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Photoreceptor Membrane Shedding and Assembly Can Be Initiated Locally Within an Insect Retina

Abstract. *Photoreceptors of locust compound eyes add new receptor membrane at dusk and shed membrane at dawn. When part of an eye is masked before dusk, premature assembly of new membrane is initiated in the masked ommatidia but not in the adjacent unmasked ommatidia. Similarly, masking some ommatidia just before dawn prevents normal shedding only in the masked ommatidia. Therefore, the shedding and assembly phases of photoreceptor membrane turnover can be initiated by a change in the state of illumination of individual ommatidia.*

The photoreceptor membrane (PRM) in vertebrates (*1*) and arthropods (*2*) turns over according to a daily cycle. During turnover, the amount of shedding of the rod outer segments in vertebrates and the rhabdomeres in arthropods normally reaches a peak after dawn, under the influence of the onset of light and of endogenous factors (*1–5*). Assembly of new rod outer segment disk is also greatest during the morning (*6*). However, in some arthropods, assembly of new microvillar PRM is restricted to a few hours just after dusk and, like shedding, appears to be controlled both endogenously and by a change in the ambient lighting (*2, 5, 7*).

Attempts to localize the control mechanisms of PRM turnover have shown that in various animals both shedding and assembly are controlled separately in each eye. Shedding in the frog *Rana* is prevented in one eye if that eye is masked at dawn, while shedding in the unmasked eye is not affected (*8*). When one eye of a crab is masked in the afternoon, new PRM is soon assembled in that eye but not in the other (*7*). Initiation of shedding, as well as synchronization of its circadian timing by the central nervous system, occurs unilaterally in rat eyes (*9*). Similarly, *Limulus* lateral eyes are affected independently by efferent input that is necessary for normal turnover (*4*). Isolation of single eyes in vitro does not prevent light-stimulated shedding of PRM in *Xenopus* eyes (*10*), or assembly of PRM in a locust (*11*) or crab eyes (*12*).

In the present experiments, I have attempted to localize the control of PRM shedding and assembly to within discrete regions of single compound eyes of a locust. Whereas all the photoreceptors of a vertebrate eye share one lens system, the compound eyes of insects such as locusts are composed of many optically isolated units known as ommatidia. I have taken advantage of this arrangement by using simple masking experiments to ascertain if shedding or assembly can be initiated locally in the retina without affecting adjacent regions.

Each locust ommatidium has eight receptor cells that contribute to a rhabdom of photoreceptive microvilli and share the same visual field (*13*). As in many arthropods, locust rhabdoms vary in size as a result of daily turnover (*5, 14*). Shedding by pinocytosis from the bases of the microvilli reduces their cross-sectional area four- to fivefold at dawn, while at dusk, assembly of newly synthesized PRM increases it by a corresponding amount (*5*); the night rhabdom is larger than the day rhabdom mainly because its microvilli are twice as long (*5, 14*). A change in rhabdom cross-sectional area is therefore a convenient and clear indication of the occurrence of PRM shedding or assembly.

When locusts are placed in darkness 5 hours before the normal time of dusk, premature initiation of the assembly of new PRM increases rhabdom cross-sec-

