Vision: How to Catch Fast Signals With Slow Detectors

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The visual system is equipped with highly sensitive but slow detectors, yet it can resolve light changes up to 60 Hz. Processes taking place in retinal circuits go beyond the intrinsic limits of the transduction machinery by an unconventional exploitation of voltage-dependent conductances, cleverly lined up to generate a cascade of band-pass amplification stages.

Vision in humans relies on two distinct retinal sensors for light, which were originally classified on a purely morphological basis as cone and rod photoreceptors. The duplicity theory of vision assigned them distinct functional roles: cones operate in the presence of bright light in the environment, whereas rods take over in dim light. In particular, the ability of rods to work as photon-counting devices sets the sensitivity limit of the entire visual system. To attain this limit, rods trade off sensitivity with speed, and, as a consequence, the temporal resolution (i.e., the ability to follow faithfully temporal changes in light intensity) of their phototransductive cascade is poor.

The observation that rod signals are transmitted via two distinct functional pathways, with different light sensitivity and temporal fidelity, suggests that the temporal resolution of rod vision may not simply mirror that of phototransduction. In the following sections, we will discuss the trade-off between sensitivity and speed that occurs in phototransduction as well as the role played by the gating of voltage-dependent currents in matching the dynamic properties of the rod phototransductive cascade with those of rod-mediated vision at the perceptual level.

The light sensitivity of the visual system is limited by the photon-counting ability of retinal rods

The idea of retinal rods as very sensitive detectors, endowed with photon-counting ability, is rooted in the results of a psychophysical study (11) aimed at establishing the sensitivity limits of the human visual system. In that study, the authors investigated the relationship between stimulus energy and reliability in perceiving light signals. They estimated that absorption of ~10 photons by the retina was sufficient to evoke a sensation of light. Considering that these 10 photons were spread over a retinal area occupied by an average of 500 rods, it turns out that the probability of 2 photons hitting the same rod is very low. The remarkable conclusion was therefore that the lower sensitivity limit of each rod was set by the quantal nature of light rather than by the biological substrate, because a rod may reliably detect the absorption of a single photon.

The intriguing interpretation of this work, that mammalian rods may operate as photon counters, had to wait for nearly 40 years to be confirmed by direct measurements. By using the suction pipette technique (2), Baylor et al. (4) were able to demonstrate that primate rods generate a measurable change in the dark current in response to the absorption of a single photon.

The photon-counting ability of rods is a consequence of both the tight packing of the photon-catchng protein rhodopsin in the disk membrane and of the high amplification occurring in the phototransductive cascade. A drawback in the design of the transductive cascade is that it privileges sensitivity at the expense of speed.

The trade-off between speed and gain in retinal rods

At the molecular level, the high gain of phototransduction in rods depends on the frequency of the encounter of an active rhodopsin molecule with the GTP-binding protein transducin. Specifically, a single activated rhodopsin molecule may diffuse in the disk membrane and sequentially activate several transducin molecules, thus providing the first amplification step of the visual cascade. It is important to note that increasing the number of rhodopsin molecules in the disk improves the ability of rods to catch photons but may also cause some crowding of proteins, with the consequent decreased chance of an encounter between active rhodopsin and transducin. The optimal performance must then result from the trade-off between increased photon catch (sensitivity) and encounter rate (amplification). Furthermore, the deactivation mechanisms also rely on diffusion-limited reactions between active rhodopsin and rhodopsin kinase and between phosphorylated rhodopsin and arrestin. Because the slowest steps in the phototransductive cascade are those controlling deactivation, increasing the photon catch will also slow down the kinetics of phototransduction. Recent evidence from transgenic animals heterozygous for a null mutation of the rhodopsin gene and expressing half the normal complement of rhodopsin indicate that rods trade off sensitivity with speed (5).

Analysis of the power spectrum of the dim flash response suggests that the transductive cascade of human rods may be modeled as a low-pass filter (13). In particular, the cascade in toad rods (3) was found to be consistent with a two-pole low-pass filter of similar critical frequency \( f_c \), i.e., the frequency at which the amplitude drops to a value of 0.5 of the maximum. The \( f_c \) is related to the time constant of the response relaxation,
and therefore (assuming that the decay of the light response in human rods has 2 similar time constants ranging from 200 to 600 ms at 37°C) it may vary between 0.8 and 0.3 Hz.

Do the kinetics of phototransduction set the temporal resolution of the visual system?

On a purely intuitive basis, one expects the performance of a measuring device to reflect to a large extent that of its sensors. Along this line of thinking, the temporal resolution of the visual system should mirror the slow kinetics of its light detectors. In general agreement with this notion, the temporal resolution is higher at high (photopic) than at low (scotopic) luminance levels, as expected from the prevalence of the quick cone response under photopic and of the slow rod response under scotopic conditions, respectively.

