Cones Survive Rods in the Light-Damaged Eye of the Albino Rat

Abstract. *Exposure to constant light causes extensive rod photoreceptor damage but spares the photopic system in albino rats. The rod branch of the dark-adaptation curve shows considerable elevation in threshold; the cone branch is hardly affected. Longer exposure and chromatic adaptation suggest that there are three cone mechanisms with peaks near wavelengths of 450, 520, and 560 nanometers.*

Continuous exposure to intense light causes severe retinal damage in rats. The amplitude of the electroretinogram (ERG) is drastically reduced (*1, 2*), and both light and electron microscopic assessments show photoreceptor damage which, with long-term exposure, can progress to complete disintegration of rods (*1, 3*). Behavioral studies on animals exposed to light whose retinas were apparently without intact rods show that these animals can nonetheless perform light-dark and pattern-discrimination tasks nearly as well as normal rats (*4*). What remaining retinal structure now serves the (rod) photoreceptor function?

Although it has long been acknowledged that the rat's eye possesses cones (*5*), the cones are so few that often little physiological function is ascribed to them. However, there has been persistent (*6*) and recently clear-cut (*7*) physiological evidence that the vision of rats is not solely determined by the rhodopsin rods. This report provides two lines of evidence that continuous exposure to intense light first damages the rhodopsin-containing rods while sparing the photopic mechanisms in the albino rat. (i) I traced the return in the dark of the eye's sensitivity after 60 seconds of bright light adaptation. In the normal albino, the dark-adaptation curve is composed of two branches (*8*). The spectral sensitivity of the mechanism underlying the late branch is consistent with rhodopsin rods. The spectral sensitivity of the early branch shows a relative increase in sensitivity for wavelengths greater than 550 nm. After light damage, the same animal's dark-adaptation curve shows a large threshold increase for the rod-mediated portion with a small threshold increase for the cone-mediated portion. (ii) With the scotopic system damaged by light, chromatic adaptation reveals the different photopic mechanisms. Three different photopic mechanisms with spectral sensitivities conforming to single pigment nomograms with peaks at wavelengths of 450, 520, and 560 nm are required to fit the data. Thus it is likely that the less affected cones mediate vision in light-damaged rats.

Fifteen albino (Sprague-Dawley) rats (weight, 150 to 350 g) were used; they had been dark-reared since birth, and previous exposure to light had been limited to brief intervals for feeding and cage cleaning. For each animal, immediately prior to light exposure, a baseline series of experiments were conducted. These same experiments were repeated after the damage. To avoid the known strong age-dependency of the light-damage effect, assessment of damage was made by comparing baseline results and those obtained after exposure for each animal separately (*9*).

Unanesthetized, unrestrained animals were placed in a white-walled cage with a wire mesh top. Two fluorescent lamps (General Electric Cool White, No. F15T8-CW, 15 watts) were placed 25 cm from the cage floor. The measured illumination at the cage floor was 100 ft-ca (1 ft-ca = 11 lu/m^2). The animals were exposed to this illumination for 12 to 24 hours and dark-adapted for 24 hours or more before testing. The light intensity and exposure duration were similar to that used by Noell *et al.* (*1*) and by Gorn and Kuwabara (*2*). These durations of exposure were chosen because they were sufficient to cause serious damage but did not completely disrupt electrophysiologically measurable visual function. The ERG's from damaged animals were high enough to allow careful and replicable measurement under these experimental conditions.

The ERG recording procedures (*10*) were standard (*11*). The test light was a 150-watt xenon arc lamp that fully illuminated the surface of a diffuser (one-third of a Ping Pong ball) placed over the eye. Calibrated neutral-density filters and narrow-band interference filters (Baird Atomic; half-bandwidth, 10 nm) were used in the test beam. The duration of the test stimulus was 20 msec; the adapting light was the full intensity of the xenon arc lamp applied for 60 seconds, which bleached at least 90 percent of the pigment (*12*). A tungsten lamp (General Electric, 18A/T10/2P) optically combined with the test light provided backgrounds. Broad-band interference filters with peak transmissions at wavelengths of 400, 550, and 600 nm were used in the background beam.

