In addition to the HCN1 channels that mediate the h current, the Kx current also performs signal filtering in rod photoreceptors. This current is known to be mediated by potassium channels and has similarities to the neuronal M current and EAG potassium channels. Although it is known that in filtering the light response of rods, $I_h$ and $I_{Kx}$ undergo complementary conductance changes, the qualities and significance of these changes are not clear. Here we present an analysis demonstrating the filtering effect of HCN1 channels in salamander rods when $I_{Kx}$ is blocked, and a simulation of the rod light response showing the magnitude and time course of the conductance changes by both currents. From this analysis, we propose that the purpose of opposing conductance changes by $I_h$ and $I_{Kx}$ may be to optimize the lateral propagation of signals through gap junctions in the rod network.

Introduction

During a photoreceptor light response many simultaneous membrane currents are summed by the cell to generate the voltage response. In turn, the voltage change of the cell alters the gating of each conductance through the voltage sensors of the channel. Through this nonlinear action of voltage on gating, and the action of gating on voltage, different classes of ion channels influence one another’s activity. Therefore, it is generally imprecise to classify the function of a single class of ion channel in a neuron outside the context of its peers. In this research addendum we describe one such interaction between $I_h$, mediated by HCN1 channels, and $I_{Kx}$, mediated by M-like potassium channels, in influencing the membrane impedance of a rod during its light response.

In our work on HCN channels in salamander photoreceptors, we characterized their biophysical properties and investigated their functional role in tiger salamander (Ambystoma tigrinum) rods and cones. From investigations of the gating kinetics and immunohistochemical staining, we showed that HCN1 channels are responsible for the $I_h$ current observed in these cells. We used the HCN antagonist ZD7288 to block HCN1 channels in rods and cones, and demonstrated that this increases the amplitude and duration of rod and cone light responses. When considered in the frequency domain, HCN block reveals the low-pass filter characteristic of the photocurrent. This low-pass characteristic comes from the slowness of the photocurrent, whose gating depends on a complex cascade of molecular interactions. In contrast, the opening of voltage gated channels such as HCN channels depends on the motion of charged voltage sensors and their interactions with the pore, which is generally a much faster process. In normal physiological conditions, HCN channels reduce response amplitudes at low frequencies, flattening the frequency response of the cell to light stimuli. This flattening allows the voltage response to be frequency independent over a wider range of stimulus frequencies, enabling the synapse to avoid saturation at low frequencies while still passing higher frequency signals. From a signal processing standpoint, this compensatory effect by HCN channels is analogous to...
high-pass filtering. Other studies previously described high-pass filtering in the rod network and postulated that it could be a way for the network to increase the signal to noise ratio for transient signals by spreading them over a larger area.\(^6\)\(^8\) By dividing transient signals into networked rods, multiple parallel rod to bipolar cell synapses can be used, increasing the signal-to-noise ratio at the bipolar cell layer. In addition to their role in the rod network, other studies have also shown how high-pass filtering due to Ih is important in increasing the speed of the individual photoreceptor light response.\(^9\)\(^10\)

Although HCN1 channels appear to be major players in the rod and cone light response, they are not the only source of filtering by voltage-gated ion channels. Another current called I\(_h\) has also been shown to be involved in high-pass filtering of rod light responses, especially responses to dim stimuli.\(^11\) I\(_h\) is similar to the M-current in neurons in that it is a potassium conductance that is partially activated at resting potential, is further activated by depolarization, and is largely non-inactivating.\(^2\) Kx channels also appear to be similar to EAG and Kcnv2 potassium channels, but its exact molecular origin is presently unknown.\(^12\)\(^13\) While both I\(_{\text{ks}}\) and I\(_h\) are known to mediate high-pass filtering in rods, an important difference between the two is that during a light response (in which the rod membrane hyperpolarizes), Kx conductance decreases, while h conductance increases. Due to their different reversal potentials (-30 mV for I\(_h\) and -75 mV for I\(_{\text{ks}}\)), the net current change caused by I\(_h\) and I\(_{\text{ks}}\) gating is inward during a light response, tending to counteract the initial hyperpolarization phase of the response.\(^11\)\(^14\) This reactive depolarizing effect leads to high-pass filtering of the input signal.

