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Light and Efferent Activity Control Rhabdom Turnover in *Limulus* Photoreceptors

Abstract. *Photosensitive membrane structures in the retinular cells of the* Limulus *lateral eye are broken down and renewed daily. The first light onset causes a rapid, synchronous disassembly and buildup of the rhabdom in each photoreceptor cell. The entire process is complete within 30 minutes. Blocking the efferent input to the retina from the brain blocks the turnover of the rhabdom, and mimicking the efferent input by optic nerve stimulation restores it.*

Vertebrate photoreceptors periodically shed and renew the parts of the cells that transduce visual stimuli. Rods shed packets of membrane discs from the tips of the outer segments soon after the onset of light (*1*), and cones shed packets of outer segment discs soon after the onset of darkness (*2*). Disc shedding is followed by renewal, a process of disc assembly that occurs at the base of the outer segment and generally requires a longer period than shedding (*3*). Diurnal changes in retinal illumination appear to control the turnover of outer segment membrane (*1*, *4*), although in constant darkness disc shedding exhibits an endogenous, circadian rhythm in several species (*1*, *3*). No hormonal controlling factors have been found in rats (*5*). Indeed, recent evidence from frogs suggests that control of the turnover process in vertebrates is endogenous to the retina (*6*).

We report here an analogous turnover of photosensitive membrane structures in an invertebrate, *Limulus polyphemus*. We show that the onset of light triggers a rapid breakdown and buildup of the rhabdomeres of all retinular cells in the lateral compound eyes. In addition, we report that an essential requirement for rhabdom turnover is efferent activity transmitted from the brain to the retina by fibers in the lateral optic nerve.

Turnover in *Limulus* retinular cells was first detected in our experiments with freshly collected horseshoe crabs in Woods Hole, Massachusetts. During the afternoon of the day of collection, we placed seven animals in seawater aquaria and kept them in the dark throughout the night. The following day we transferred six of the animals to aquaria in direct sunlight and fixed the lateral eyes after various durations of exposure (*7*). The eyes of the seventh animal were fixed in the dark.

Figure 1 shows the sequence of light-induced changes in the rhabdomeres of retinular cells. In each electron micrograph the plane of section is perpendicular to the optic axis of the ommatidium, and the view is near the central region of the rhabdom (*8*). Figure 1A shows the rhabdom of the animal that was not exposed to light. Included are portions of the well-defined microvillar arrays from the rhabdomeres of three neighboring retinular cells and a portion of the central ring surrounding the dendrite of the eccentric cell. Figure 1B shows that a 15-minute exposure to sunlight triggered an extensive breakdown of the light-sensitive rhabdomeres. The microvilli were almost completely disassembled, and whorls of microvillar membrane formed lamellar bodies in the nearby palisade region. After 30 minutes of exposure (Fig. 1C), the rhabdom was nearly restored to its original state (Fig. 1A). By this time no lamellar bodies were present, and numerous small multivesicular bodies (MVB) lined the rhabdom rays (Fig. 1D). After 1 hour of sunlight the small MVB began to coalesce, forming larger MVB (Fig. 1E), which moved away from the rhabdomere. After 4 hours, several large MVB formed along the peripheral margin of the rhabdom (Fig. 1F). After 8 hours, large dense inclusion bodies were present in the peripheral region of the photoreceptor cells (Fig. 1G).

The onset of light causes a rapid, synchronous turnover of rhabdom in all the photoreceptor cells of the lateral eye. Because of the high rate of breakdown and buildup of the microvillar array, it has not yet been possible to determine the exact percentage of rhabdom involved in a given experiment. Nonetheless, all experiments exhibit evidence of turnover after light onset, but not all show the extent indicated in Fig. 1 (*9*). A useful indicator of turnover is the appearance of MVB near the rays of the rhabdom (*10*). We have no direct evidence that the MVB contain all of the membrane from the disassembled microvilli; in fact, the size and density of MVB appear inadequate to account for all membrane removed from the rhabdom. However, we conclude from the sequence in Fig. 1 that the disassembly and reassembly of the rhabdom most likely represents a process of membrane turnover.

