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BEHAVIORAL CORRELATES OF CIRCADIAN RHYTHMS IN THE *LIMULUS* VISUAL SYSTEM

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ABSTRACT

A clock in the brain of *Limulus* generates circadian rhythms in retinal sensitivity. We examined the relation between behavioral responses to light and circadian changes within the visual system. Our first experiment recorded unconditioned movements of the telson (tail) elicited by a constant level of illumination of the lateral eyes at different times of the day while an animal remained in the dark. Under these conditions, tail movements followed the same pattern of response as the electroretinogram (ERG) recorded from the lateral eyes. That is, the probability of tail movement was directly proportional to the amplitude of the ERG and both exhibited a circadian rhythm. In the second experiment we conditioned the reflexive movements of the tail and gills by pairing illumination of the lateral eyes with an aversive stimulus, and then measured the level of illumination necessary to elicit responses at different times of the day. Results show that animals maintained in darkness are about 10 times more sensitive at night than during the day. The day-night change in visual sensitivity measured behaviorally is consistent with that measured physiologically (Barlow *et al.*, 1980). The daily rhythm of visual performance could thus be attributed to the known rhythm in retinal sensitivity generated by a circadian clock located in the brain.

INTRODUCTION

The visual system of the horseshoe crab, *Limulus polyphemus,* exhibits circadian rhythms in sensitivity. Illumination of the lateral eyes elicits larger receptor potentials, larger ERGs, and higher optic nerve discharges at night than during the day (Barlow *et al.*, 1977; Kaplan and Barlow, 1980). Such high nighttime responses result from changes in lateral eye structure (Barlow *et al.*, 1980), excitation (Barlow *et al.*, 1977), inhibition (Batra and Barlow, 1982), noise (Kaplan and Barlow, 1980), and metabolism (Chamberlain and Barlow, 1979, 1984) mediated by the efferent neural output of a circadian clock located in the brain (Barlow, 1983).

What behavior is served by such intricate cellular mechanisms for increasing retinal sensitivity at night? *Limulus* mates primarily at night (Cavanaugh, 1975; Howard *et al.*, 1984), and recent field observations indicate that vision plays a role in this behavior (Barlow *et al.*, 1982, 1984). Horseshoe crabs use visual contrast cues during mating: males can discriminate between cement castings of the female carapace and other forms. The degree of discrimination depends on the form and contrast of the castings and the time of day. Even during the new moon under starlight alone, the animals can use vision to detect high-contrast targets. It seems likely that the circadian increase of retinal sensitivity at night underlies this remarkable visual performance.

The objective of this study was to assess the day-night changes in visual performance

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under controlled laboratory conditions. We measured reflexive responses to lateral eye illumination to determine whether the probability of their occurrence follows the known circadian rhythm of retinal sensitivity. In a second experiment, we measured conditioned responses to determine whether the magnitude of the day-night change in visual performance corresponds to that of retinal sensitivity.

EXPERIMENT 1: REFLEXIVE RESPONSE TO LIGHT

Cole (1923) and Northrop and Loeb (1923) reported that *Limulus* reflexively turns toward a source of light. Both studies utilized this reflexive, visually guided behavior to test Loeb's photochemical theory of animal phototropism. Cole reported that when one lateral eye was occluded, *Limulus* constantly moved in the direction of the uncovered eye, forming circles around a light source. According to Loeb's theory, these reflexive "circus movements" represent an automatic orientation for equating the intensity of illumination on both lateral eyes. Northrop and Loeb found that *Limulus,* tethered to a corner of an aquarium by a string tied to its tail, would move toward one glass wall or the other, depending on the relative levels of illumination of light sources placed behind the walls. The animal's "automatic change in direction" was viewed as support for Loeb's phototropic theory. Although these reflexive responses of *Limulus* to light are robust, they are not well suited for our studies because the movements of the animal make it difficult to determine with precision the level of retinal illumination.