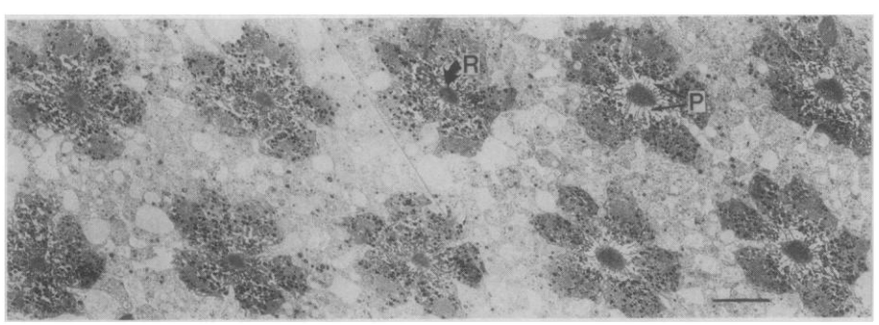

Fig. 1. Electron micrograph of a transverse section of ommatidia across the boundary between masked (right) and unmasked (left) parts of an eye. The ventral half of the eye was masked with tape 5 hours before dusk, and the eye was fixed 4 hours later. Over the central region of a locust compound eye, rhabdom size is normally the same among different ommatidia. Masking, however, has induced the four rhabdoms on the right to enlarge (they average 13 μm^2 cross-sectional area) by assembling new microvillar membrane. The four rhabdoms on the left have remained in the day state (average cross-sectional area is 5.3 μm^2). The two central rhabdoms may be in an intermediate state (6.5 μm^2) (*19*). The masked rhabdoms are surrounded by a "palisade" (*P*) of endoplasmic reticular vacuoles. Scale bar, 10 μm. *R*, rhabdom.

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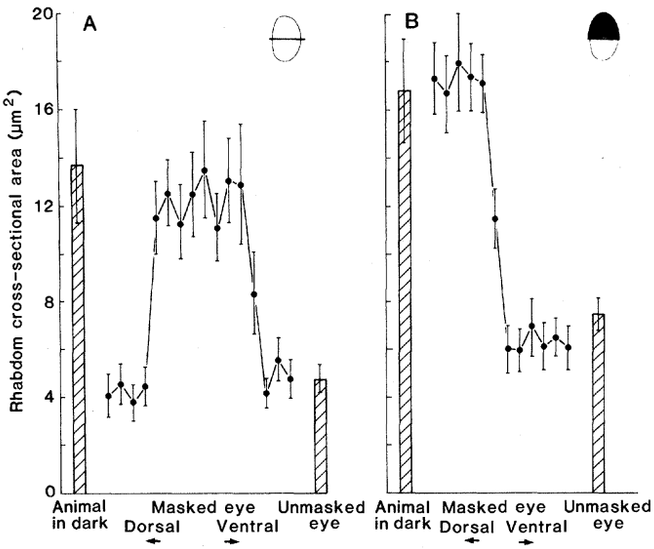

Fig. 2. Graphs of rhabdom cross-sectional area across the boundaries between masked and unmasked regions of an eye. Each point represents the mean of five rhabdoms from a horizontal row of ommatidia. The histograms represent measurements of controls. The mean size of 30 rhabdoms sampled from the unmasked eye contralateral to the masked one is on the right. On the left is the mean of 30 rhabdoms measured from three animals (ten rhabdoms from one eye of each animal) that were placed in darkness (A) or retained in dim red light (B) during the period that the other animals were masked. All bars extend ±1 standard deviation. Measurements were made from a transverse section taken 50 to 100 μm from the distal ends of the rhabdoms, where their diameter is nearly uniform (*5*). The centers of the *x*-axes of the graphs correspond to the centers of the sections. The interommatidial angle along the vertical axis of the eye is only about 1°; therefore, all rhabdoms were considered to be in true transverse section. (A) The eye was masked with a 0.3-mm horizontal strip of black tape 5 hours before dusk and fixed after 4 hours. A 0.3-mm strip should cover eight to ten horizontal rows of facets. This number corresponds to the number of ommatidia affected by the masking. Masked rhabdoms underwent assembly of new membrane and thus enlarged to the size of rhabdoms in locusts that were placed entirely in darkness (left histogram). In contrast, adjacent unmasked rhabdoms remained at a day-state size, comparable to that of rhabdoms in the other eye, which was left completely unmasked (right histogram). (B) The dorsal half of the eye was masked just before dawn, and the eye was fixed 1 hour after lights-on. Unmasked rhabdoms shed their membrane and thus diminished in size, while the masked rhabdoms remained in the night state, comparable to the rhabdoms of animals that were not exposed to light at dawn (left histogram). Localization of the effects of masking, as shown by (A) and (B), were also found with a vertical boundary, formed by masking the anterior or posterior halves of eyes.