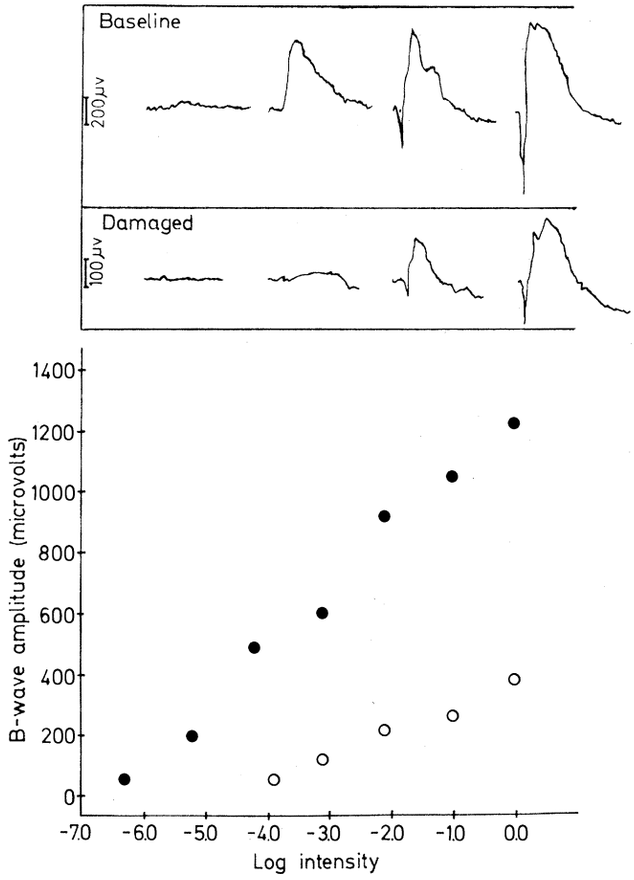
Figure 1 (top) shows typical ERG responses to 20-msec test flashes of increasing intensity for normal and damaged conditions in the same animal. For low intensities, there is a positive b-wave; at higher intensities, there is an initial negative a-wave followed by a b-wave. In this animal, after exposure to 12 hours of constant light the intensity required to yield a 50-μv threshold b-wave increased by 2.5 log units; and the full intensity of the xenon arc gave a 400-μv ERG b-wave (measured as trough of a-wave to peak of b-wave) as compared to a 1240-μv response prior to damage (Fig. 1, bottom). There was also a decrease in the slope of the curve of ERG response as a function of intensity. The maximal ERG after light damage for animals described by these typical results ranged between 360 and 480 μv, a threefold reduction from the normal. The dark-adapted threshold was elevated from 1 to 2.5 log units and the dark-adapted spectral sensitivities (shown in Fig. 2) were always rhodopsin mediated.

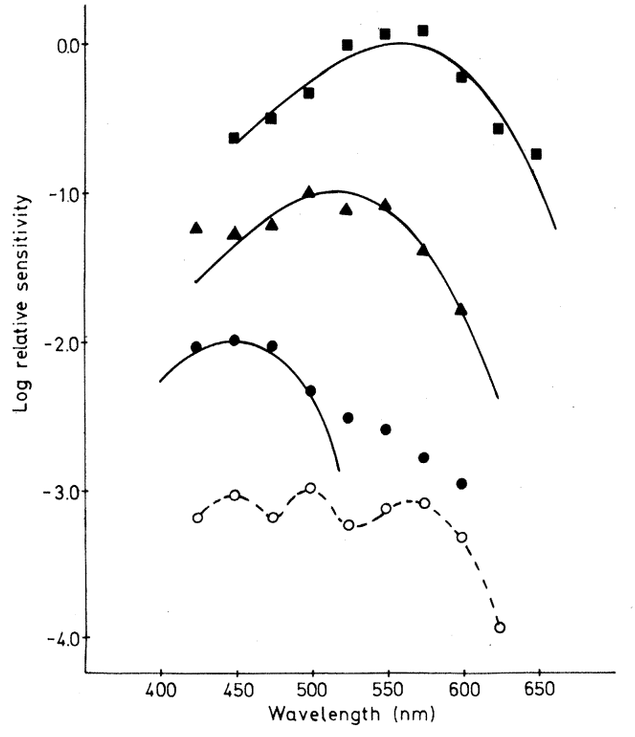
Figure 2 shows dark-adaptation curves for another animal before and after exposure to 12 hours of constant light, which increased the absolute threshold by 1.31 log units and reduced the maximal response from 1060 to 360 μv. Each data point represents the intensity of the xenon arc light needed to produce a 50-μv response. Before exposure (closed circles) there is an initial sharp fall in threshold followed by a period when the threshold is stable, and then, near 20 minutes, a fast decline in threshold. This suggests that two mechanisms that adapt at different rates are involved. To show this, I measured spectral sensitivities in the dark-adapted state and on the plateau of the first branch (*13*) (insets in Fig. 2). The smooth curves are for a Dartnall nomogram pigment peaking at 502 nm. The dark-adapted spectral sensitivity linked to the second (rod) branch implicates a rhodopsin-mediated mechanism. The spectral sensitivity for the mechanism underlying the first branch has a long wavelength increase consistent with cone function and is similar to that found previously for the albino rat under conditions of light adaptation (*6, 7*).