**Results**

To extend our previous studies of the h-current, we examined the contribution of I\(_h\) to the rod light response when the Kx conductance, calcium conductance, and other potassium conductances are blocked. To do this, we recorded the rod light response with 5 mM Co, 5 mM Ba and 20 mM TEA present in the bath.\(^14\)\(^16\) Five flashes of light of increasing intensity were delivered to a rod and the voltage response was recorded. Then I\(_h\) was blocked with 50 \(\mu\)M ZD7288 and light responses were recorded again.

In the presence of Co, Ba and TEA, the rod’s light response is smaller and occurs from a more depolarized potential than in normal ringer solution (Fig. 1A). Compare this with the simulation of a normal light response in Figure 2A. The depolarization in darkness is consistent with a reduction in the outward potassium current (due to I\(_{\text{ks}}\) and other uncharacterized potassium conductances) that normally counteract the inward dark current. A small transient “nose” in the light response is seen due to the presence of I\(_h\) (Fig. 1A).

When I\(_h\) currents are then blocked with ZD7288, the light response is seen to increase in magnitude and the transient “nose” is abolished (Fig. 2B). This demonstrates that I\(_h\) can play a role light response recovery even when I\(_{\text{ks}}\) is blocked. In both the solutions with and without ZD7288, an overshoot is seen following the recovery phase of the light response (Fig. 1A and B). This is a known effect of TEA on the rod light response first observed by Fain et al. but unlike in other studies, the overshoots we observed failed to generate regenerative spikes due to the block of calcium currents with Co.\(^16\)\(^22\) The ionic current that causes this overshoot is not completely clear. Although our model could account for much of the shape of the waveforms in Figure 1A and B when Kx and h-conductances were blocked (data not shown), it failed to account for this overshoot. One potential source of the overshoot could be an uncharacterized effect of TEA and/or Co, on the photocurrent, which the model did not include.

Although others have noted the complementary conductance changes by I\(_h\) and I\(_{\text{ks}}\) during a light response, the magnitude and time courses of these changes are unknown. To evaluate the simultaneous contributions of I\(_h\) and I\(_{\text{ks}}\) to the rod light response, we simulated the rod light response by solving differential equations describing voltage gated channels and the photocurrent numerically (see Methods and Appendix 1). The model was stimulated with five flashes of light of increasing intensity, and the time courses of the
Appendix 1

Cell Voltage and Currents

\[
\dot{V} = -\frac{1}{C_m} (i_{couple} + i_{i_t} + i_t + i_{c_a} + i_{o_c} + i_{pho})
\]

\[
i_{couple} = g_c A V
\]

\[
i_{i_t} = g_{i_t} m_{i_t} (v - E_{i_t})
\]

\[
i_t = g_t v (v - E_t)
\]

\[
i_{c_a} = g_{c_a} n_{c_a} (v - E_{c_a})
\]

\[
i_{o_c} = g_{o_c} v (v - E_{o_c})
\]

\[
i_{pho} = I_{dark}
\]

where (for \( n = 3 \) rods in a linear network)... 

\[
\begin{bmatrix}
V_1 \\ V_2 \\ V_3
\end{bmatrix} =
\begin{bmatrix}
1 & -1 & 0 \\
-1 & 2 & -1 \\
0 & -1 & 1
\end{bmatrix}
\begin{bmatrix}
F_1 \\ F_2 \\ F_3
\end{bmatrix}
\]

