

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.

**I**N 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<sub>10</sub> 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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the light beams carrying the target and background field to be focused in a small region of the observer's pupil, while the two fields themselves were imaged on the retina (7). Fixation was aided by a tiny illuminated cross. Unlike Aguilar and Stiles, who used only a long-wavelength background [for which the sensitivity difference between the rods and cones is smallest (5)], we used background wavelengths of 450, 520, 560, or 640 nm.

Five normal observers (A through E), one typical, complete achromat (author K.N.), and a blue-cone monochromat took part in the experiments. They were fully informed

about the general nature of the experiments and the possible consequences (none). Before we began a series of measurements, each observer was dark-adapted for 30 min and the pupil of his experimental eye was dilated with 0.5% tropicamide (Mydracyl Roche). A measurement consisted of setting a neutral density wedge so that the target was just visible, and the mean of ten settings was taken. After each measurement, the background intensity was increased and the observer was light-adapted to the new field for 3 min before the new threshold was set. As a control for observer bias, the measurements were repeated for one observer (A) by

means of a two-alternative, temporal forced-choice staircase procedure. No important differences were found between the two procedures (see Table 1).

One can assess the extent of rod isolation for each background intensity by comparing in Fig. 1 the thresholds measured during steady field adaptation (filled circles) with those measured during the plateau that terminates the cone phase of recovery from a white (3100 K) bleaching light of  $7.7 \log_{10}$  photopic troland-seconds (open circles). Only at higher background intensities, where the two curves intersect, are the cones more sensitive to the target than the rods. As expected, rod isolation appears best for the Aguilar and Stiles conditions. On the 640-nm background, the rod-cone transition takes place at an intensity above  $2.0 \log_{10}$  scotopic trolands (td), which is about  $1.5 \log_{10}$  units higher than that at which the rods yield the incremental threshold to the cones on the 450-nm background. But what about the effect of background wavelength on the shape of the rod incremental threshold curve?

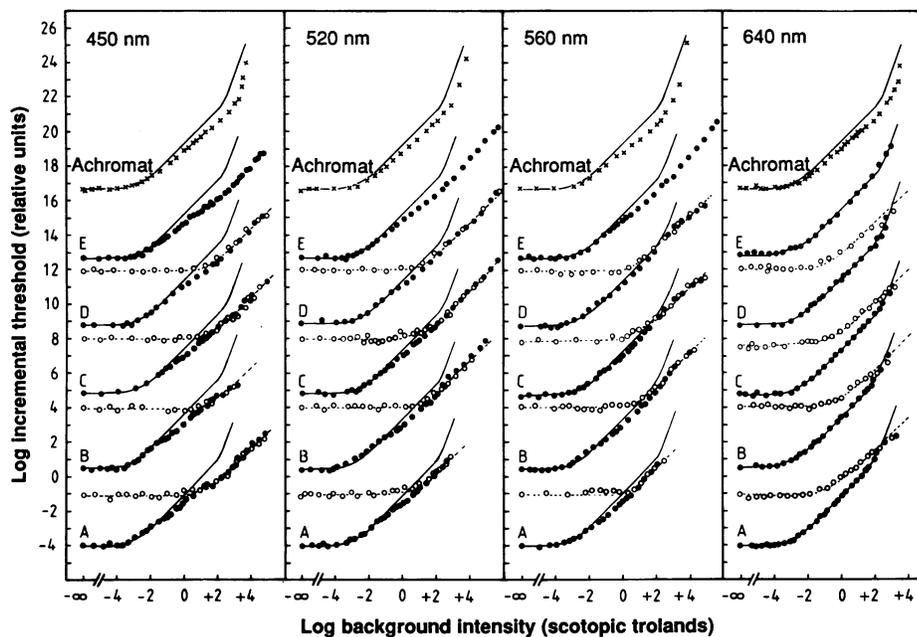
Table 1 presents, for each background wavelength, the slope of the curve between background intensities of  $-2.0$  and  $0.0 \log_{10}$  scotopic td. (We calculated the slope over the same region of background intensity for each wavelength. An upper limit of  $0.0 \log_{10}$  scotopic td was chosen because it was always at least  $0.5 \log_{10}$  unit below the intensity at which the cones first detected the flash.) If the rods adapt independently of the cones, then all the curves should have

**Table 1.** Rod incremental threshold responses for a 200-ms, 6°-diameter target. Absolute thresholds and slopes are given for five normal observers, A through E; a typical, complete achromat; a blue-cone monochromat; and the "mean observer" of Aguilar and Stiles (1).