Figure 1 shows several types of reflexive light responses that can be recorded from immobilized animals. The recording of heart rate in (a) taken from an earlier study (Barlow and Palfai, 1971) shows that the onset of illumination of the lateral eyes produces a transient increase of the ongoing rate and cessation of illumination causes a transient decrease. The recording in (b) taken from the current study shows that illumination of the lateral eyes can stop gill ventilation and produce a different rate of ventilation after light offset. The electrophysiological record in (c) shows that the offset of illumination of the lateral eyes can elicit an "off" discharge in some single fibers of the abdominal nerve cord (R. Barlow and D. Goodman, unpub. obs.). As indicated in trace (d), illumination of the lateral eyes can elicit reflexive movements of the telson (tail). Tail movements also can be elicited by illumination of the ventral and median eyes (Wasserman, 1973).

Of these four reflexive responses to light, we chose to study tail movements and gill ventilation because they are readily observed, easily recorded, and do not require surgery. Because the animal is immobile, we could also record the electroretinogram (e) simultaneously with tail movement for a convenient measure of retinal sensitivity during the reflexive response to light.

Materials and methods

The experiment was carried out in Syracuse with adult male *Limulus* (20 to 25 cm across the carapace) shipped from the Gulf Specimen Company, Panacea, Florida. The animals were freshly caught in the spring, shipped immediately, and tested shortly after arrival in Syracuse. Animals transported to Syracuse during the summer from the Marine Biological Laboratory, Woods Hole, Massachusetts, and stored until fall or animals shipped to Syracuse during the fall did not respond.

Animals were held firmly on a platform by means of Plexiglas clamps placed around the rim of the carapace. The platform was placed in an aerated seawater aquarium such that the tail was free to move, and a bead thermistor (Model

