. However, our conclusions regarding the contribution of these currents to light responses are based on their general order of magnitude and should not depend critically on the details of their implementation.

Results

Passive Model: A passive model, containing no explicit voltage-gated conductances, was able to qualitatively account for the main features of cone responses to brief light flashes (fig. 2). For dim flashes, voltage responses are hyperpolarizations similar in duration to the underlying photocurrents. For brighter flashes, however, voltage responses develop an initial sharp transient that decays to a plateau potential several mV below the dark resting potential. Such effects have been observed in primate cones in response to similar stimuli [1, 2]. In the model cone, the attenuation of the voltage response at bright flash intensities is due to negative feedback from horizontal cells. During the initial response to a bright light flash, the light sensitive conductances within the cone outer segments approach zero, causing both cones and horizontal cells to become hyperpolarized. The resulting reduction in synaptic inhibition from horizontal cells causes the cone membrane potential to repolarize towards the resting potential in the absence of a dark current, assumed to be -40 mV. Repolarization is not complete, however, because of residual inhibition from horizontal cells.

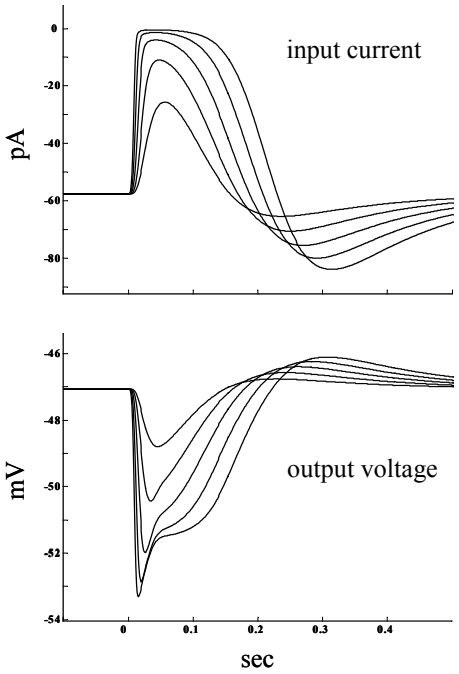

Fig. 2. A passive membrane model of the outer retina reproduces the non-linear Input/Output properties of primate cones [1, 2]. Photocurrent (top) and membrane voltage (bottom) in response to 10 msec full-field light flashes of variable intensity.

In a previous computational study [18], it was shown that in response to a sustained hyperpolarizing current, intended to represent a light stimulus, negative feedback from horizontal cells repolarizes the membrane potential of the cone back towards its resting potential in the dark. Our results suggest, however, that when current injection is replaced by a light-sensitive decrease in a photoconductance, feedback from horizontal cells will not contribute to repolarizing the cone unless the resting potential of the isolated cone inner segment is sufficiently depolarized. Another important distinction is that in the present model the transfer function characterizing negative feedback from horizontal cells onto cones is nearly linear (fig. 1), whereas in the previous study this transfer function was very non-linear, producing small oscillations in the responses to large current injections. Such oscillations are not evident in the light responses generated by the present model because at the peak of the light response the gain of the cone-horizontal cell circuit is small.

Examination of the photocurrent shows that there is a rebound phase of opposite polarity beginning approximately 200 msec after the onset of the flash stimulus. The depolarizing portion of the photocurrent response does not affect the membrane potential of the cone as strongly as the initial hyperpolarizing response, however, because negative feedback from the horizontal cells opposes depolarization of the cone above the dark potential. Since the dark potential of the cone is very close to the steeply rising portion of its synaptic output function, negative feedback from the horizontal cells is recruited very strongly by a depolarization of the cone above the dark potential. In contrast, feedback from the horizontal cell is recruited only weakly during the primary phase of the flash response when the cone is hyperpolarized and the synaptic output function of the cone is relatively insensitive to changes in membrane potential. This simple model therefore suggests that the feedback from horizontal cells plays a major role in shaping the voltage responses of primate cones to brief light flashes, and that the non-linearity in the synaptic transfer

function from cones to horizontal cells is necessary to prevent oscillations that would otherwise result from the delayed negative feedback.