Rhabdom turnover is not solely an effect of light exposure. Under natural conditions of illumination, the first light of dawn triggers turnover; a subsequent period of darkness and light on the same day does not (*11*). Efferent optic nerve activity that modulates lateral eye sensitivity exhibits a circadian rhythm (*12*). The efferent fibers discharge impulses only during the animal's subjective night. They are not active during the day, even in darkness. Our results show that rhabdom turnover occurs at the first light onset after a normal nighttime period of enhanced lateral eye sensitivity. However, no turnover was detected with animals that exhibited little or no circadian rhythm in visual sensitivity.

We further investigated the possible influence of efferent activity on rhabdom turnover by cutting one lateral optic nerve in each of five animals. The animals had been entrained to normal diurnal changes in illumination. Nerve transection was performed shortly before dusk, and the animals were stored in the dark overnight. The following day the lateral eyes were fixed after 15 minutes of light exposure. In each case the eyes

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with intact optic nerves showed evidence of turnover (as in Fig. 1B); the eyes with cut optic nerves did not (as in Fig. 1A). Blocking the efferent input to the photoreceptors blocked turnover.

Rhabdom turnover can be restored by delivering pulses of current to the cut optic nerve during the night. The procedure of the preceding experiment was repeated for two animals with the exception that, when the animals were placed in the dark, current pulses were delivered to the distal end of the cut optic nerve to mimic the normal efferent activity (*13*). At dawn the following morning the current pulses were turned off, at noon the animals were exposed to light, and 15 minutes later the lateral eyes were fixed. Electron micrographs show that rhabdom turnover occurred in the retinular cells of both eyes in each animal. Thus, mimicking the efferent input to the retina at night enables the first onset of light the following day to trigger turnover.

Perhaps the circadian efferent activity that modulates lateral eye sensitivity and structure (*12*) is the same as that which controls rhabdom turnover. Although all of our evidence is consistent with this suggestion, we have not completely eliminated the possibility that another source of efferent activity is responsible for controlling rhabdom turnover.

We have shown that the photosensitive rhabdom in *Limulus* retinular cells undergoes a rapid, synchronous turnover at the first light onset each day. The microvillar array is almost completely broken down and then reconstructed within 30 minutes of light onset. Cutting the optic nerve abolishes turnover, and shocking the cut optic nerve to mimic the efferent input restores turnover. Therefore, turnover of the array photoreceptor membrane requires both the onset of light and an efferent input to the retina from the brain. (The period of darkness before light onset and the duration of efferent optic-nerve activity necessary for turnover have not yet been determined.)

Membrane turnover in vertebrate rods and rhabdom turnover in *Limulus* retinular cells share several characteristics. In both cases, light onset triggers a rapid, synchronous breakdown of photoreceptor structure. Shedding of rod outer segment discs is complete after about 1 hour of light exposure in the rat and frog (*1*), and breakdown of the rhabdom occurs in less than 30 minutes in *Limulus*. In contrast, renewal of the rhabdom in *Limulus* is complete within 1 hour of light onset, whereas addition of discs to rod outer