Although intuitively sound, the notion of a correspondence between photoreceptor kinetics and temporal resolution of vision is not always correct and actually contrasts with some experimental findings. The first indication comes from the psychophysical measurements (6) of the dependence of critical flicker frequency (CFF, a good indication of the temporal resolution of vision) on the intensity of the background light in a rod monochromat subject lacking cone vision (12). As shown in Fig. 1A, the relation between CFF and retinal illumination in scotopic trolands (1 scotopic troland roughly corresponds to a 507-nm light producing the photoexcitation of 5 rhodopsin molecules per rod per second) has two limbs, separated by a plateau region between 1 and 10 trolands. It is interesting to note that the function is bimodal in both the normal and the monochromat subject, indicating that the different kinetics of rods and cones are not sufficient to explain the temporal properties of the visual system. Changes in the temporal properties of rod vision with the average luminance are also evident at frequencies lower than the CFF. As shown in Fig. 1B, at low intensities the system has the properties of a low-pass filter and becomes tuned at the brightest intensities (12).

The simplest explanation for the dependence on background light of both CFF and filtering properties of rods is that signals travel along two distinct pathways with different kinetics. The reasonable conclusion is that the temporal resolution of rod vision does not simply reflect the kinetics of rod phototransduction but also depends on processing taking place in the associated retinal circuits. Considering the estimated $\tau_{c}$ of rod phototransduction of 0.3–0.8 Hz (see above), signal processing by the retinal circuitry may explain the significant improvement of the temporal resolution of rod vision shown in Fig. 1B, with an $\tau_{c} > 10$ Hz.

The functional identification of two distinct pathways, both carrying rod-generated signals, eventually stirred an interest in their anatomic substrate. The slow, sensitive rod pathway goes through the synapse between rods and depolarizing “on” rod bipolar cells, which in turn connect to cone on bipolars via All amacrine cells and to “off” bipolars through a hyperpolarizing chemical synapse (reviewed in Ref. 16). The fast, insensitive rod pathway probably goes through the rod-cone electrical synapse, which has recently been shown to be functional in primates (17).

**FIGURE 1.** A: relationship between temporal acuity and illuminance for a contrast of 0.95 for a rod monochromat (●) and a normal subject (○). B: plot of the relationship between stimulus frequency and contrast sensitivity (threshold) for different background intensities for a rod monochromat (○, ▲, △, ▽) and a normal subject (□, △, ▽). Note that for the highest intensity the relation became tuned. Data are from Ref. 12.

This intrusion of rod-generated signals into the cone pathways is a neat example of multiplexing in biology, but how may it contribute to vision? A possible clue comes from the observation that subjects with only rods and cones with blue pigments may experience color vision at dusk or dawn (mesopic conditions), suggesting that rods contribute to color vision in twilight (14) by feeding their signals into cones.

Where are the compensatory mechanisms in the slow rod pathway localized, and how do they operate?

At this point, one may wonder where and how these compensatory mechanisms operate. In particular, both the localization and the molecular nature of the underlying components of these two rod-mediated pathways need to be defined. The analysis of human and cat electroretinograms (ERGs) has provided useful insight into the mechanisms involved in the elaboration of rod-generated visual signals at early stages of retinal processing. Stockman et al. (18) found evidence for the existence of fast and slow pathways in the human visual system by ERG recordings. The notion that the ERG mostly reflects currents generated in photoreceptor outer segments and in depolarizing rod bipolar cells suggests that the compensatory mechanisms start to operate at the level of rods and/or bipolar cells.

In their work, Gargini et al. (9) isolated pharmacologically the individual contribution to ERG of rods and bipolars by using 2-amino-4-phosphorobutyric acid (APB) and N-methyl-DL-aspartate (NMDLA; an agonist selective for the glutamate receptor of depolarizing rod bipolars and a blocker of signals generated by retinal elements postsynaptic to bipolars, respectively). As shown in Fig. 2A, the ERG response to a 5-Hz stimuli is still large in the presence of NMDLA, whereas in APB, when rods only contribute to the ERG, the response to a 5-Hz stimulus is already approaching its minimum value. The low temporal resolution of the ERG in APB is in general agreement with the estimates of the temporal resolution of phototransduction previously proposed.
Therefore, these results indicate that some compensatory mechanisms must operate between the outer segment of rods, where phototransduction takes place, and second-order neurons that are postsynaptic to rods. As for the precise localization of the compensatory mechanisms, it should be noted that, in scotopic conditions, where the rod input prevails, a CFF < 10 Hz has been reported for monkey horizontal cells with mixed rod-cone (also called H1) input (20). This value is higher than expected from the kinetics of phototransduction yet lower than the CFF of the depolarizing rod bipolar. These results are thus consistent with the notion that both rods and rod bipolar cells play a role in the improvement of the temporal resolution of the slow pathway above the limits imposed by the kinetics of the phototransductive cascade.