Subject	Absolute threshold ( $\log_{10}$ scotopic td)	Slope (wavelength)			
		450 nm	520 nm	560 nm	640 nm
A	-3.97	0.69	0.69	0.71	0.95
A (2AFC)*	-3.95	0.71	0.75	0.81	0.96
B	-3.60	0.76	0.80	0.72	0.97
C	-3.23	0.78	0.81	0.86	0.99
D	-3.22	0.73	0.71	0.76	0.92
E	-3.27	0.78	0.77	0.79	0.95
Mean	-3.46	0.75	0.76	0.77	0.96
SD	0.33	0.04	0.05	0.06	0.03
t Test		7.00†	6.73†	6.33†	
Achromat	-3.30	0.75	0.78	0.79	0.77
Blue-cone monochromat	-3.24	0.82			0.81
Aguilar and Stiles	-3.23				0.95

\*Temporal two-alternative forced-choice procedure. These values are not included in the mean and SD determinations. †Scheffé post hoc comparisons t test ( $P = 0.01$ ,  $df = 3, 16$ ) = 3.98.

**Fig. 1.** The effect of background wavelength on the form of the curve of rod incremental threshold versus background intensity. The target conditions were chosen to favor the rods relative to the cones (for details, see text). Four background wavelengths were used: 450, 520, 560, or 640 nm. The filled circles (observers A through E) below the point where they intersect with the open circles are rod thresholds; above the intersection they are cone thresholds (or in some cases mixed rod and cone thresholds). The open circles represent cone thresholds measured for the same stimulus conditions during the plateau that terminates the cone phase of recovery from a white bleaching light. (The cone plateau thresholds were not measured for observer E.) The crosses indicate the rod-only threshold responses of a typical, complete achromat. All the curves are correctly placed with respect to the axis of the abscissae, but the axis of the ordinates is correct (in  $\log_{10}$  scotopic td) only for the lowest curve in each of the four panels; the other curves are displaced upward in intervals of  $4.0 \log_{10}$  units. Each data point is a mean based on at least three sets of measurements made on different days. The solid line drawn through each incremental threshold function is the mean function of Aguilar and Stiles (1, 9) shifted along the ordinate axis to correct for differences in absolute threshold. The function, which has a slope of 0.95, has also been fitted to the cone plateau thresholds (dashed line), demonstrating that for the given target conditions the cones, regardless of background wavelength, obey Weber's law.



the same slope in this region. In other words, backgrounds having the same intensity in scotopic trolands, and thus equated for the rate of rod quantal absorptions, should have identical effects.

But this clearly is not the case. For all five normal observers, rod thresholds on the 640-nm field, which nearly obey Weber's law (that is, a slope of 1.0 on log-log coordinates), rose more steeply than those on the other fields. This was confirmed by a one-way analysis of variance ( $F$  ratio = 22.84;  $P < 0.01$ ;  $df = 3,16$ ) and Scheffé post hoc comparisons between the mean slopes (8): The slope on the 640-nm background was significantly steeper ( $P < 0.01$ ) than the slopes on the other wavelength backgrounds. The difference is easy to see in Fig. 1 because each curve for observers A through E has been fitted with the mean response function from Aguilar and Stiles (1, 9), whose slope (0.95) exactly matches that of the 640-nm data ( $0.96 \pm 0.03$ ; mean  $\pm$  SD) but not those of the 450-nm ( $0.75 \pm 0.04$ ), 520-nm ( $0.76 \pm 0.05$ ), and 560-nm ( $0.77 \pm 0.06$ ) data. Curves measured with smaller ( $0.1^\circ$  or  $1.0^\circ$ ) and briefer (10 or 100 ms) targets displayed similar wavelength dependencies, although, in general, the slopes were less steep than those measured with the larger, longer target (10).