Active Model: We next investigated the contribution of voltage-gated conductances to the light responses of cones and horizontal cells. Voltage-clamp studies of the isolated primate cone inner segment identified five voltage-gated currents, an A-current, a delayed rectifier, a sustained calcium current, a calcium-dependent chloride current, and an anomalous rectifier or h-current [7]. Similarly, three voltage-gated conductances have been identified in adult cat type-B horizontal cells, a large A-current, a delayed rectifier and an anomalous rectifier [14]. Using the built-in capabilities of the simulator to determine up to 10 or more parameters simultaneously, it was possible to construct a model cone that was able to reproduce many of the voltage-dependent effects seen in the biological data. The currents recorded from the isolated inner segment of a primate cone generated in response to a series of 10 mV voltage steps from a holding potential of –75 mV were reasonably well fit by the currents recorded from an isolated model cone in response to an analogous stimulation protocol (fig. 3). For each of the voltage-gated currents in the model, the parameters characterizing the activation and (if present) inactivation functions and associated time constants, were determined by first fitting voltage-clamp studies designed to isolate the current in question. The maximum conductances for the different currents were then determined simultaneously by fitting the whole cone voltage-clamp series with the activation parameters of the individual currents held constant. A similar procedure was used to implement voltage-gated currents in horizontal cells, the details of which are less important for the conclusions derived here.

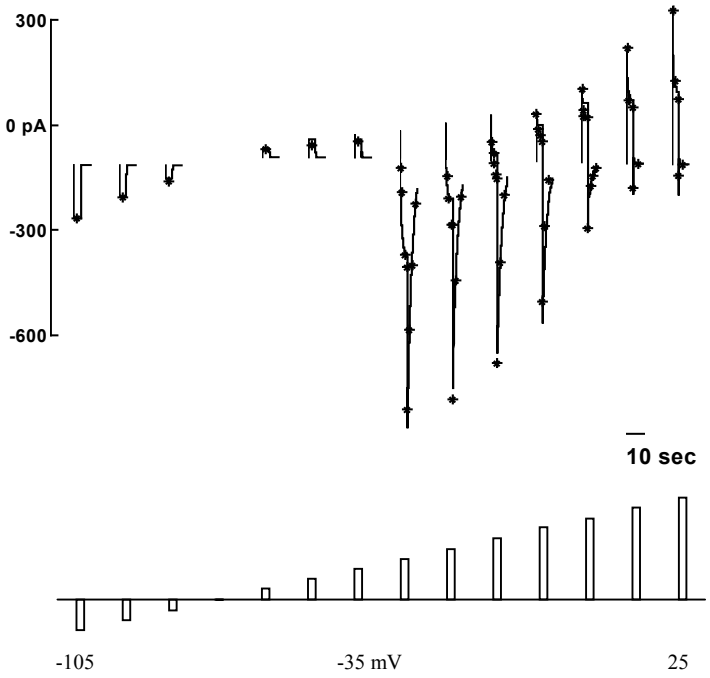

Fig. 3. Voltage-clamp protocol used to fit model cone responses (lines) to experimental data (*). Bottom: Voltage-clamp protocol.

Voltage-gated currents contributed little to the output of the model cone in response to a 10 msec impulse representing a brief light flash (fig. 4). The largest contribution to the flash response was from the photocurrent (photo), which dropped from its dark level of approximately -30 pA to nearly zero. The next largest contributor to the flash response was due to synaptic feedback from horizontal cells (hc). In the passive model, synaptic feedback from horizontal cells mediated a rapid repolarization of the cone, and this was true in the active model as well. In contrast, none of the voltage-gated currents (h, L, Cl[Ca], DR) changed by more than a few mV during the flash response, less than the light-induced modulation of the leak current (leak). (The A-current was negligible in these experiments and was therefore omitted). Furthermore, the voltage-gated currents that were present tended to cancel each other out. In particular, the outward current through the delayed rectifier was reduced by approximately the same amount as the in-

ward current through the non-inactivating calcium channel, resulting in very little net change. Thus, our results indicate that voltage-gated conductances do not contribute significantly to the input/output properties of primate cones during the response to brief light flashes.