**Gating of voltage-dependent conductances implements band-pass amplification in the slow pathway**

The improvement of the temporal resolution of depolarizing rod bipolar cells above that of the phototransductive cascade occurs through band-pass amplification. This has been demonstrated in the cat retina, as illustrated in Fig. 2B, where the gain of the transfer function peaks at ~8 Hz (9).

In general, band-pass amplification is the expression of a negative feedback with a delay. Two distinct biological mechanisms that may implement a negative feedback are adaptation to light and/or gating of voltage-dependent conductances by light-induced hyperpolarization. In the turtle, for instance, the marked band-pass characteristics in the response of luminosity-type, cone-driven, horizontal cells have been interpreted and modeled as an expression of a negative feedback caused by light adaptation (19).

On the other hand, a role of voltage-dependent conductances in the implementation of band-pass amplification in mammals is indicated by the effect of selective blockers of the hyperpolarization-activated current (I_h), such as cesium and/or zatebradine (8). As shown in Fig. 2B, zatebradine affects the transfer function of the ERG by a 30% reduction of the maximum gain.

One may raise an objection about the functional significance of feedback mechanisms generated by a large response that induce either adaptation or voltage-dependent phenomena. The objection stems from studies in amphibia indicating that the operative range of the rod synapse spans 3–5 mV at most (1) because of the deactivation of calcium channels. Assuming a similar voltage dependence of calcium channels in amphibia and mammals, the rod bipolar synapse should saturate and start clipping signals at light intensities lower than those required for significant adaptation or gating of voltage-dependent currents. Specifically, this may challenge the role of I_h in filtering the light response of rods, because this current is usually activated by membrane hyperpolarizations more negative than the working range of the synapse.

**FIGURE 2.** A: relationship between the normalized amplitude of the first harmonic of the photoreceptor component of cat electroretinogram (ERG) (NMDLA + APB-isolated component; ○). The ERG responses were evoked with stimuli whose luminance was modulated sinusoidally at different temporal frequencies. Amplitude of bipolar cell component (●) was computed by vectorial subtraction of the ERG signal in NMDLA + APB from that in NMDLA (dotted line). Vertical bars are SE. B: gain of the synaptic transfer between photoreceptor and bipolar cell component in control conditions (○) and in the presence of the hyperpolarization-activated current (I_h) blocker zatebradine (●). Note the reduction in gain in the presence of zatebradine. Data in A and B are from Refs. 9 and 8, respectively.

**FIGURE 3.** A: effect of 3 mM cesium on the membrane voltage recorded in current-clamp mode in response to inward current injections of −5 or −15 pA. B: inward relaxation measured in voltage-clamp mode to hyperpolarizing voltage steps to −50 or −80 mV. In A and B, thin traces are control records, thick traces are in the presence of 3 mM CsCl, and dotted traces are recovery from CsCl. Modified from Ref. 7.
Properties and functional roles of hyperpolarization-gated currents in mammalian rods

The properties and the functional roles of voltage-dependent currents gated by membrane hyperpolarization have recently been investigated in guinea pig rods by using the perforated-patch technique to avoid changes in the properties of the conductances as a consequence of the washout of critical intracellular components (7). Two distinct voltage-dependent conductances have been identified. The first is an M-like potassium conductance ($I_{kM}$) carrying a background outward current to balance the inward dark current, thus contributing to setting the membrane potential in darkness. The second is activated by membrane hyperpolarization and has similar permeabilities for sodium and potassium ($I_{kx}$). As shown in Fig. 3B, cesium has minimal effects on the inward relaxation of membrane current induced by a voltage step from −35 to −50 mV but fully blocks relaxation at −80 mV. This indicates that the relaxation at −50 mV is mostly due to the deactivation by membrane hyperpolarization of $I_{kM}$, which is insensitive to cesium. Accordingly, current-clamp recordings illustrated in Fig. 3A indicate that cesium nearly fully blocks the membrane rectification induced by a −15-pA stimulus but hardly reduces rectification induced by a −5-pA current step, suggesting that dark current suppression up to 5 pA mainly gates $I_{kM}$.

Considering a dark current suppression of 0.7 pA in response to the absorption of a single photon, and assuming linearity, absorption of five photons would reduce the dark current by 3.5 pA, which, with an input resistance of 1 GΩ, would cause a voltage drop of 3–5 mV. Therefore, light intensities covering the working range of the slow and sensitive rod pathway (1 scotopic troland or 5 photoisomerizations; see Fig. 1) would gate mainly $I_{kM}$. Accordingly, the rod-to-rod bipolar synapse may transmit light intensities spanning the working range of the slow rod pathway that will mainly gate $I_{kM}$.

