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Light Sensitivity of the Rhinophores and Eyes of *Aplysia*

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Summary. The rhinophores (posterior tentacles) of the head of *Aplysia* (Fig. 1) are well known as chemical and tactile sensors. Here, they are shown to be excellent photoreceptors too.

1. Illumination of the rhinophore of an isolated preparation of the rhinophores-cerebral ganglion-eye (Fig. 2) evoked neuronal activity in cerebral neurons and efferent activity in the optic nerve (Fig. 3).

2. Evoked activity in the ipsilateral optic nerve was a phasic burst of unitary spikes at "on". The number of spikes was approximately proportional to the log of intensity (Fig. 4). After the phasic "on" response the normally ongoing efferent activity originating in the cerebral ganglion and recorded in the optic nerve was suppressed briefly but resumed if the light was continued for minutes (Fig. 3).

3. The spectral sensitivity of the rhinophore and eye are similar. Both have a peak near 500 nm and sensitivity is 1 log unit less at 405 nm and 1.7 log units less at 660 nm than at 500 nm (Fig. 6).

4. The greatest photosensitivity of the rhinophore is at the tip in the sensory groove (Fig. 2) directed laterally and upward from the head.

5. The rhinophores may serve as extraocular photoreceptors to the circadian systems or modulators of ocular photosensitivity and they may also function in simple visual discriminations since orienting movements of the rhinophores were evoked by illumination of the head of intact *Aplysia*.

Introduction

The light sensitivity of *Aplysia* is known to be mediated by the eyes (Waser, 1968; Jacklet, 1969a) and many relatively unspecialized structures such as the dermis (Dijkgraaf, 1935); the siphon (Lukowiak and Jacklet, 1975) and central neurons (Arvanitaki and Chalazonitis, 1949; Block and Smith, 1973; Brown and Brown, 1973). Now, Chase (1979) has shown that the rhinophores are photoreceptors, which cause phasic neuronal activity in the rhinophore nerve in response to light, although they were better known as chemical and tactile receptors (Jahan-Parwar, 1972; Audesirk, 1975).

Knowledge of the organization of photoreceptors of *Aplysia* is important for understanding the mechanisms of behavior and specifically with regard to photic entrainment of known circadian rhythms. The circadian rhythm of neuronal activity from the eye (Jacklet, 1969b) can be entrained to red light cycles in whole animals if the optic nerve is intact to the cerebral ganglion but not if the nerve is cut (Block et al., 1974). Thus, it seems that the eye oscillator is subject to input from red sensitive extraocular photoreceptors whose pathway of influence includes the optic nerve and cerebral ganglion. Also, the circadian rhythm of locomotory activity can be entrained to red or white light cycles in eyeless *Aplysia* (Lickey and Wozniak, 1979; Lickey et al., 1977) supporting the conclusion that extraocular photoreceptors provide input to the circadian locomotor system.

In the present study the rhinophores are shown to be excellent photoreceptors which distribute light information to the cerebral ganglion and the eyes. This apparently redundant arrangement of sending photic information to the eyes may have implications for entrainment of circadian clocks and visual discrimination by the animal.

Methods

Preparations of a rhinophore and eye each attached by their nerves to the cerebral ganglion (Fig. 2) were dissected out and placed

Abbreviations: *CAP*, compound action potential; *ASW*, artifical sea water

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in a recording chamber containing 10 ml of artificial sea water (ASW) at 20–22 °C. Electrical recordings were made from the optic nerve en passant with a tubing electrode (PE 10 tubing), amplified with a Tektronix 122 and displayed on a Tektronix 5113 oscilloscope and a Grass polygraph. The rhinophore or optic nerve could be cut at appropriate times to disconnect the sensory receptors without changing the recording situation. The preparation was housed in a dark box and the stimulating light was led in by optical fibers from a 6 V tungsten source. Interference filters with 10 nm half widths were used to select wavelengths and neutral density filters were used to attenuate the intensity in 0.5 log unit steps. Light was pulsed by an electromechanical shutter. Intensity was measured by placing the sensor of a radiometer/photometer (United Detector Corporation, Model UDT, 40 ×) in the eye position and reading the intensity in $\mu W/cm^2$ and lux. Sensitivity at 405, 420, 459, 500, 553, 606 and 660 nm was determined by noting the intensity needed for a just detectable or criterion response (i.e., 1 CAP within 5 s). The logarithm of the reciprocal of the intensity at each wavelength was compared to 500 nm to obtain a relative measure. Chemical synapses in the pathway from rhinophore to optic nerve were tested by exposing preparations to 102 mM Mg–0.1 mM Ca ASW.

