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Modulation of Rod-Cone Coupling by Light

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Although electrical coupling between rods and cones in the retina has been assumed to be static, it has now been shown that rod-cone coupling can be strengthened by light. Increment threshold measurements reveal that cone input to rods increases progressively as background light becomes brighter. Current injection into cones produces larger responses in adjacent rods in the presence of background light than in darkness. Weak coupling under dark-adapted conditions facilitates synaptic transmission of small rod signals, and strong coupling under light-adapted conditions enhances transmission of large cone signals.

IN THE VERTEBRATE RETINA, RODS AND cones are electrically coupled to each other by gap junctions (*1*), and the coupling is thought to be weak and static (*2*). However, electrical coupling between horizontal cells in the fish and turtle retinas can be modulated by light or by neurotransmitters (*3*). Moreover, anatomical analysis has indicated that signal transmission from photoreceptors to bipolar cells would be enhanced if the strength of rod-cone coupling varied with light adaptation conditions (*4*). We therefore studied the effect of steady background light on rod-cone coupling in the tiger salamander retina. We measured (i) increment threshold functions of the rods and cones to 500- and 700-nm light stimuli and (ii) the influence of background light on the voltage responses of rods to current injections into neighboring cones. We also studied the voltage dependence of this light-induced modulation of the rod-cone coupling.

Rods and cones were recorded separately or simultaneously under visual control with infrared illumination in superfused, flat-mounted, isolated retinas from the larval tiger salamander (*Ambystoma tigrinum*) (*5*). In this retina, there is primarily one type of rod (peak spectral sensitivity around 520 nm) and one type of cone (peak spectral sensitivity around 620 nm) (*2, 5*). We first studied the effect of background light on the cone and rod responses to 700- and 500-nm test lights (Fig. 1A). The intensities of the two test lights were adjusted so that they evoked responses of the same amplitude in darkness. In the presence of background light, the two cone responses were of similar amplitude, indicating that cones receive little influence from other cells. This does not, however, imply that background light does not affect rod-cone coupling, because rod responses are suppressed when background light is present and thus they cannot influence cones, regardless of any change in coupling. In contrast to the cone responses, rod responses to the 700- and 500-nm test light in the presence of background light were of different amplitude: the response to 700-nm light was larger than that to 500-nm light. This finding is consistent with the notion that the cone contribution to rod responses is greater in the presence of background light than in darkness.

We then determined the intensity of 500- and 700-nm lights necessary to obtain a 2-mV criterion response as a function of the intensity of a background stimulus (I_B) of 500 nm. This increment threshold data for a cone is shown in Fig. 1B. Under dark-adapted conditions ($I_B = -\infty$), the threshold intensities for both 500- and 700-nm flashes are similar, because the cone pigment in this retina is about equally sensitive to these two wavelengths (*5*). As I_B increases, the cone response threshold did not change until background light exceeded −5. As I_B increased further, the cone became responsive to the background light, adaptation occurred, and thresholds became elevated. The 500- and 700-nm functions closely correspond to each other throughout the whole range of I_B intensity. This finding indicates that only the cone visual pigment, acting according to the photochemical principle of univariance (*6*), governed the behav-

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ior of the cone. At first glance, this might suggest that cones receive little influence from rods by means of electrical coupling or any other means. However, because rods are nearly saturated at the I_B intensities that make up this cone increment threshold curve (Fig. 1A), this observation more probably reflects the different dynamic ranges of rods and cones.

The increment threshold curves for rods are shown in Fig. 1C. In the totally dark adapted eye ($I_B = -\infty$), rods are almost 3 log units (a factor of almost 1000) more sensitive to 500- than to 700-nm light, an observation that reflects the sensitivity of the rod photopigment (*5*) (Fig. 1A). As I_B increases, the 500-nm increment threshold function for the rod becomes increasingly steeper than the 700-nm function, and results at high intensities ($I_B > -4$) suggest a convergence of these functions at a common point. Hence, unlike the response of the cone (Fig. 1B), the rod does not obey the photochemical principle of univariance, and as I_B increases, the rod response is increasingly influenced by the cone pigment, which results in a more gradual elevation of increment threshold functions for 700- than for 500-nm light. The fact that the two functions approach each other as I_B increases suggests that rod-cone coupling increases as background intensity becomes brighter (Fig. 1C).

We next investigated the influence of the rod and cone membrane voltage on this change in coupling strength. In the presence of steady background illumination, the rods and cones do not stay at their peak response voltages but recover partially to plateau levels more positive than the peak response voltages (*2, 5*). Figure 1D shows the difference in this plateau voltage from the dark-adapted voltage as a function of I_B. The rod response plateau saturated when I_B was -4, and the cone did not show any response while I_B was less than -5.5. This result shows that the rod membrane voltage is constant when the background is brighter than -4 and that the cone membrane voltage is constant when the background is dimmer than -5.5. However, the increment threshold functions in Fig. 1C suggest that the strength of rod-cone coupling increases progressively through the whole range of background light intensity (between -8 and -3). In other words, the strength of rod-cone coupling changes within the background ranges when both the rod (between -4 and -3) and cone (between -8 and -5.5) membrane voltages are constant. It is therefore unlikely that the background-induced change in rod-cone coupling is mediated by voltage-dependent mechanisms in either rods or cones.