tional area to about three-fourths that of the normal night state (*5*). In the first experiment, young adult *Valanga irregularis* that had been reared under cyclic lighting (*15*) had part of one of their large, light-colored eyes covered with black tape 5 hours before "dusk." Half of the eye (three animals) or a 0.3-mm horizontal strip across the middle of the eye (three animals) was masked. The other eye was left unmasked and served as a control. For additional control, three animals were placed entirely in the dark. Eyes were fixed for electron microscopy 4 hours after they were masked (*16*). Examination of sections of the eyes showed that the rhabdoms of masked ommatidia—and only those of the masked ommatidia—had enlarged and therefore undergone assembly of new PRM (Figs. 1 and 2A). Another indicator of the occurrence of PRM assembly is a characteristic disarray of the rhabdoms during the process of assembly (*5*). In other eyes, fixed 0.5 to 1 hour after they were masked, the rhabdoms of masked ommatidia were found in this state.

When locusts are maintained in darkness past dawn, rhabdom size diminishes gradually, but there is no significant change in size for the first few hours (*5*). In a second experiment, this fact was used to localize initiation of normal shedding. Seven animals were kept overnight under a dim red safelight (Kodak 1A, 15-W bulb). Just before exposure to light at "dawn," half of one eye was masked in each of four animals. Again, the unmasked eye served as a control, as did one eye from each of three animals that were not exposed to light. Eyes were fixed 1 hour after lights-on. The rhabdoms of ommatidia that were not masked had shed their membranes and were smaller than those of masked ommatidia, which were still in a night state (Fig. 2B). In addition, the characteristic secondary lysosomal products of shedding (*17*) were evident in the photoreceptor cells of the unmasked ommatidia and absent in the others.

A further effect of dark-light adaptation was also correlated with the masked or unmasked condition of the ommatidia. Masked rhabdoms were surrounded by a clear "palisade" of endoplasmic reticular vacuoles, and the unmasked rhabdoms, by dense cytoplasmic contents (Fig. 1); the first state is characteristic of dark adaptation and the second of light adaptation (*18*). This finding shows that the masked ommatidia were indeed in darkness, while the unmasked ommatidia were light-adapted.

Thus, changes in rhabdom size indicate that the initiation of shedding at dawn and the initiation of assembly at the onset of darkness are localized to the individual ommatidium (*19*). This result raises the possibility that the individual photoreceptor cells may have autonomous control over the daily turnover of their transductive membrane (*20*). The possibility that efferent nervous input to the photoreceptors (*21*) is a source of extraretinal control has not been eliminated by the present experiments. However, this input, although required for turnover in *Limulus* (*4*), has been found to be unnecessary for the assembly of PRM in the crab, *Leptograpsus* (*12*), which has a pattern of turnover similar to that in the locust (*7*).

Changes in the sensitivity of photoreceptors and the position of photoreceptor cell pigment granules during dark-light adaptation in arthropods are local effects (*22*). These changes appear to be mediated through changes in intracellular Ca^{2+} concentration (*23*). Since PRM assembly and shedding are likewise local events and associated with dark and light adaptation, perhaps they are controlled by the same signal.

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Moreover, the cells of one ommatidium appear to be electrically coupled [P. G. Lillywhite, *J. Comp. Physiol.* **125**, 13 (1978)]. For a description of ommatidial structure, see M. Wilson, P. Garrard, S. McGinness, *Cell Tissue Res.* **195**, 205 (1978).