Discussion

By making the following three biologically reasonable assumptions, we could account for the voltage-responses recorded from primate cones to brief light flashes: 1) the transfer function describing synaptic input from cones to horizontal cells is strongly non-linear [8], 2) horizontal cells make feedback inhibitory synapses onto cones governed by a transfer function that is nearly linear [9], 3) the resting potential of the isolated cone inner segment is relatively depolarized, presumably reflecting the action of cGMP-gated channels that have been identified in the cone inner segments of lower vertebrates [12, 13].

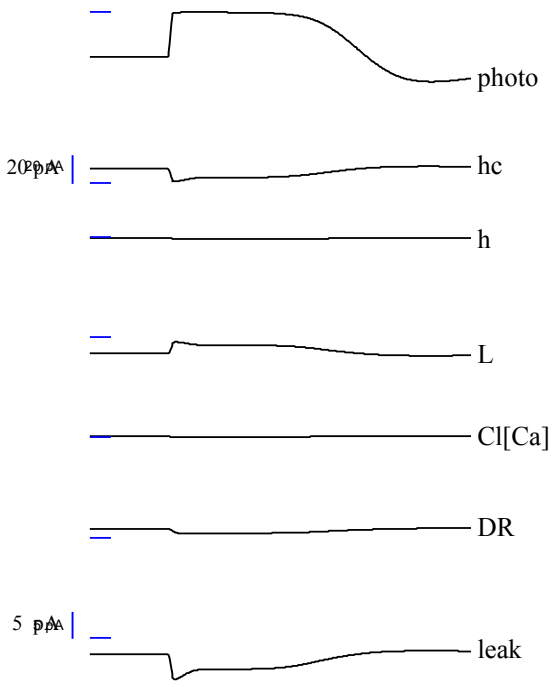

Fig. 4. Currents in model cone during flash response. Upper scale bar (20 pA): photo: photocurrent, hc: synaptic current. Lower scale bar (5 pA): h: anomalous rectifier, L: non-inactivating calcium current, Cl[Ca]: calcium-dependent chloride current, DR: delayed rectifier, leak: leak current. Horizontal scale bars (25 msec) at zero current.

Although it was possible to account for the input/output properties of primate cones using a purely passive membrane model, we sought to understand how known voltage-gated conductances might also contribute to this transformation. Voltage-clamp studies of the primate cone inner segment have identified five voltage-gated currents [7], all of which were included in the present model. Similarly, the model also included the three voltage-gated conductances that have been identified in adult cat type-B horizontal cells [14]. Including these voltage-gated channels into the model did not significantly affect the input/output properties of the cone as measured by the responses to brief light flashes. Space constraints prohibit a detailed description of how each of the voltage-gated currents was implemented, but given the limited amount of published experimental data for each current, it is unlikely that our implementation correctly accounted for the full dynamic range of primate cones. However, given that we achieved a relatively good fit to published data, the fact that none of the voltage-gated currents in the model approached even the same order of magnitude as the photo and synaptic currents makes it unlikely that our conclusions would be substantially modified by changing the details of our implementation.

In lower vertebrates, it has been reported that the repolarizing phase of the cone's light response is due in part to the action of voltage-gated currents [19]. A selective-cationic conductance activated at hyperpolarized potentials, identified as an h-current, was shown to account for the repolarization following the onset of a bright light, while a potassium current activated by membrane depolarizations, termed Kx, contributed to the repolarization following onset of a dim light. The time course of these effects are much slower, however, than the very rapid repolarization seen in primate cones. It is thus unclear to what extent mechanisms identified in the lower vertebrate can be applied to the mammalian outer retina.