We also tested the effect of background illumination on the voltage responses of a rod (V_{rod}) to current injections into an adjacent cone (I_{cone}) (Fig. 2). In darkness, an injection of -1 nA of current into the cone elicited a sustained hyperpolarization of about 2.3 mV in the rod. A steady background light (500 nm, -4.25) was then introduced to the retina, which hyperpolarized the rod to a plateau level about 6.5 mV below the resting potential. The rod responses to the same current injection be-

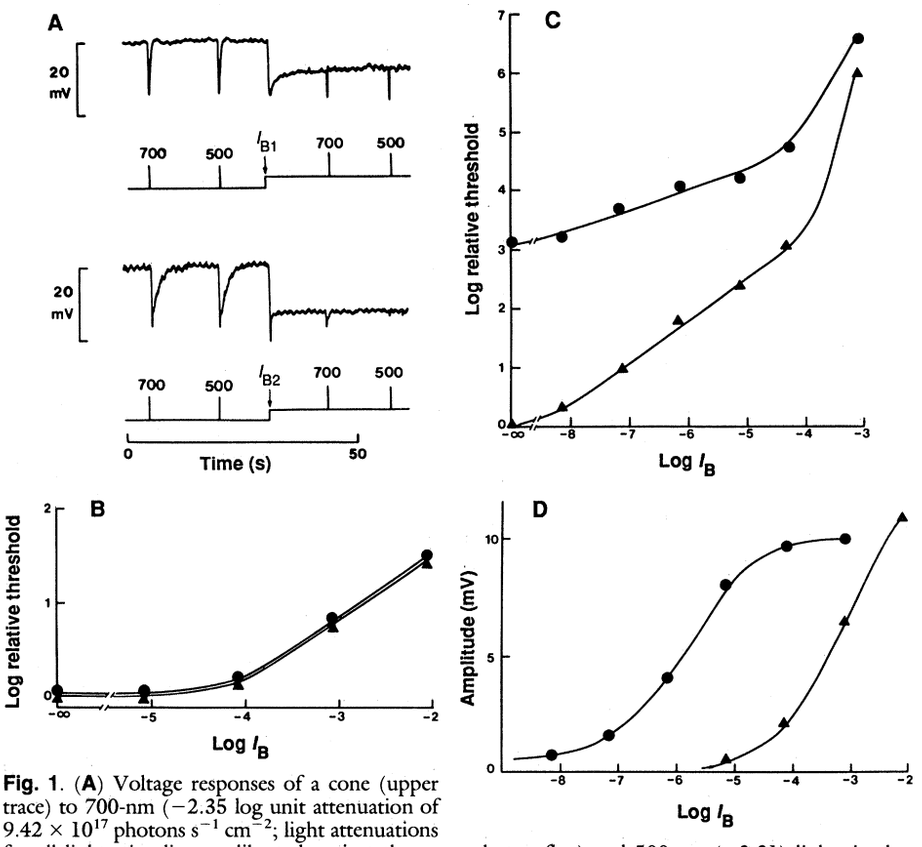

Fig. 1. (**A**) Voltage responses of a cone (upper trace) to 700-nm (-2.35 log unit attenuation of 9.42×10^{17} photons s^{-1} cm^{-2}; light attenuations for all light stimuli are calibrated against the same photon flux) and 500-nm (-2.31) lights in the absence and presence of background light ($I_{B1} = -3.12$, 500 nm); and voltage responses of a rod (lower trace) to 700-nm (-1.31) and 500-nm (-4.32) lights in the absence and presence of background light ($I_{B2} = -4.13$, 500 nm). The duration of all test lights was 100 ms. The 700- and 500-nm lights were adjusted for each cell so that they produced equal response amplitudes under dark-adapted conditions. (**B** and **C**) Increment threshold function of a cone and a rod, respectively, for 500-nm (▲) and 700-nm (●) lights. (**D**) The voltage-intensity (V-log I_B) functions of the rod (●) and the cone (▲) response plateaus. All cells were recorded from dark-adapted retinas. The criterion voltage for the increment threshold functions was 2 mV, and the duration of test lights was 100 ms in (B) and (C). The background light was introduced steadily to the retinas, and its intensity (in log units) was calibrated to the same absolute values for (B) through (D) (log I_B unattenuated = 6.8×10^{15} photons s^{-1} cm^{-2}, 500 nm, for all three abscissas labeled with log unit attenuations). Similar results were obtained from nine other rods and six other cones.