Variables and Constants

\[\begin{align*}
V & = \text{membrane voltage (mV)} \\
I & = \text{Currents given in pA}
\end{align*}\]

\[C_m = 35 \cdot 10^{-3} \, \text{nF}\]

\[g_c = 0.5 \, \text{nS}\]

\[g_{i_t} = 1.5 \, \text{nS}\]

\[g_t = 2 \, \text{nS}\]

\[g_{c_a} = 0.5 \, \text{nS}\]

\[g_{o_c} = 0.2 \, \text{nS}\]

\[E_{i_t} = -74 \, \text{mV}\]

\[E_t = -30 \, \text{mV}\]

\[E_{c_a} = -74 \, \text{mV}\]

\[E_{o_c} = -55 \, \text{mV}\]

\[E_{pho} = 40 \, \text{mV}\]

Photocurrent (from Hamer, 2000)

\[F = (cG/cG_{dark})^{G}\]

\[\dot{R} = \dot{\phi} - \left(1/\tau_R\right)R\]

\[\dot{E} = \dot{\phi} (R - (1/\tau_E)E)\]

\[cG = n_c G_0 (c/c_0)^{n_c} - cG_{dark} - cG_{light} + EF_j\]

Outer Segment Calcium Concentration and Buffer Activity

\[\dot{c} = k_c F - \gamma c c_{o_c} - c_c\]

\[\dot{c_c} = k_c (c - c_{o_c}) c - k_c c_c\]

Variables and Constants

\[\begin{align*}
R & = \text{Number of activated rhodopsin molecules} \\
E & = \text{Number of activated PDE molecules} \\
cG & = \text{cGMP concentration in outer seg. (\text{µM})} \\
c & = \text{Calcium concentration in outer seg. (\text{µM})} \\
c_{o_c} & = \text{Buffered calcium concentration in outer seg. (\text{µM})} \\
cG_{dark} & = 2 \, \text{µM} \\
cG_{light} & = 2.201 \\
\tau_R & = 0.416 \, \text{s} \\
\gamma & = 1734.72 \, \text{s}^{-1}
\end{align*}\]

Note: All numerical values are given as they were entered in the simulation. Units are provided for reference.
Appendix 1

\( \tau_e = 1.195 \text{ s} \)

PDE inactivation time const.

\( A_{\text{out}} = 4.461 \text{ mM/s} \)

Maximum cGMP synthesis rate

\( K_{\text{in}} = 0.219 \text{ mM} \)

Half-activation level of guanylate cyclase by Ca

\( n_{\text{out}} = 2.855 \)

Ca activated guanylate cyclase Hill coeff.

\( \beta_{\text{out}} = 0.136 \text{ s}^{-1} \)

cGMP hydrolysis rate in darkness

\( \beta_{E} = 1.68 \times 10^{-5} \text{ s}^{-1} \)

PDE dependent cGMP hydrolysis rate

\( b = 0.780 \text{ mM s}^{-3} \text{pA}^{-1} \)

Photocurrent to Ca flux

\( J_c = 72.296 \text{ pA} \)

Photocurrent model dark current

\( \gamma_c = 99.67 \text{ s}^{-1} \)

Ca exchanger extrusion rate

\( c_{\text{min}} = 0.005 \text{ mM} \)

Minimum outer segment Ca level

\( k_1 = 0.166 \text{ mM}^{-1} \text{s}^{-1} \)

Ca buffer binding rate

\( \beta_{\text{dark}} = 0.136 \text{ s}^{-1} \)

Total Ca buffer concentration

\( \beta_{E} = 1.68 \times 10^{-5} \text{ s}^{-1} \)

Ca buffer unbinding rate

\( n_h = 1 - (1 - m_h)^4 + 4m_h(1 - m_h)^3 \)

h-current kinetics

Probability of 2 of 4 particles being open

\( \alpha_h = 18(1 + e^{+50(1/12)}) \)

Probability of one particle being open

\( \beta_h = 18(1 + e^{+50(1/19)}) \)

\( m_c = \alpha_c(1 - m_c) - \beta_c m_c \)

\( \alpha_c = 0.66e^{+50(2/19)} \)