Results

The rhinophores are erect paired cephalic appendages, familiar as chemical and tactile receptors, which are responsible for the appearance of *Aplysia* that prompts its common name, sea hare. The eyes are just anterior and lateral to the rhinophores (Fig. 1). The sensory groove at the tip of each rhinophore is normally directed upward and laterally suggesting that it could be useful in detecting directional sensory signals. It was noted in observations of freely moving dark adapted animals that illumination of the head provoked orienting movement of the rhinophores and in some cases startle-like withdrawal of the head.

Branching of the rhinophore nerve near the sensory groove as described by Hanström (1928) was verified by dissection. Histological sections of the nerve at the branch points revealed neurons and a complex of fibers and numerous sense cells in the adjacent sensory groove.

Rhinophore Photosensitivity

Recording en passant from the optic nerve (Fig. 2) revealed 2 kinds of ongoing activity in darkness (Fig. 3); large afferent compound action potentials (CAP) whose source is the eye and smaller unitary efferent spike activity which originates in the cerebral ganglion (Eskin, 1971). The efferent activity occurred in random bursts and modulated the otherwise regular CAP frequency. A 1 s flash of light on the rhinophore evoked a phasic burst of efferent activity (Fig. 3A). A longer pulse of light evoked a similar burst at the onset of illumination (Fig. 3B) but not

Fig. 1. Photograph of the head of *Aplysia* showing an eye (short arrow) at the base of the erect rhinophores (medium arrow). The sensory groove at the tip of each rhinophore is directed upward and laterally. The anterior tentacle (long arrow) also has a similar groove. Rhinophore 2 cm long

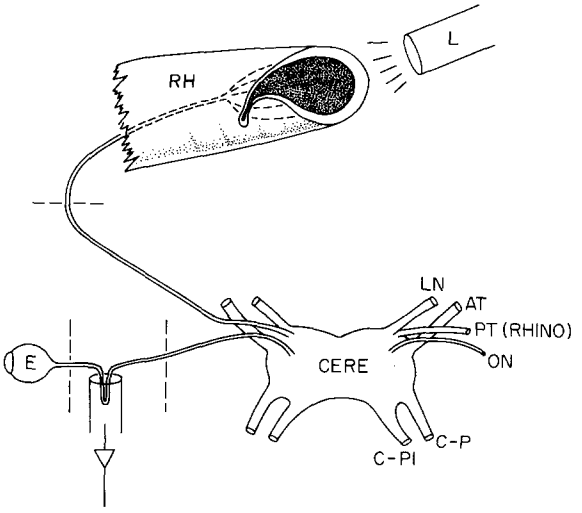

Fig. 2. Diagram of the rhinophore(RH)-eye(E)-cerebral ganglion (*CERE*) preparation showing the *en passant* recording from the optic nerve (*ON*). Selected nerves of the bilaterally symmetrical cerebral ganglion are labelled: *LN*, labial nerve; *AT*, anterior tentacular; *PT*, posterior tentacular (rhinophore); *C-P*, cerebral-pedal; *C-PL*, cerebral-pleural. The rhinophore and optic nerves were cut at the dotted lines to determine the sources of light (*L*) evoked activity