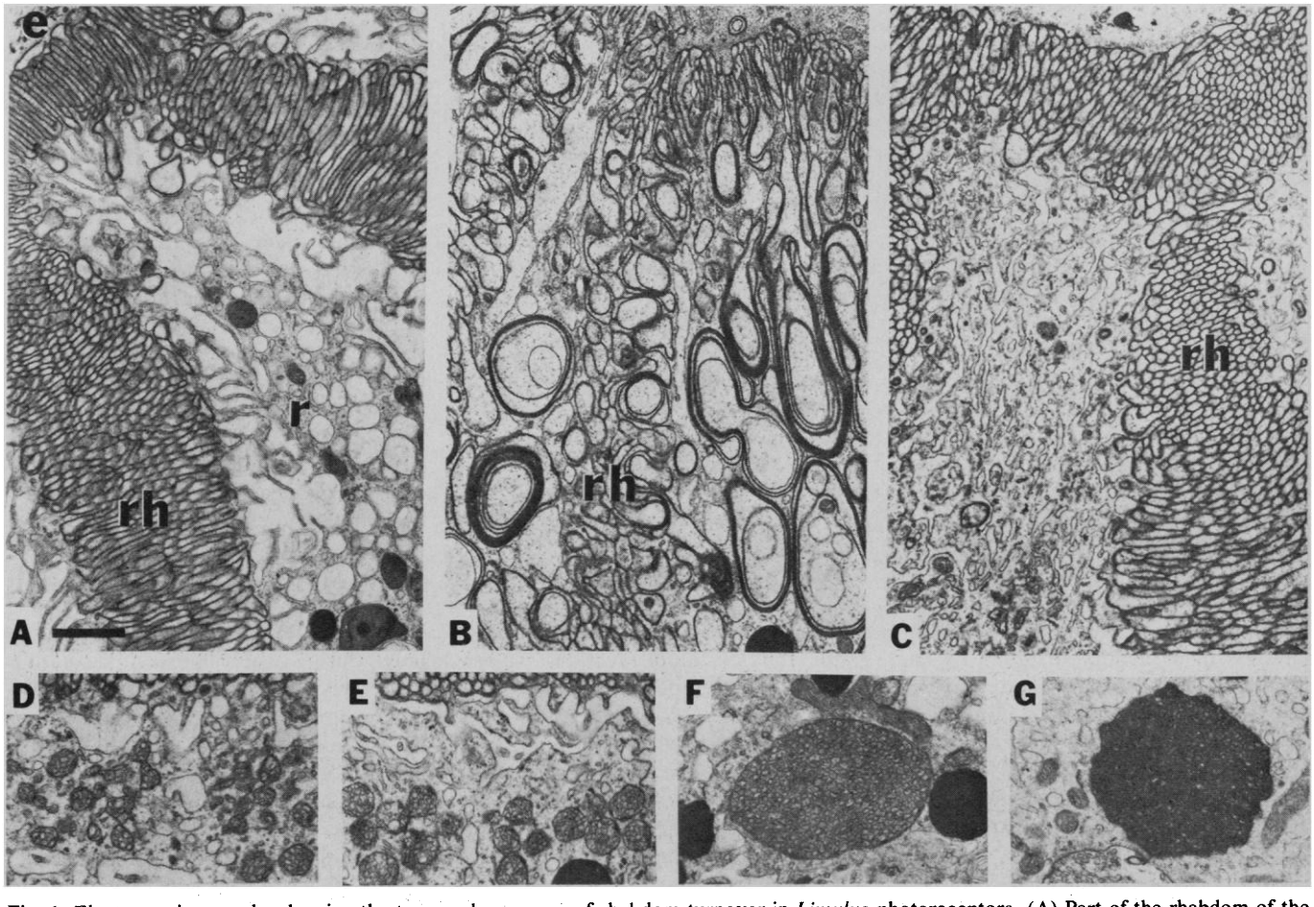

Fig. 1. Electron micrographs showing the temporal sequence of rhabdom turnover in *Limulus* photoreceptors. (A) Part of the rhabdom of the lateral eye of an animal that was not exposed to light. The well-organized microvillar array of the rhabdomeres (*rh*) of adjacent retinular cells is also present at the border between eccentric cell dendrite (*e*) and the retinular cell (*r*). (B) Breakdown of the rhabdom after 15 minutes of continuous exposure to sunlight. The microvillar array is disrupted and much of its membrane has been incorporated into lamellar bodies which fill the retinular cell cytoplasm. (C) Renewal of the rhabdom after 30 minutes of continuous exposure to sunlight. The microvillar array has been reconstructed. Numerous multivesicular bodies (MVB) formed from lamellar bodies appear in the retinular cell cytoplasm. Microvilli in portions of the rhabdom have been cut transversely. (D) MVB lining a rhabdomere after 30 minutes of continuous exposure. (E) MVB have coalesced and moved away from the rhabdomere after 1 hour. (F) Large MVB have moved to the periphery of the rhabdom after 4 hours. (G) Dense inclusion bodies formed by the condensation of large MVB appear in the periphery of retinular cells after 8 hours. (Bar represents 1 micrometer.) Lateral eyes were fixed in situ by intraretinal injection. After 30 minutes, the eyes were excised and immersed in fixative at room temperature for 5 hours, then refrigerated overnight. The retinas were cut into small pieces, rinsed, and placed in 1 percent OsO_4 for 1 hour. The pieces were then rinsed, dehydrated in alcohols, and embedded in Epon-Araldite. Thin sections were stained with uranyl acetate and lead citrate. Electron micrographs were made with a Siemens Elmiskop 101.

segments in *Xenopus* tadpole requires about 8 hours (*3*). In both cases, turnover requires an extended period of darkness before light onset. During the period of darkness, *Limulus* retinular cells require an efferent input from the brain. In the absence of cyclic lighting, rod shedding in rats and *Xenopus* tadpoles (*1*, *3*) exhibits a circadian rhythm, indicating the possible existence of an extraocular control mechanism. The control of rod shedding in frog, however, lies within the eye (*6*). In sum, turnover of photoreceptive organelles in the retinas of diverse animals appears to share several common characteristics, but the control mechanisms do not.