\( \beta_c = 0.66e^{+50(3/19)} \)

\( \beta_{\text{dark}} = 9e^{-(v+10(1/40))} \)

kx-current kinetics

Two-state noninactivating kinetics

\( \alpha_{hk} = 5(100 - v)e^{100(1-(1/42))} \)

\( \beta_{hk} = 9e^{-(v+10(1/40))} \)

\( \alpha_{hv} = 0.15e^{-(v+10(1/74))} \)

\( \beta_{hv} = 0.4125e^{v+10(1/74)} \)

kv-current kinetics

Gating with 3 activation and 1 inactivation particles

Activation particle

\( \alpha_{mkv} = 5(100 - v)e^{100(1-(1/42))} \)

\( \beta_{mkv} = 9e^{-(v+10(1/40))} \)

\( \alpha_{hhv} = 0.15e^{-(v+10(1/74))} \)

\( \beta_{hhv} = 0.4125e^{v+10(1/74)} \)

\( n_{mk} = m_h h_u \)

\( m_h = \alpha_h(1 - m_h) - \beta_h m_h \)

\( \alpha_h = 300e^{100(1/21)} \)

\( \beta_h = 100e^{-(v+10(1/21))} \)

\( \alpha_{hk} = 5(100 - v)e^{100(1-(1/42))} \)

\( \beta_{hk} = 9e^{-(v+10(1/40))} \)

\( \alpha_{hv} = 0.15e^{-(v+10(1/74))} \)

\( \beta_{hv} = 0.4125e^{v+10(1/74)} \)

Ca-current kinetics

Gating with 1 activation and 1 inactivation particle

Activation particle

Note: All numerical values are given as they were entered in the simulation. Units are provided for reference.
voltage, h and Kx conductances at each flash intensity were evaluated. During a light response the voltage (Fig. 2A) causes an increase in h conductance and a decrease in Kx conductance (Fig. 2B). These complimentary conductance changes tend to counterbalance one another during the flash response, resulting in a reduced net conductance change whose amplitude is time dependent (Fig. 2B, green traces). With large stimuli, the faster response kinetics of I_h cause a small transient conductance increase, followed by a longer lived conductance decrease due to I_Kx. Smaller stimuli cause a more synchronous activation of I_h and I_Kx (Fig. 2B, green traces). In our model, the net conductance change due to both currents deviates no more than 0.3 nS from the resting level, whereas each individual conductance changes by nearly 0.6 nS. Although the conductance increase by I_h and decrease by I_Kx are not perfectly synchronized, together they halve the maximal conductance change of one current individually.

Discussion

It has been observed using current pulse injection that, in contrast to cones, rods do not undergo an appreciable conductance change during a light response.23 One explanation for this observation was that during a light response, an increase in h conductance counteracts the conductance decrease from the photocurrent.23 This hypothesis does not account for the then unknown Kx conductance, and overlooks an important property of the photocurrent. During a light response, the photocurrent, which is actually a shutting off of the inward dark current, causes hyperpolarization of the cell membrane. The dark current's instantaneous I-V relation is nearly flat throughout the rod's physiological voltage range, from -20 to -80 mV, in both light and darkness (Baylor and Nunn, Fig. 6).24 This property means that from the standpoint of the rod, the photocurrent acts as a current source whose magnitude depends on light, and not on the membrane potential. The counterintuitive consequence is that although the photocurrent is mediated by a closing of the ion channels carrying the dark current, the voltage-independence of the current through these channels means that it does not contribute to a membrane conductance change during a light response. There may, however, be some slow conductance change associated with the voltage dependence of the Na-Ca-K exchange pump.25 It is important to note that unlike the rod, the cone dark current I-V relation is not flat, and therefore cones do undergo a conductance decrease when exposed to light.26

With the dark current ruled out as a source of conductance change, we conclude that the lack of observed net conductance change during a rod light response is likely due to the coordinated counterbalancing of h and Kx conductances, as we show with our simulation (Fig. 2). While the opposite conductance changes by I_h and I_Kx were first investigated some time ago,7,8 the question remains as to what, if any, advantage these complementary changes would confer during a light response. One theory is that the two different conductance changes are a consequence of having two separate mechanisms (I_Kx and I_h) for filtering small and large signals.11 Alternatively, we propose that the answer to this question may lie with the fact that rod photoreceptors are coupled to one another through gap junctions.