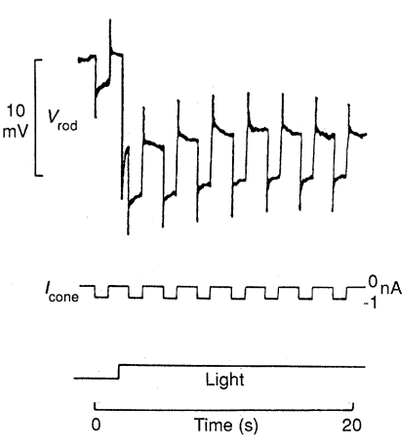

Fig. 2. Voltage responses of a rod (V_{rod}) to current injection into an adjacent cone (I_{cone}) (-1 nA, 1 s in duration) in the absence and presence of background light ($I_B = -4.25$, 500 nm). The response amplitudes were measured as the sustained hyperpolarizing levels, that is levels before the current pulse was switched off. Similar results were obtained from three other rod-cone pairs. The average rod response to an injection of -1 nA of current in cones in darkness was 2.0 ± 0.4 mV, and that in the presence of background light was 3.9 ± 0.6 mV. The time for reaching steady-state voltage responses was 22 ± 6.5 s after the onset of background light.

came larger, with an initial value of about 4.2 mV, which declined to a steady-state value of about 3.6 mV. Similar results were observed in cones when current was injected into adjacent rods. In agreement with the results shown in Fig. 1, rod-cone coupling in the presence of background illumination is stronger than in darkness.

Alternatively, cone input to rods could be enhanced by background light because light shuts down the photosensitive channels in the photoreceptor outer segments and thus reduces current shunting (*7*). This is unlikely, however, because background light fails to change the amplitude of rod responses when current is injected into adjacent rods (*8*). Mechanisms underlying the light-induced change in rod-cone coupling are unclear. Chemical synapses observed between rods and cones (*9*) and feedback synapses from horizontal cells to cones (*10*) may be involved.

We have shown in the tiger salamander retina that background light enhances cone-to-rod signals but not rod-to-cone signals because rod responses are suppressed. However, Nelson (*11*) suggested that in the cat retina, the opposite is true: rod signals can be seen in cone and cone bipolar cells but not vice versa. A possible explanation for this difference is that the rod/cone ratio of the tiger salamander retina is approximately 1 (*2, 9*), whereas that of the cat retina is on average 63/1 (varies from 10/1 in the central region to about 200/1 in the peripheral region) (*4, 12*). Hence in cats, a cone receives influence from many rods, but each rod receives small influence from cones; in salamanders, rods and cones have about an equal chance of contacting each other.

An adaptation-induced change in coupling strength may be advantageous for signal transfer between photoreceptors and second-order retinal cells. Under dark-adapted conditions, it is important for dim images to excite rods. Weak rod-cone coupling is desirable because it prohibits shunting of small rod signals into cones and thus enhances the efficacy of signal transmission to the second-order cells (*4*). In the presence of background light, rod responses to light saturate but are still important for second-order neurons to convey information regarding the presence of bright images. Strong rod-cone coupling is desirable because it allows large cone signals to spread into adjacent rods. Because rod and cone signals converge in the salamander retina (*9, 13*), enhanced coupling permits rods to supplement cones; this interaction leads to larger postsynaptic responses because the photoreceptor output synapses are rectified in favor of small presynaptic signals (*14*).

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The Incremental Threshold of the Rod Visual System and Weber's Law

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The incremental threshold of the isolated rod visual system is believed, under certain conditions, to obey Weber's law (that is, to increase in direct proportion to the intensity of the background). This relation was tested at several background wavelengths, over an intensity range for which the target was seen only by the rods. Although the slope on long-wavelength background approximates unity (that is, Weber's law on log-log coordinates), it averages less than 0.8 on short- and middle-wavelength backgrounds. This is the same value as that found for the thresholds of a typical, complete achromat—who lacks cone vision—regardless of background wavelength. These results force the conclusion that Weber's law for incremental threshold detection is achieved not by the rods alone but only by the rods acting together with the cones.

IN A CLASSIC AND FREQUENTLY CITED experiment, Aguilar and Stiles (*1*) measured the detection threshold of the human rod system from darkness to saturation. To isolate the responses of the rods from those of the cones, they presented a target chosen to favor the rods upon a long-wavelength background chosen to maximally desensitize the cones relative to the rods. Over four $\log_{10}$ cycles of background intensity, they found that the rod threshold for the target increased in direct proportion to the intensity of the adapting field [that is, it obeyed Weber's law (*2*)]. Aguilar and Stiles attributed this behavior to the activity of rods alone, on the assumption that rods and cones adapt independently (*3*). But, consistent with earlier reports (*4*), we find that the rate of increase of the rod incremental threshold depends on the background wavelength: Weber's law prevails on long-wavelength backgrounds but not on short- and middle-wavelength ones.

Our experimental conditions were essentially the same as those used by Aguilar and Stiles (*1*). A target 6° in diameter, exposed for 200 ms every 2000 ms, was centered 12° from the fovea in the nasal field of view and presented in the center of an adapting field or background 18° in diameter. To favor the rods, we used a target wavelength of 520 nm [because the ratio of the rod sensitivity to the cone sensitivity is large at this wavelength (*5*)] and its entry point in the pupil was 3 mm off center [because oblique entry light is much less effective for the cones than for the rods (*6*)]. The entry point of the background was central. Both the target and the background were presented in Maxwellian view, an imaging technique that allowed

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