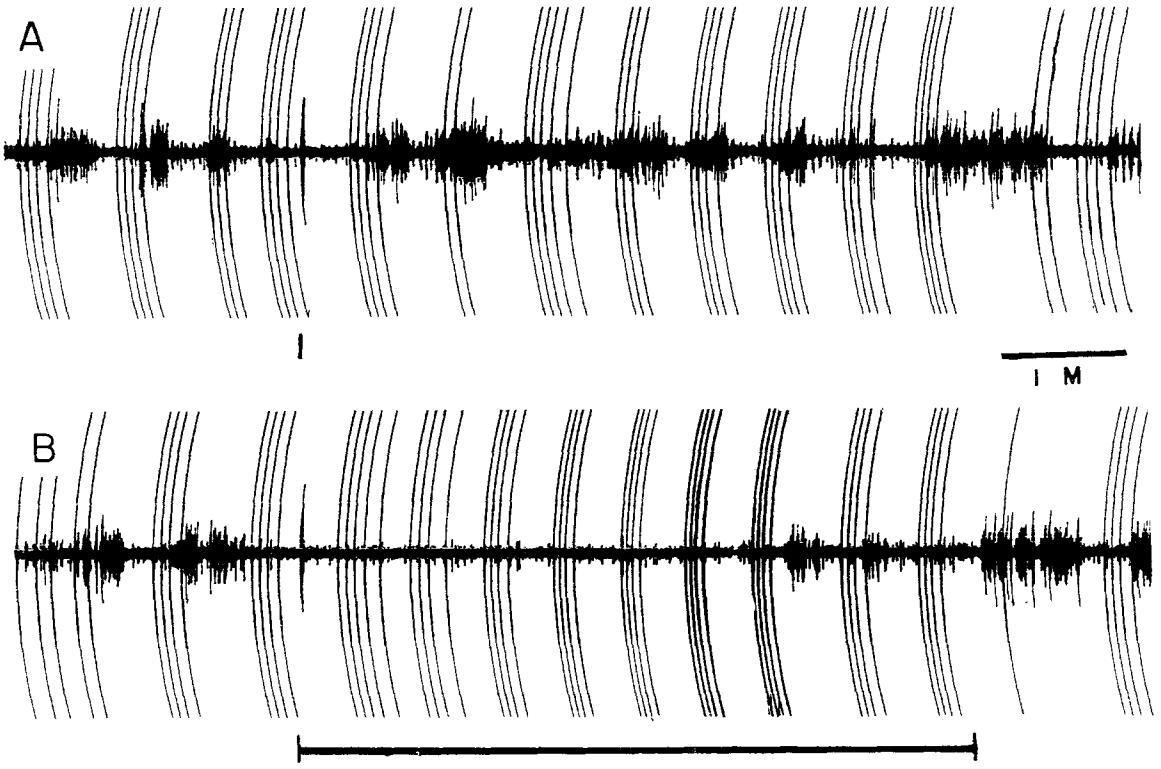

Fig. 3A and B. Ongoing afferent CAP activity and efferent spike activity from the optic nerve of a rhinophore-cerebral-eye preparation. In **A** a 1 s flash (500 nm, 18 μW) evoked a burst of efferent activity. In **B** a 5.5 min flash (500 nm, 18 μW) evoked a burst of efferent activity and later suppressed ongoing activity. Efferent activity about 20 μV, CAP about 150 μV (saturated pen response)