In the rod network (Fig. 3), signals propagate to adjacent rods through gap junctions in order to cancel random noise in individual cells and increase the number of parallel channels used in the rod to bipolar cell synapse.27 One commonly overlooked aspect of the rod network is that the degree to which signals propagate through the network is dependent not only on the strength of the signal itself and the coupling impedance, but also on the membrane impedances of the cells in the network. With high membrane impedance
**Methods**

Patch clamp recordings were made from dark adapted tiger salamander (*Ambystoma tigrinum*) rods at room temperature. External solution consisted of (in mM) 108 NaCl, 2.5 KCl, 1.2 MgCl₂, 2 CaCl₂, 5 HEPES (Sigma-Aldrich) titrated to pH 7.7 with HCl. 20 TEA-Cl, 5 BaCl₂, and 5 CoCl₂ were added to the bath to block voltage gated potassium, Kx, and calcium conductances, respectively. Where indicated, 50 μM ZD 7288 (Tocris) was added to the bath to block HCN channels. Internal solution consisted of 106 K-glucionate, 5 NaCl, 2 MgCl₂, 5 EGTA and 5 HEPES, titrated to pH 7.4 with KOH. Whole-cell recordings were made from salamander rods in the whole mount retina, using an EPC-10 patch-clamp amplifier (HEKA) in current clamp mode. Light stimuli were generated with a custom voltage to current source buffer circuit driving a 530 nm green Luxeon V LED (Philips).

Simulations were computed in MATLAB (Mathworks) using the ode15s numerical solver. The differential equations and constants for the model are given in appendix 1. Equations for voltage gated channels come from studies previously published by Liu and Kourennyi with the Iₜ model as published by Barnes and Hille. Although more accurate models of HCN channel kinetics exist, the Barnes model describes Iₜ kinetics relatively well (unpublished data), and requires only one state variable, greatly decreasing computational complexity. Equations and constants for the photocurrent come from studies by RD Hamer, originally from Nikono et al. Initial conditions for the model were determined by allowing the system to relax to steady-state without any light input.

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**References**

1. Wollmuth LP. Mechanism of Ba²⁺ block of M-like K channels of rod photoreceptors of tiger salamanders. J Gen Physiol 1994; 103:45-66.
2. Kourennyi DE, Barnes S. Regulation of M-like K⁺ current, Iₜ, by Ca²⁺-dependent phosphorylation in rod photoreceptors. Am J Physiol 1997; 272:1844-53.
3. Barrow AJ, Wu SM. Low-conductance HCN1 ion channels augment the frequency response of rod and cone photoreceptors. J Neurosci 2009; 29:5841-53.
4. Mánnikko R, Pandey S, Larsson HP, Elinder F. Hysteresis in the voltage dependence of HCN channels: conversion between two modes affects pacemaker properties. J Gen Physiol 2005; 125:305-26.
5. Mánnikko R, Elinder F, Larsson HP. Voltage-sensing mechanism is conserved among ion channels gated by opposite voltages. Nature 2002; 419:837-41.
6. Dertwiler PB, Hodgkin AL, McNaughton PA. A surprising property of electrical spread in the network of rods in the turtle's retina. Nature 1978; 274:562-5.
7. Owen WG, Torre V. High-pass filtering of small signals by retinal rods. Ionic studies. Biophys J 1983; 41:325-39.
8. Torre V, Owen WG. High-pass filtering of small signals by the rod network in the retina of the toad, *Bufo marinus*. Biophys J 1983; 41:305-24.
9. Gargini C, Demontis GC, Bisti S, Cervetto L. Temporal fidelity in the visual system. Archives italiennes de biologie 1999; 137:299-309.
10. Demontis GC, Longoni B, Barcaro U, Cervetto L. Properties and functional roles of hyperpolarization-gated currents in guinea-pig retinal rods. J Physiol 1999; 515:813-28.
11. Beech DJ, Barnes S. Characterization of a voltage-gated K⁺ channel that accelerates the rod response to dim light. Neuron 1989; 3:573-81.
12. Frings S, Brühl N, Drezg A, Angèle A, Hagen V, Kaupp UB, Baumann A. Characterization of ether-à-go-go channels present in photoreceptors reveals similarity to Iₚ, a K⁺ current in rod inner segments. J Gen Physiol 1998; 111:585-99.
13. Czirjak G, Tóth ZE, Enyedi P. Characterization of the heteromeric potassium channel formed by Kᵥ2.1 and the retinal subunit Kᵥ8.2 in Xenopus oocytes. J Neurophysiol 2007; 98:1213-22.