Other invertebrate retinas exhibit evidence of turnover of photoreceptor membrane, but the detailed mechanisms may differ from those we report for *Limulus*. For example, in mosquito larvae, membrane turnover is a continuous process that depends on the state of adaptation of the retina (*14*). In shrimp and crayfish (*15*), lamellar bodies and MVB appear in the retinular cells after prolonged exposure to light, but the temporal sequence of turnover is not clear. The retina of the spider *Dinopis* may be a special case in that the first light of dawn triggers almost a complete destruction of the rhabdom, but membrane synthesis occurs in a rapid burst many hours later at nightfall (*16*).

Why do photoreceptors periodically break down and renew their photosensitive membranes? Perhaps some aspect of visual transduction is irreversible, or the membrane that supports transduction lacks long-term stability. A better understanding of the turnover process should elucidate photoreceptor function. Studies of the *Limulus* lateral eye may prove useful because rhabdom turnover is controlled in part by efferent activity transmitted from the brain.

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10. We have inferred the temporal sequence of structures formed after the breakdown of the rhabdom from the temporal and spatial distribution of these structures and various lengths of light exposure. We have not yet carried out incorporation studies to show progressive labeling of breakdown structures.
11. The time of occurrence of the first light onset does not significantly affect rhabdom turnover. Animals first exposed to light in the early morning, midday, or late afternoon all showed similar turnover. For turnover to occur at first light onset, the preceding period of darkness must include a period of efferent activity.
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Erythrosin B Inhibition of Neurotransmitter Accumulation by Rat Brain Homogenate

Abstract. *A mixture of seven food dyes inhibited the accumulation of eight neurotransmitters or neurotransmitter precursors by rat brain homogenate. At a low concentration (1 microgram per milliliter), erythrosin B (FD&C red 3) was the only dye that inhibited dopamine accumulation. Erythrosin also was effective in decreasing the accumulation of all the other transmitter substances, suggesting that the inhibition is nonspecific and probably secondary to general membrane alteration.*

Food dyes are color additives utilized solely for esthetics. Except for recent concern about the carcinogenic and teratogenic potential of certain dyes, these substances have been considered to be relatively nontoxic (*1*). Questions have been raised however, about the possible behavioral effects of these and other additives in the human diet. Although not scientifically validated, there have been many claims that food dyes produce hyperactivity and other behavioral abnormalities in susceptible children (*2*). Either a pharmacologic or toxic or an immunologic or allergic mechanism could mediate such responses. Recent evidence indicates that one of these food dyes, erythrosin B (FD&C red 3), has synaptic effects at the frog neuromuscular junction (*3*). A concentration of 10 μM increased the frequency of spontaneous miniature end-plate potentials in this preparation. Our experiments were undertaken to evaluate possible chemical effects of these dyes on mammalian central nervous system (CNS) transmitters. Erythrosin B inhibited the accumulation of these substances by a CNS preparation.

The membrane transport systems for neurotransmitters or neurotransmitter (NT) precursors by CNS tissue are ac-

Table 1. Effect of dye mixture on NT accumulation.

NT	Uptake (pmole/g)*		Percent of control	P†
	Control	Dye		
Choline	79 ± 8	45 ± 10	57	<.01
Dopamine	238 ± 25	124 ± 18	52	<.001
γ-Aminobutyric acid	2072 ± 180	1146 ± 365	55	<.01
L-glutamic acid	5769 ± 205	2759 ± 471	47	<.001
Glycine	314 ± 44	115 ± 20	36	<.001
l-Norepinephrine	260 ± 28	128 ± 8	49	<.001
Serotonin	512 ± 14	359 ± 40	70	<.01
Taurine	41 ± 7	23 ± 3	56	<.02

*Uptake represents the mean ± standard deviation of quadruplicate samples in picomoles of NT accumulated per gram of tissue (wet weight) after 5 minutes of incubation at 25°C. †Significantly different from control, using the unpaired two-tailed t-test and with 6 d.f.

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