If voltage-gated currents indeed do not contribute to the flash response in primate cones, then one might ask what physiological function they do perform? Examination of how the different currents are activated at various potentials reveals that their major contribution occurs outside the normal physiological range, and would act in such a manner so as to drive the membrane potential back into the physiological range. Thus, we suggest that the major contribution of voltage-gated currents in the primate cone may be to ensure that the membrane potential always remains near an optimal operating point.

References

1. Schnapf, J.L., et al., *Visual transduction in cones of the monkey Macaca fascicularis.* Journal of Physiology, 1990. **427**: p. 681-713.
2. Schneeweis, D.M. and J.L. Schnapf, *Photovoltage of rods and cones in the macaque retina.* Science, 1995. **268**(5213): p. 1053-6.
3. Travis, B.J., *A computational model of one pathway in the cat sub-cortical auditory system.* Mathematics and Computers in Simulation, 1996. **40**: p. 81-99.
4. Wässle, H., B.B. Boycott, and J. Röhrenbeck, *Horizontal cells in the monkey retina: Cone connections and dedritic network.* European Journal of Neuroscience, 1989. **1**: p. 412-435.
5. Picaud, S., et al., *GABAA and GABAC receptors in adult porcine cones: evidence from a photoreceptor-glia co-culture model.* J Physiol (Lond), 1998. **513**(Pt 1): p. 33-42.
6. Kamermans, M., et al., *Hemichannel-mediated inhibition in the outer retina.* Science, 2001. **292**(5519): p. 1178-80.
7. Yagi, T. and P.R. Macleish, *Ionic conductances of monkey solitary cone inner segments.* Journal of Neurophysiology, 1994. **71**(2): p. 656-65.
8. Attwell, D., *The photoreceptor output synapse*, in *Progress in Retinal Research*, N.N. Osborne and G. Chader, Editors. 1990, Pergamon Press: New York. p. 337-362.
9. Schwartz, E.A., *Depolarization without calcium can release gamma-aminobutyric acid from a retinal neuron.* Science, 1987. **238**(4825): p. 350-5.
10. Attwell, D., F.S. Werblin, and M. Wilson, *The properties of single cones isolated from the tiger salamander retina.* Journal of Physiology, 1982. **328**: p. 259-83.
11. Baylor, D.A., A.L. Hodgkin, and T.D. Lamb, *The electrical response of turtle cones to flashes and steps of light.* J Physiol (Lond), 1974. **242**(3): p. 685-727.
12. Rieke, F. and E.A. Schwartz, *A cGMP-gated current can control exocytosis at cone synapses.* Neuron, 1994. **13**(4): p. 863-73.
13. Savchenko, A., S. Barnes, and R.H. Kramer, *Cyclic-nucleotide-gated channels mediate synaptic feedback by nitric oxide.* Nature, 1997. **390**(6661): p. 694-8.
14. Ueda, Y., A. Kaneko, and M. Kaneda, *Voltage-dependent ionic currents in solitary horizontal cells isolated from cat retina.* Journal of Neurophysiology, 1992. **68**(4): p. 1143-50.
15. Lohrke, S. and H.D. Hofmann, *Voltage-gated currents of rabbit A- and B-type horizontal cells in retinal monolayer cultures.* Visual Neuroscience, 1994. **11**(2): p. 369-78.
16. Huguenard, J.R. and D.A. McCormick, *Simulation of the currents involved in rhythmic oscillations in thalamic relay neurons.* J Neurophysiol, 1992. **68**(4): p. 1373-83.
17. Press, W.H., et al., *Numerical Recipes*. 1986, Cambridge: Cambridge University Press.
18. Smith, R.G., *Simulation of an anatomically defined local circuit: the cone-horizontal cell network in cat retina.* Visual Neuroscience, 1995. **12**(3): p. 545-61.
19. Barnes, S., *After transduction: response shaping and control of transmission by ion channels of the photoreceptor inner segments.* Neuroscience, 1994. **58**(3): p. 447-59.