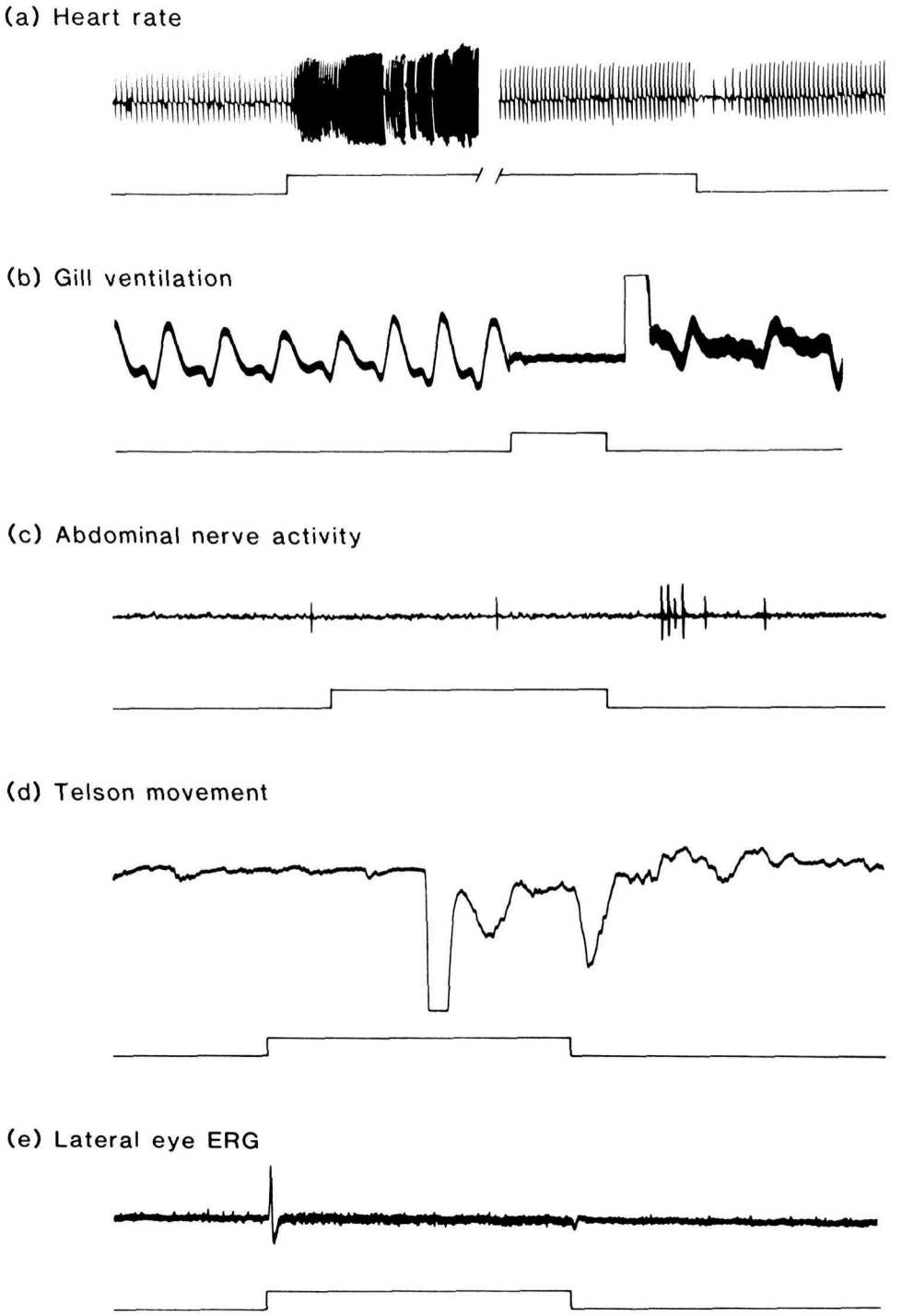

FIGURE 1. Reflexive responses to illumination of the *Limulus* lateral eye. (a) Heart rate, recorded via a pressure transducer implanted beneath the carapace (Barlow and Palfai, 1971), increased from 8 min^{-1} to more than 30 min^{-1} after light onset at 9:40 a.m. Solid line indicates retinal illumination. A transient

P60DA202M, 2K, ±20% at 25°C, Thermometrics, Edison, New York) encased in a waterproof housing (Powers and Easter, 1978) was attached to the tail with elastic bands. A small current was passed across the thermistor and the voltage drop across the device was monitored on a chart recorder. Movements of the tail momentarily cooled the thermistor, producing changes in resistance which were detected as changes in potential on the chart recorder. Complete experiments were carried out with three animals.

One lateral eye of each animal was illuminated with a light pipe (1.2 cm dia) placed 1 cm from the corneal surface parallel to the optic axis of the eye. The level of illumination was adjusted so that detectable tail movements occurred with a probablity of 0.8 for test flashes delivered during the animal's active phase. The light stimulus was a train of five monochromatic flashes (λ = 520 nm) 100 ms in duration at a rate of 5/s. Each flash delivered about 10^7 photons/cm^2 at the surface of the cornea in the experiment reported in Figure 2. The fixed intensity stimulus was presented every 15 min for four days beginning several days after the animal was placed in the dark. A "response" was defined as any detectable deflection in the thermistor record during or immediately following the train of flashes. The percent of trials that produced responses was computed every hour and averaged over the four days.

To measure retinal sensitivity, ERG responses were recorded from the test eye of the animal during the fourth day of the experiment. The technique for recording the ERG was identical to that reported previously (Barlow, 1983) except that the reference electrode was placed on the surface of the carapace near the eye instead of through a hole in the carapace. The placement of electrodes was carried out under infrared illumination. The ERG amplitude was recorded together with the tail response every 15 minutes for the next 24 hours.

Results

Figure 2 shows the amplitude of the ERG and the probability of tail movements for one animal as a function of time of day. The probability of occurrence of detectable tail responses varied from about 0.05 to 0.80 over the 24-h cycle, while the animal remained in total darkness. Tail movements were highly correlated with ERG amplitude ($r = .84$; $P < .001$), and both responses changed systematically with time of day. Other animals yielded similar results.

Note that both rhythms in Figure 2 were shifted in phase about 6 h with respect to the solar day. Previous studies of the circadian rhythm of the ERG show that maximal retinal sensitivity is centered about midnight for animals placed in darkness after exposure to solar lighting (Barlow, 1983). But for the animal in Figure 2, the period of elevated sensitivity was centered about 6 a.m., probably due to the time of day the animal was placed in a darkened container for shipping. It is interesting that both the behavioral and physiological responses exhibited the same phase shift. Apparently the rhythms of both responses are related.