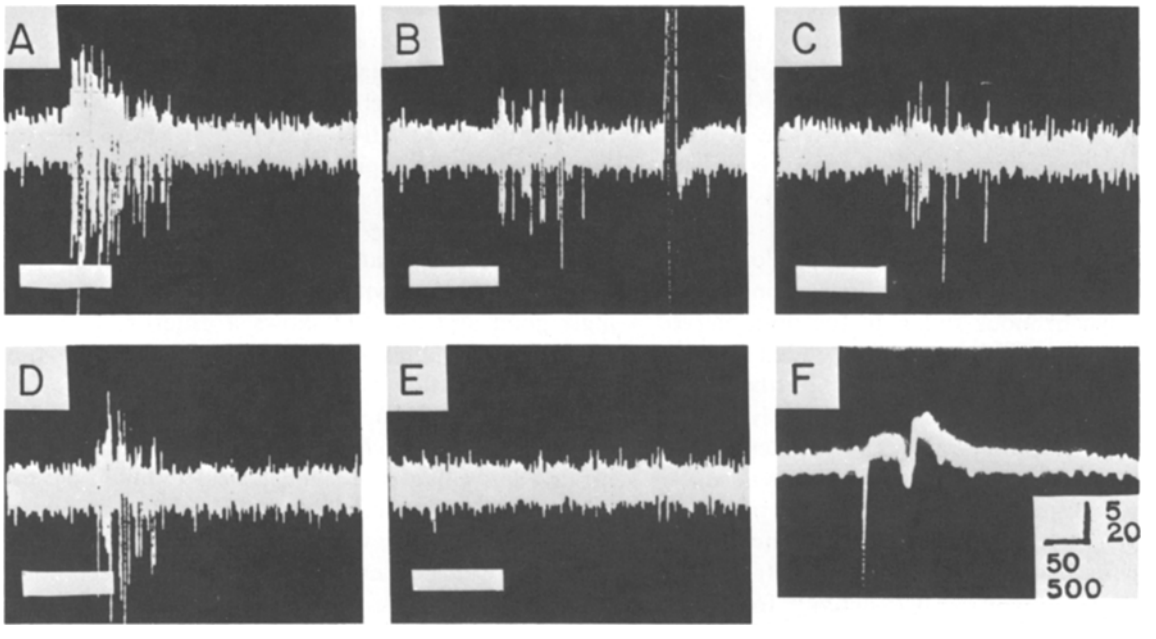

Fig. 4A–F. Evoked efferent activity in the optic nerve. 1 s flashs at 500 nm in **A–E**; at 18 μW in **A**; −2 log in **B**; −2.5 log in **C**; −1 log in **D** and **E**. **E** after rhinophore nerve was cut. Optic nerve responses to electrical stimulation of rhinophore nerve in **F** at 1 ms, 20 V. Scales 5 μV and 500 ms for **A–E**, 20 μV and 50 ms for **F**

tonic activity during the light. The ongoing spontaneous efferent activity was suppressed for a few minutes but recovered to near normal levels before the light was turned off. No "off" activity was noted but the ongoing efferent activity resumed when the light was off. The source of the "on" burst was the cerebral ganglion and not the eye since cutting the optic nerve between the eye and recording site (dotted line, Fig. 2) did not eliminate the unitary activity but it did eliminate the CAP. During the long light pulse in Fig. 3B when the efferent activity was suppressed the CAP activity became more regular and frequent. The same effect was produced by cutting the optic nerve between the recording site and the cerebral ganglion; the CAP became more frequent and regular (Eskin, 1971). These results suggest that the light evoked input from the rhinophore causes an initial burst of activity in cerebral neurons efferent to the eye, and then suppresses their ongoing activity for some time.

Oscilloscope recordings of the response to 1 s pulses of light at 500 nm are shown in Fig. 4. In A the rhinophore received 18 μW of light and responded with a brisk sustained burst after a latency of about 500 ms. Reducing the intensity by 1 log unit with a neutral density filter reduced the quantity of evoked spikes by about 1/2 (Fig. 4D) and attenuation of the intensity by 2 log units reduced the quantity of spikes by about 1/2 again (Fig. 4B). In this preparation, the intensity at 2.5 log units (or about 0.06 μW at 500 nm) was the threshold intensity needed to provoke a reliable response (Fig. 4C). When the rhinophore was illuminated with the same intensity of light (Fig. 4D), but after the rhinophore nerve was cut there was no response in the optic nerve (Fig. 4E) showing the source of the light evoked response was the rhinophore. In one preparation the ASW was changed to $loCa^{++}-hiMg^{++}$ ASW and the evoked photic response was obliterated but recovered when normal ASW bathed the preparation. This shows chemical synapses are in the pathway but it does not identify the site as central or peripheral or both. Electrical stimulation of the proximal rhinophore nerve evoked synchronous spikes in the optic nerve (Fig. 4F) whose amplitude could be graded by the stimulus voltage indicating a population of fibers was provoked into activity. The latency of the response was 50 ms suggesting that most of the latency for the light evoked response was due to photic transduction and not transmission time from the point of recording. Long latencies (400 ms) were noted by Chase (1979) for light responses recorded from the rhinophore nerve. Ongoing CAP activity from the eye could be slightly reduced in frequency by electrical stimulation of the rhinophore nerve at 4–5/s, but this was a weak influence which fatigued rapidly.

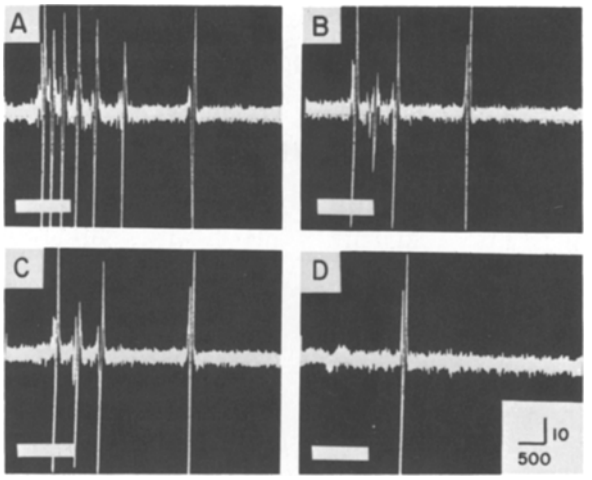

Fig. 5A–D. Evoked CAP in the optic nerve by 1 s flashes in an isolated eye preparation. **A**, 500 nm at 18 μW; **B**, 500 nm at −1 log; **C**, 660 nm at 57 μW; **D**, 660 nm at −1 log