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15. Lights-on ("dawn") was at 0600, lights-off ("dusk") at 1800. Illuminance from daylight-fluorescent plus incandescent lighting was 1000 lux in the center of each white-sided cage (36 by 36 by 46 cm). Temperature outside the cage was 20° to 35°C. Animals were fed bran and wheat. At least 24 hours before fixation (including the period when eyes were masked), animals were kept at 25° ± 2°C and 48 ± 2 percent humidity, under the same cyclic lighting as that used during rearing.
16. Before primary fixation, dissection was limited to one or two slices through the surface of the eye around the central region, where the inter-ommatidial angle is constant. Whole heads were then quickly immersed in primary fixative: 2.5 percent glutaraldehyde plus 2 percent paraformaldehyde buffered in 0.08*M* NaH_2PO_4-NaOH plus 0.06*M* D-glucose. After 3 to 12 hours, the eyes were trimmed, fixed in OsO_4, dehydrated, and embedded in Araldite. Sections for electron microscopy were stained with uranyl acetate and lead citrate. They were cut so that the ommatidia at the boundary between masked and unmasked areas were in transverse section. Measurements of rhabdom cross-sectional areas were made with an image analyzer (Kontron MOP-AMO3), from electron micrographs magnified 3300 times.
17. Shed membrane is aggregated as vesicles in multivesicular bodies, which degrade through multilamellar bodies to residual bodies (*5*). This pathway is common to most arthropods [E. Eguchi and T. H. Waterman, *Cell Tissue Res.* **169**, 419 (1976); A. D. Blest, L. Kao, K. Powell, *ibid.* **195**, 425 (1978); A. D. Blest, S. Stowe, D. G. Price, *ibid.* **205**, 229 (1980); G. S. Hafner, G. Hamond-Soltis, T. Tokarski, *ibid.* **206**, 319 (1980); (*4*)].
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19. Usually at the boundary between masked and unmasked ommatidia, a row of ommatidia had rhabdoms of an intermediate size. These ommatidia were probably incompletely masked, so that their rhabdoms received an intermediate light intensity. Rhabdom size has been shown to be modulated according to "intermediate" light intensities in a mosquito [R. H. White and E. Lord, *J. Gen. Physiol.* **65**, 583 (1975)]. In the shedding experiment, the intermediate-sized rhabdoms are a result of a smaller amount of shedding, since no rhabdomeral disarray was found to indicate that full shedding followed by some assembly of new PRM had occurred. The intermediate size in the assembly experiment, however, could have resulted from either assembly of less PRM, or full assembly followed by some shedding.
20. Early experiments, in which polarized or monochromatic light was used to selectively stimulate some of the photoreceptors in a crab, *Libinia* [E. Eguchi and T. H. Waterman, *Z. Zellforsch. Mikrosk. Anat.* **84**, 87 (1968)], and in a honey bee [F. G. Gribakin, *Nature (London)* **233**, 639 (1969)], respectively, provided evidence that breakdown of microvillar PRM can be restricted within an ommatidium to the individual photoreceptors. In the bee, the breakdown did not appear to constitute normal shedding, since it was associated with swelling of the microvilli rather than the common form of internalization by pinocytosis [E. Eguchi and T. H. Waterman, *Z. Zellforsch. Mikrosk. Anat.* **79**, 209 (1967); R. H. White, *J. Exp. Zool.* **169**, 261 (1968)]. In *Libinia*, the extent of shedding that did not coincide with the daily rhythm was determined (by relative numbers of secondary lysosomes; no size change was manifest), rather than the initiation of a cell's normal shedding response as in the present report.
21. Efferent neurons project to the lamina in insect and crustacean compound eyes [N. J. Strausfeld and D. R. Nässel, in *Handbook of Sensory Physiology*, H. Autrum, Ed. (Springer-Verlag, New York, 1981), vol. 7/6B, p. 1].
22. Local control has been shown in photoreceptor cell pigment (and other photomechanical) movements in *Limulus* lateral eye [M. E. Behrens, *J. Comp. Physiol.* **89**, 45 (1974)]; pigment in fly [D. G. M. Beersma, thesis, Rijksuniversiteit Groningen (1979)]; and pigment-palisade interplay in locust [this report (Fig. 1)]. Retinomotor movements during dark and light adaptation in fish retinas are also controlled locally [S. S. Easter and A. Macy, *Vision Res.* **18**, 937 (1978)].
23. This was established for photoreceptor sensitivity in the *Limulus* lateral eye [J. E. Lisman and J. E. Brown, *J. Gen. Physiol.* **59**, 701 (1972)]; the honey bee drone [C. R. Bader, F. Bauman, D. Betraud, *J. Gen. Physiol.* **67**, 475 (1976)]; and in the fly [H. Muijser, *J. Comp. Physiol.* **132**, 87 (1979)]; and for photoreceptor cell pigment movements in the fly [K. Kirschfeld and K. Vogt, *Naturwissenschaften* **67**, 515 (1980)].