Subsequent to light damage (open circles, upper set, in Fig. 2) the dark-adaptation curve is pushed to higher thresholds, but the two branches are not shifted equally. The rhodopsin rod branch is elevated by 1.31 log units, while the cone branch is elevated by only 0.2 log unit—the rods have been damaged more severely than the cones. As a consequence of the differential decrease in sensitivity, the break in the dark-adaptation curve occurs at 50 minutes. When the curve is replotted (open circles, lower set) after a shift of 1.31 log units (the decrease in dark-adapted sensitivity), the rod branches fall atop one another.

In addition, the dark-adapted and cone plateau spectral sensitivities measured after light damage (open circles) superimpose their respective counterparts prior to damage. These two observations provide strong evidence that the first branch for the damaged eye reflects the same nonrhodopsin mechanism underlying the first branch of the undamaged eye and that the second branch in both cases is mediated by a rhodopsin mechanism.

When rats were exposed to constant light for longer periods of 15 to 24 hours, the dark-adapted spectral sensitivities were drastically modified. Figure 3 shows the dark-adapted spectral sensitivity (open circles) with three peaks. I used colored backgrounds to selectively de-





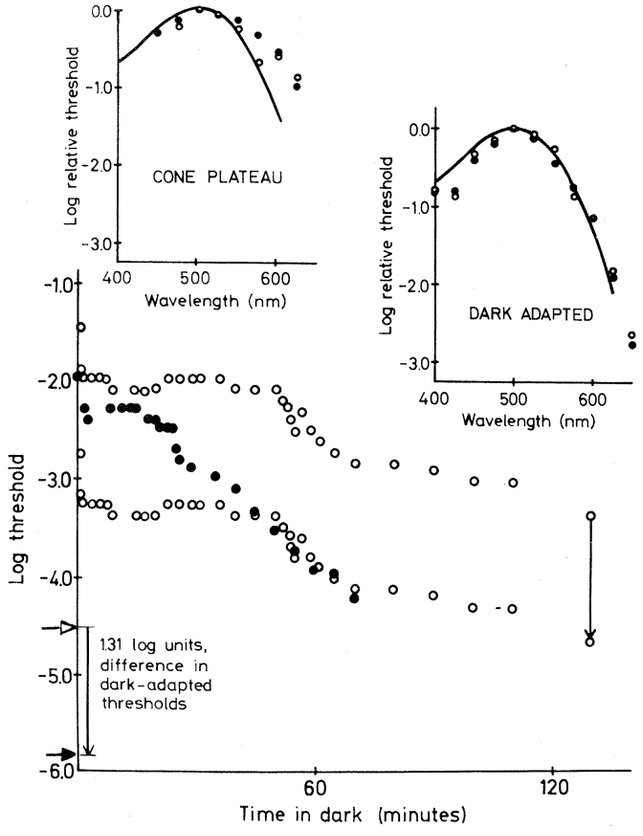


Fig. 1 (upper left). At the top are typical ERG's from a normal albino rat (*Baseline*) and from the same animal after 12 hours of constant light exposure (*Damaged*). The records show a decrease in b-wave amplitude at each intensity level. Shown below is a plot of b-wave amplitude as a function of intensity for both baseline (closed symbols) and damaged (open symbols) conditions. The figure shows a 2.5 log unit decrease in absolute sensitivity and a decrease in maximal ERG amplitude from 1240 to 400 μv. Fig. 2 (upper right). The bottom part of the figure shows dark-adaptation curves for the normal albino rat (closed symbols) and for the same animal after 12 hours of constant light exposure (upper curve, open symbols). Each point represents the relative test energy required to produce a 50-μv response. The closed arrow on the ordinate marks the absolute dark-adapted threshold for the normal animal; the open arrow 1.31 log units above marks that for the damaged. After light damage, the first branch has been elevated by 0.2 log unit while the second branch has been elevated by much more. When the curve for the damaged animal is replotted after a shift of 1.31 log units (lower curve, open symbols), the second branches superimpose. The dark-adapted spectral sensitivity (right inset) conforms to the rhodopsin rods: the curve is the Dartnall nomogram for a peak at a wavelength of 502 nm. The spectral sensitivity measured on the cone plateau (left inset) has a second peak near 600 nm. The scotopic mechanism has been damaged over a log unit more than the photopic. Fig. 3 (lower left). The open circles show the dark-adapted spectral sensitivity for an animal whose absolute threshold was raised by 1.6 log units after 15 hours of constant light exposure; whereas typically the dark-adapted spectral sensitivity conforms to a single pigment with a peak at a wavelength of 502 nm, now there are multiple peaks. Chromatic adaptation to 550-nm light shows a peak at 450 nm (closed circles), 600-nm light adaptation shows a 520-nm peak (triangles), and 400-nm adaptation shows a 560-nm peak (squares). Under chromatic adaptation, spectral sensitivities fit modified Dartnall nomograms consistent with three different pigments (shown as solid curves).

press the sensitivity of one mechanism so as to reveal others (*14*). The logic of the method lies in choosing a wavelength near the peak sensitivity of the mechanism one wishes to depress which is also far off the peak of the mechanism one wishes to express. Chromatic adaptation to light of a wavelength of 550 nm gave the next curve (closed circles) showing a peak near 450 nm. Adaptation to 600 nm light gave a spectral sensitivity with a peak near 520 nm (triangles), and adaptation to 400 nm light gave a spectral sensitivity peaking near 560 nm (squares). The smooth curves are modified Dartnall nomograms for peaks at wavelengths of 450, 520, and 560 nm (*15*). These curves provide evidence that when the rhodopsin rod mechanism is severely damaged by light other mechanisms mediated by pigments normally associated with cones survive in the albino rat (*16*).

While this work was in progress, LaVail (*17*) provided anatomical evidence that cones survive rods after long exposure to constant light in the Fischer albino rat. The percentage of cones detected by light and electron microscopy increased from 1.5 percent of all photoreceptors in the rat to about 60 percent with very long exposure. These findings are in conflict with earlier reports (*4*) that purport to find no photoreceptors surviving after exposure to lights of comparable intensity and shorter durations. The evidence presented here, showing greater initial damage to the rhodopsin rod system after much shorter exposure times, is consistent with LaVail's anatomical results and constitutes evidence linking anatomically described cones to a function distinct from that of rods.

In summary, two lines of physiological evidence show that the rods of the albino rat are initially more susceptible to the damaging effects of constant bright light than are the cones. In addition, the spectral sensitivities of the surviving mechanisms are consistent with single pigment Dartnall nomograms with peak sensitivities at wavelengths of 450, 520, and 560 nm.