To assess its functional role, it is important to know whether gating of $I_{kx}$ in this voltage range may confer band-pass properties to rods. This has been investigated using current stimuli of up to 5 pA, whose amplitude was sinusoidally modulated in time, while recording in the current-clamp mode. As shown in Fig. 4, A and B, gating of voltage-dependent currents may confer band-pass amplification to rods, with a maximum gain of 1.7 at 2 Hz. Therefore, gating of $I_{kx}$ in the limited operative range of the rod synapse by low-intensity light stimuli may provide enough band-pass amplification to improve the temporal resolution of the slow rod pathway.

But if $I_{kx}$ mostly gates outside the operative voltage of the rod synapse, then how can we explain the effect of $I_{kx}$ blockers like zatebradine and cesium? Current-clamp recordings (see Fig. 3A) show that cesium slows the recovery of membrane voltage at the end of the hyperpolarization induced by the injection of an inward current of −15 pA. This suggests that $I_{kx}$ may affect the recovery of membrane potential after large hyperpolarizations. Considering that recovery is the rate-limiting step of the rod response, the inward $I_{kx}$ activated by the large hyperpolarization may thus accelerate the rate of voltage recovery in a range of membrane potentials in which the rod synapse operates. Correspondingly, in the depolarizing rod bipolars a steady-state $I_{kx}$ is active in darkness. Switching-on light would depolarize the cell and deactivate $I_{kx}$, speeding up the rising phase of the light response.

The tuning of the fast, insensitive rod pathway.

Gating of voltage-dependent conductances in postsynaptic neurons may also play a role in improving the temporal resolution of the fast rod pathway. As mentioned above, for background intensities >1 scotopic troland the dynamic properties of rod vision change from low pass to band pass. Considering that for larger hyperpolarizations the transient component of the rod response becomes more marked, reflecting an increased tuning of its voltage response, it is tempting to speculate that, after band-pass filtering by $I_{kx}$ gating, the signal takes the fast rod pathway via the rod-cone gap junctions. This may happen because the cone synapse has a wider dynamic range than that of rods (15), owing to the fact that the cGMP-sensitive channels of cones are also localized in the synaptic terminals and have a higher calcium permeability than the rod cGMP channels. The presence of a rod component in the response to light of monkey cones indicates that the rod-cone pathway is functional, although it appears to filter out the prominent nose present in the rod response to bright light (17). These data are consistent with the limited improvement of the temporal resolution of the rod component of the response of H1 horizontal cells in primates (20) compared with rod phototransduction. Therefore, these data suggest that a substantial

FIGURE 4. A: voltage response of a guinea pig rod to a 2-s step of −4 pA. The kinetics of the response are predicted by the analog circuit on the left. B: normalized impedance ($\bullet$), ratio between the amplitude of the voltage response in C and of the current stimulus in D) and phase (O; expressed as time difference between the peaks of stimulus and response) of the current to voltage conversion, plotted as a function of stimulus frequency. The continuous line through gain values is computed from the circuit model in A, with parameters obtained by fitting the voltage decay of the response in A ($R_1 = 3.68$ GΩ, $R_2 = 4.08$ GΩ, $L = 2.2$ GHz, and $C_m = 6$ pF; see Ref. 7). Note the good agreement between computed and experimental values. Modified from Ref. 7. C and D: voltage responses (C) and current stimuli (D) of 0.4 and 4 Hz. The dotted vertical line marks the peak of stimulus amplitudes. Note the difference in amplitude and phases for the response in C. Modified from Ref. 7.
improvement of the temporal resolution of the fast rod pathway will take place in cells that are postsynaptic to cones.

Concluding remarks

The limits imposed on the temporal resolution by the amplification stages of the transductive cascade are particularly important in vision, which deals with light, the sensory stimulus capable of the fastest possible temporal changes. Indeed, coding of temporal information may not be so important in other sensory modalities, such as smell or taste, which mainly deal with the quality of the stimulus rather than with its temporal pattern. However, it is important to note that the idea of a trade-off between sensitivity and temporal resolution is not restricted to sensory transduction, because it probably applies to every signal transduction process, including those operating in the dendrites of nerve cells.

It is intriguing that the tuning provided by band-pass amplification depends on the kinetics of gating. As an extreme example, gating of voltage-dependent sodium currents may provide band-pass amplification with a peak gain of ~50 Hz (10). By analogy with the signal equalizer of a high-fidelity sound system, equipping dendrites with a variety of voltage-dependent conductances that gate with different kinetics may implement a sort of equalizer for subthreshold signals, in addition to regulating the firing threshold of neurons. This unconventional picture of the role played by the gating of voltage-dependent currents in processing visual signals may thus turn out to be a general feature of signal processing by a variety of other neurons.

References