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**Figure 3.** An electrical model of the rod network. Signal propagation in the rod network depends not only on the strength of resistances coupling adjacent photoreceptors, but also on the membrane resistance of individual photoreceptors. With increasing membrane conductance, more current flows through the rod membrane (downward arrow), dissipating signals more readily in the rod network. For the indicated rod, the sum of the lateral and membrane currents must equal the current input.
14. Hestrin S. The properties and function of inward rectification in rod photoreceptors of the tiger salamander. J Physiol 1987; 390:319-33.
15. Bader CR, Bertrand D, Schwartz EA. Voltage-activated and calcium-activated currents studied in solitary rod inner segments from the salamander retina. J Physiol 1982; 331:253-84.
16. Fain GL, Quandt FN. The effects of tetraethylammonium and cobalt ions on responses to extrinsic current in toad rods. J Physiol 1980; 303:515-33.
17. Satoh TO, Yamada M. A bradycardiac agent ZD7288 blocks the hyperpolarization-activated current (Ih) in retinal rod photoreceptors. Neuropharmacology 2000; 39:1284-91.
18. Liu X, Kourennyi DE. Effects of tetraethylammonium on Kx channels and simulated light response in rod photoreceptors. Annals of Biomedical Engineering 2004; 32:1428-42.
19. Barnes S, Hille B. Ionic channels of the inner segment of tiger salamander cone photoreceptors. J Gen Physiol 1989; 94:719-43.
20. Altomare C, Bucchi A, Camatini E, Baruscotti M, Viscomi C, Moroni A, DiFrancesco D. Integrated allosteric model of voltage gating of HCN channels. J Gen Physiol 2001; 117:519-32.
21. Nikonov S, Engheta N, Pugh EN. Kinetics of recovery of the dark-adapted salamander rod photoreceptor. J Gen Physiol 1998; 111:7-37.
22. Fain GL, Gerschenfeld HM, Quandt FN. Calcium spikes in toad rods. J Physiol 1980; 303:495-513.
23. Baylor DA, Matthews G, Nunn BJ. Location and function of voltage-sensitive conductances in retinal rods of the salamander, *Ambystoma tigrinum*. J Physiol 1984; 354:203-23.
24. Baylor DA, Nunn BJ. Electrical properties of the light-sensitive conductance of rods of the salamander *Ambystoma tigrinum*. J Physiol 1986; 371:135-45.
25. Jarvinen JLP, Lamb TD. Inverted photocurrent responses from amphibian rod photoreceptors: role of membrane voltage in response recovery. J Physiol 2005; 566:455-66.
26. Yau KW. Phototransduction mechanism in retinal rods and cones. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 1994; 35:9-32.
27. Zhang J, Wu SM. Physiological properties of rod photoreceptor electrical coupling in the tiger salamander retina. J Physiol 2005; 564:8-49-62.