Spectral Sensitivity

An important question raised by inquiry into the function of the rhinophores is how does the spectral sensitivity of the rhinophore compare with the eye. Waser (1968) showed a broad irregular spectral sensitivity for the eye that has recently been challenged by Menzel (1979). To clarify this question the spectral sensitivity of the rhinophore and eye were measured. Rhinophores or eyes were first dark adapted for at least 30 min and then given 1 s flashes every 10 min in a random sequence of wavelengths. A typical threshold response for the rhinophore is shown in Fig. 4C. This measurement was difficult to make in some preparations because of the ongoing efferent activity from the cerebral ganglion. It was also noted that the photic sensitivity of the rhinophore deteriorated much more rapidly than the sensitivity of the eye although they were kept under the same conditions. Figure 5 shows responses of the isolated eye to 1 s pulses of light at 500 nm (5A, B) and at 660 nm (5C, D). The sensitivity at 660 nm (red) was surprisingly good and Fig. 5D shows a criterion response used in determining the spectral sensitivity (in this case, 1 CAP within 5 s of the flash onset).

The results from 5 eyes and 2 rhinophores are shown in Fig. 6. Each point on the top curve is the mean relative log of the reciprocal of intensity for 5 eyes and the vertical bars at 405, 420 and 660 nm mark the standard deviation. Significant variation occurred only at these extreme wavelengths. Each point on the bottom curve is the mean for 2 rhinophores. This curve is plotted 0.5 log units below the eye curve because each of the rhinophores measured was 0.5 log units less sensitive than the eye (included in the upper

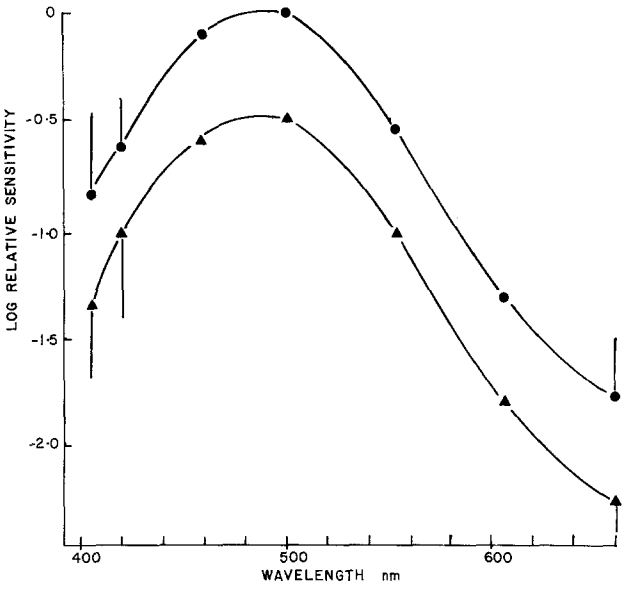

Fig. 6. Spectral sensitivity of the eye (top curve) and the rhinophore (bottom curve). The mean and S.D. are shown for 5 eyes and 2 rhinophores. Rhinophores were compared to eyes from the same animal for relative sensitivity. Sensitivity is the reciprocal of the intensity ($\mu W/cm^2$, 1 s flashes) needed for a criterion response at each wavelength compared to 500 nm

curve) from the same animal. Measurements were made on several other rhinophores but they were judged to be inferior (due to deterioration) to the 2 excellent measurements shown. The 2 curves (eyes and rhinophores) are remarkably similar and perhaps based on a single photopigment although the sensitivity below 400 nm is not known and no partial bleaching experiments were performed.

Discussion

In their description of the sense cells of the rhinophore Emery and Audesirk (1978) identified 2 morphological types, both ciliated, and an intraepithelial cell with numerous long cilia. Presently, it is not known which cells are responsible for transduction of the various chemical, tactile (Jahan-Parwar, 1972) and photic stimuli (Chase, 1979) to which the rhinophores are responsive. Ciliated receptors in the retina of *Aplysia* have been described by Hughes (1970) but most of the photoreceptors are microvillous (Jacklet et al., 1972). Still, this gives only a weak clue to the actual cells involved since photoreceptors can be quite unspecialized. On the other hand the excellent sensitivity of the rhinophore, rivaling the eye, speaks for a specialized cell.