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8. Low-intensity adapting lights will give a single, branched dark-adaptation curve. Infrequent data points may also spuriously give a single, smoothly descending curve. Dodt and Echte (*6*) have previously identified the change in spectral sensitivity during dark adaptation following intense light adaptation.
9. Repeated recordings did not by themselves cause damage. When control animals were recorded multiple times without intervening light exposure, there was no decrement of the response. After each experiment, the eye was ophthalmoscopically examined and flushed with mammalian Ringer's. Prophylactic doses of tetracycline were routinely administered in the drinking water to reduce infection.
10. Animals were dark-adapted 24 hours or more, then anesthetized with intraperitoneal injections of sodium pentobarbital (5 mg per 100 g of body weight) initially. To maintain anesthesia for periods longer than an hour, additional doses of 2.5 mg at a time were administered. The animal was mounted in an upright position by earbars. An electric blanket controlled by a temperature probe maintained the animal's temperature at 37°C. Under dim red illumination, the eyelids of the left eye were gently pulled back by sutures that were secured so the eyeball was exposed. The pupil was dilated fully with atropine. A cotton wick electrode with saline solution leading to a chlorided silver wire was placed at the edge of the cornea. An indifferent electrode was placed in a small cut in the cheek. The recorded signals were amplified by a Tektronix 122 preamplifier with a 0.2-second time constant and were displayed on a storage oscilloscope and photographed when desired by an oscilloscope camera.
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12. This estimate of pigment bleached was made by using Cone's (*11*) calibration. The intensity of my bleaching source was converted to Cone's absolute intensity units by using his own procedure of sliding the b-wave amplitude curves until they matched his.
13. The cone plateau is that portion of the first branch where the threshold is stable.
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Centrifugal Fibers to the Eye in a Nonavian Vertebrate: Source Revealed by Horseradish Peroxidase Studies

Abstract. *A source of efferent fibers to the eye of snakes of the genus* Thamnophis *has been identified by the use of the retrograde transport of horseradish peroxidase. Cell bodies of the contralateral nucleus of the ventral supraoptic decussation accumulate horseradish peroxidase after intraocular but not intraorbital injections. Intraocular injections also result in anterograde transport of horseradish peroxidase to retinofugal axon terminals. Intraorbital injections result in accumulation of horseradish peroxidase in the cell bodies of the cranial nerve nuclei of extraocular muscles.*

The existence of efferent fibers to the avian retina has been known since Cajal (*1*) first described these fibers in the retina. Efferent fibers in the retina have been described for several other vertebrate classes as well (*2*). However, it is only in the avian species that the cells of origin of the efferent fibers, the cells of the isthmo-optic nucleus, have been clearly demonstrated by means of anatomical techniques (*3*, *4*). That the source of the efferents to the retina has not been described in any other vertebrate class is surprising because the technology to identify the cells of origin of axon terminals has, over the past few years, become routine in many laboratories (*5*, *6*). In this report we describe a source of efferents to the eye of garter snakes as revealed by the use of the retrograde transport of horseradish peroxidase in neurons.

Fifteen garter snakes (ten *Thamnophis sirtalis sirtalis*, and five *Thamnophis radix*) weighing from 14 g to 92 g were anesthetized with a short-acting barbiturate (*7*). Type VI horseradish peroxidase (HRP) (0.1 to 8.0 μl of a 50 percent solution in saline) was injected unilaterally into the vitreous body or extraocular space through glass micropipettes with tip diameters between 30 and 40 μm. The micropipettes were inserted from the corneoscleral junction at the periphery of the eyeball into the vitreous body behind the lens for intraocular injections and into the orbital cavity for extraocular injections. Slow delivery of HRP through a microdrive system took from 5 to 20 minutes depending on the volume to be delivered. Animals were killed 24 hours to 5 days after injection; however, those killed after 2 to 3 days yielded the best results. The snakes were killed by intracardiac perfusion with cold solutions (4°C) of 0.18*M* sodium cacodylate buffer (*p*H 7.2) followed by fixative 1 (1 percent paraformaldehyde, 1 percent glutaraldehyde, in 0.1*M* sodium cacodylate buffer), and then fixative 2 (2 percent paraformaldehyde and 1 percent glutaraldehyde in 0.1*M* sodium cacodylate buffer). Brains were removed, stored, and processed according to the procedures described by Colman *et al.* (*8*) in which an unbuffered incubation medium containing *o*-dianisidine (3,3′-dimethoxy-