24. Helpful comments on the manuscript were provided by S. Laughlin, S. Stowe, and D. Blest.

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Role of Serotonergic Input in the Regulation of the β-Adrenergic Receptor–Coupled Adenylate Cyclase System

Abstract. *The action of desipramine on the norepinephrine-sensitive adenylate cyclase system and the density of β-adrenergic receptors in rat cortex was studied after selective lesioning of serotonergic neurons with 5,7-dihydroxytryptamine. In animals with lesions desipramine failed to reduce the density of β-adrenoceptors but decreased the response of adenosine 3′,5′-monophosphate to isoproterenol and norepinephrine to the same degree as in animals without lesions. The results demonstrate a functional linkage between serotonergic and noradrenergic systems in the rat cortex, with β-adrenergic receptors and neurohormonal sensitivity of the adenosine 3′,5′-monophosphate–generating system being under separate regulatory control.*

Various prototypes of clinically effective antidepressant drugs have been shown to down-regulate the noradrenergic adenosine 3′,5′-monophosphate (cyclic AMP)–generating system and its β-adrenergic receptor population in the brain (*1–3*). The ability of antidepressants to reduce the sensitivity of the system and the density of β-adrenergic receptors depends on an intact noradrenergic neuronal input (*4–6*). However, an increased availability of norepinephrine (NE) appears to be only one prerequisite for the regulation of the receptor system. Thus, iprindole, which does not increase the availability of NE, nevertheless reduces both the sensitivity to NE and the density of β-adrenergic receptors (*4, 7, 8*). Conversely, cocaine, which increases the availability of NE, does not change the density of β-adrenergic receptors (*3, 9*). Since the terminal fields of serotonergic projections in the cortex overlap those of noradrenergic projections, we studied the consequences of selective lesioning of serotonergic neurons on the regulation by desipramine of the NE receptor–coupled adenylate cyclase system in the rat cortex. Our results demonstrate that desipramine fails to decrease the density of β-adrenergic receptors in the absence of serotonergic input while still reducing the sensitivity of the cyclic AMP–generating system to NE and the β-adrenergic agonist isoproterenol.

As subjects we used male Sprague-Dawley rats (250 to 300 g) kept under

Table 1. Effect of DHT lesions of the central serotonergic system on the recognition and action functions of the NE receptor–coupled adenylate cyclase system. The lesions were made 10 to 12 days before treatment with desipramine (15 mg/kg, intraperitoneally) daily for 7 days. Twenty-four hours after the last desipramine injection, the animals were decapitated and the cyclic AMP responses to NE and the density of β-adrenergic receptors were determined. Each response equals the stimulated concentration of cyclic AMP minus the basal level. For the determination of specific [^{3}H]DHA binding, no fewer than five different concentrations of ligand (0.3 to 3.5 n*M*) were used. Numbers in parentheses indicate the number of animals, each sample being analyzed in duplicate (cyclic AMP) or in triplicate (DHA binding). Values are means ± standard errors.

Treatment	Cyclic AMP (pmole/mg protein)		[^{3}H]DHA binding	
	Basal concentration	Response to 100 μ*M* NE	Maximum number of sites (fmole/mg protein)	Affinity (n*M*)
No lesion; saline	18.0 ± 2.5 (10)	65.2 ± 6.3 (17)	100 ± 10 (8)	1.31 ± 0.13
No lesion; desipramine	17.4 ± 2.0 (12)	27.0 ± 3.6* (17)	68 ± 4† (8)	1.32 ± 0.07
Lesion; saline	18.1 ± 1.3 (13)	50.7 ± 4.6 (23)	131 ± 4† (7)	1.56 ± 0.08
Lesion; desipramine	17.7 ± 1.7 (13)	23.9 ± 3.1‡ (20)	133 ± 25 (7)	1.71 ± 0.21

*Significantly different from corresponding value for nonlesioned animals given saline ($P < .001$). †$P < .025$. ‡Significantly different from corresponding value for lesioned animals given saline ($P < .001$).

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