Chase (1979) showed the rhinophore response to light recorded from the rhinophore nerve had a latency of 400 ms and quickly adapted, requiring several minutes for recovery of the full responsiveness. In this study responses recorded from the optic nerve were very similar. The response is phasic with latencies of about 500 ms which leaves 100 ms for transmission time from the rhinophore nerve, through the cerebral ganglion and out the optic nerve. It is likely that chemical synapses are interposed between neurons of the pathway since $loCa^{++}-hiMg^{++}$ ASW blocks the response. Candidates for the cerebral neurons in the light pathway are cells stained by cobalt backfilling of the optic nerve by Luborsky-Moore and Jacklet (1976). These cell bodies are at the base of the rhinophore nerve in the cerebral ganglion.

The spectral sensitivity of the eye of *Aplysia* determined in this study is virtually identical to the sensitivity of the eye of *Strombus* (Gillary, 1974) which has a structure similar to the *Aplysia* eye. The eye of *Strombus* has a peak sensitivity at 485 nm and sensitivity 1 log unit less at 400 nm and 1.5 log units less at 600 nm. That is very similar to Fig. 6 and suggests that both eyes are based on the same single photopigment. The spectrum in Fig. 6 is different from the broad flat sensitivity measured by Waser (1968) for the *Aplysia* eye. Menzel (1979) has contended that Waser's curves are based on false determinations of the sensitivity. This seems to be the case, since a curve constructed from data in Fig. 2 of Waser's paper shows a typical rhodopsin curve. The *Aplysia* eye sensitivity appears to be based on a single photopigment with a peak near 500 nm in agreement with measurements from many other molluscan eyes (Menzel, 1979). The spectral sensitivity of the rhinophore is similar and its absolute sensitivity is only about 0.5 log units less than that of the eyes.

Waser (1968) found threshold intensities for the eye of about 0.01 μW for 1 min pulses. In this study 1 s pulses at 0.01 μW at 500 nm were sufficient in most preparations to provoke a response. The equivalent illuminance is about 0.01 lux. At 660 nm the threshold was about 1.5 μW which is about 0.5 lux. Thus, the eye is still quite sensitive to red light even though the relative sensitivity is quite low compared to 500 nm. The sensitivity did not change appreciably in measurements on the same eye 12 h later.

The ongoing efferent activity from the cerebral ganglion which occurs in random phasic bursts modulates the afferent CAP activity which originates in the eye and is controlled by the circadian clock (Jacklet, 1969b). Eskin (1971) first noted that the CAP frequency was lower and more irregular under the influence of the efferent cerebral activity. He also observed an inhibition of the efferent activity and

consequently more regular CAP activity if tentacular nerves to the cerebral ganglion were electrically stimulated. Those basic observations were confirmed in this study. Selective illumination of the rhinophore caused a phasic burst of efferent activity at "on" and then suppressed any ongoing efferent activity. Light evoked inputs from the rhinophore, apparently, have a biphasic effect: prompt excitation followed by longer lasting inhibition of cerebral efferents to the eye. These responses are mimicked, in part, by electrical stimulation of the rhinophore nerve. It had previously been noted by Block (1975) that illumination of the anterior tentacles evoked a burst of activity in the ipsilateral optic nerve. It seems likely that the anterior tentacular photic system may be organized along the same plan as the rhinophore system and the animal has at his disposal three fairly specialized photoreceptor systems, the eyes, the rhinophores, and the anterior tentacles.

In intact animals, the circadian oscillator in the eye is entrained by white light, but not by red light unless the optic nerve from the cerebral ganglion is intact (Block et al., 1974). The red light evoked weak responses in the isolated eyes but was insufficient to entrain the circadian rhythm in the intact animal. These observations show that an intact optic nerve is necessary for entrainment to red light and suggest that a modulatory influence from the cerebral ganglion or a signal from an extraocular photoreceptor is necessary for entrainment of the ocular oscillator to red light. The necessary signal may come from the rhinophore or it may modulate the ocular photosensitivity. Illumination of the ocelli of *Limulus* enhances the lateral eye photosensitivity (Barlow et al., 1977).

The locomotor activity rhythm of *Aplysia* is entrained by light-dark cycles (Lickey et al., 1977) or red-dark cycles (Lickey and Wozniak, 1979) even in eyeless animals suggesting that extraocular photoreceptors are coupled to the locomotion control center as well as ocular photoreceptors. The rhinophores may participate in this photic control of locomotion but since it is reported (Lickey et al., 1977, p. 129) that some eyeless animals with the rhinophore nerves cut entrain to light-dark cycles, the rhinophores can not be the only extraocular photoreceptors providing input to the locomotion control center, perhaps the anterior tentacles do.

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