How can the change in slope with background wavelength be explained? We can largely rule out the possibility that the change is caused by the cones detecting the target and distorting the "rod" thresholds, simply because slope differences are first encountered far below the cone absolute threshold. Although signals from the rod and cone systems may interact to lower the detection threshold, relative to that of the more sensitive receptor system, they only seem capable of doing so when their sensitivities differ by less than  $0.3 \log_{10}$  unit (11–13). And even then the reduction is never more than  $0.2 \log_{10}$  unit (12), which is too small to explain our effects. It follows, therefore, that the main effect of the cones must be caused by quantal absorptions from the background, either by increasing rod sensitivity against short- and middle-wavelength backgrounds or by decreasing it against long-wavelength ones.

The second alternative seems more likely for two reasons. First, because the absolute sensitivities of the rods and cones tend to converge at long wavelengths (5), long-wavelength fields will excite the cones much more strongly than scotopically equated short- or middle-wavelength ones and are consequently more likely to influence rod threshold through their effects on cones. In fact, within the region used to calculate the

rod threshold slopes ( $-2.0$  to  $0.0 \log_{10}$  scotopic td), the short- and middle-wavelength fields have little effect on cone sensitivity. The cone thresholds (open circles) on 450-, 520-, and 560-nm fields hardly differ from their absolute value before the background exceeds  $0.0 \log_{10}$  scotopic td (Fig. 1). In contrast, by  $0.0 \log_{10}$  scotopic td the cone thresholds on the 640-nm field have already begun to rise. These thresholds, which are those of the middle wavelength-sensitive cones, hide those of the long wavelength-sensitive cones, which are even more strongly adapted by the 640-nm field.

Second, our results show that the incremental threshold curves of a typical, complete achromat, who does not generate cone signals (14–16), rise less steeply than Weber's law (and the Aguilar and Stiles mean function). His slope is about  $0.77 \pm 0.02$ , regardless of background wavelength (Table 1). This is closer to the values found for the normal observers with the 450-, 520-, and 560-nm backgrounds than to the value found with the 640-nm background; and it implies that, in the normal observers, cones excited by the long-wavelength background are influencing the sensitivity of the rods to the target (4, 17). This result has been confirmed in a blue-cone monochromat, who lacks middle- and long-wavelength cones (18). For him, too, the slope is the same ( $0.81 \pm 0.01$ ) against the 450- and 640-nm backgrounds (Table 1).

Unlike the lower (absolute sensitivity) asymptote of the rod incremental threshold curve, where threshold is independent of background intensity, and the upper (saturation) asymptote, where it rises precipitously, the regular logarithmic rise in threshold cannot be accounted for by events taking place within the outer segments of the rods themselves (19). In fact, we know that it must be due to more central events, because the sensitivity of individual rods changes very little before they saturate (19). The importance of a postreceptor site for summing rod signals and regulating rod sensitivity was established psychophysically more than a quarter of a century ago by Rushton and Westheimer (20). Our own results imply that the site (or sites) involved in setting rod sensitivity receives input from cones as well as rods.

We conclude that Weber's law for the detection of rod incremental targets is attained not by the rods alone but only by the joint action of the rods and cones. On its own, the rod visual system does not exceed a slope of about 0.8 on log-log coordinates before saturating. A long-wavelength background, such as used in the classic isolation procedure (1), favors the confounding influence of the cones upon rod field adaptation

(4, 17), as will any procedure that attempts to secure rod isolation by suppressing the sensitivity of the cones. These conclusions are consistent with other psychophysical (4, 21–25) and physiological (26, 27) observations, implying that rod signals travel in pathways used by cone signals and that the adaptation of the rod system is not independent of those of the cone systems.

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