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decrease in rate occurred at the offset of illumination 12 h later at 9:40 p.m. Each segment recorded in darkness is 4 min duration. (b) Gill ventilation, monitored with a thermistor (see text), is inhibited by a 5-s flash of intense light. (c) A discharge of nerve impulses is triggered in an abdominal nerve fiber at the offset of 2-s light exposure of one lateral eye. The other nerve fiber fired before, during, and after the stimulus. The activity of both fibers was recorded with a single suction electrode. (d) Telson (tail) movements, measured with a thermistor, are elicited by a 10-s flash of moderate intensity white light. (e) An ERG (120 mV peak-to-peak) was recorded from the lateral eye with a corneal electrode at the onset of the 10-s flash that elicited the tail movements in (d).

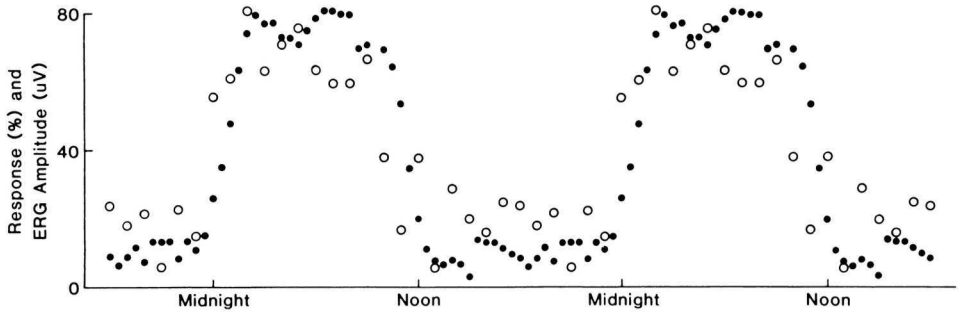

FIGURE 2. Probability of reflexive tail movements correlates with the circadian rhythm in ERG. Tail movements were elicited by constant intensity light pulses (10^7 photons/cm^2/flash) delivered every 15 min to one lateral eye while the animal remained in darkness for 4 days. Open circles give the percent of trials per hour to which the animal responded over the entire 4-day period (16 trials per point). Filled circles give the ERG amplitude recorded from the lateral eye every 15 min during the final day of the experiment. Data are double plotted.

EXPERIMENT 2: CONDITIONED RESPONSES TO LIGHT

Detailed knowledge of the physiology of the *Limulus* lateral eye from the work of Hartline and his colleagues (Hartline, 1972; Ratliff and Hartline, 1974) inspired several investigators to study behavioral responses elicited by lateral eye illumination (Smith and Baker, 1960; Makous, 1969, 1970; Wasserman and Patton, 1969, 1970; Wasserman, 1970; Barlow and Palfai, 1971). The investigators attempted to train *Limulus* to respond to light by pairing a visual stimulus with a non-visual one that alone elicited a reflexive response. These laboratory studies yielded marginal success. At best, conditioning required a large number of trials, and only a small percentage of animals were ultimately trained (Makous, 1969).

Field studies of *Limulus,* on the other hand, reveal a role for vision in the animal's mating behavior (Barlow *et al.,* 1982, 1984). Also, in Experiment 1 of this paper we found in the laboratory a correlation between the probability of reflexive visual responses and circadian changes in the sensitivity of the lateral eye. We therefore were encouraged to develop a classical conditioning paradigm for determining the magnitude of circadian changes in visual performance. Results are described here in Experiment 2. Preliminary results were presented elsewhere (Powers and Barlow, 1981).

Materials and methods

This experiment was performed during the summer at the Marine Biological Laboratory, Woods Hole, Massachusetts, where freshly collected animals are readily available. As with Experiment 1, only freshly caught animals yielded optimal results. Following the technique in Experiment 1, the animals were held firmly on a platform with a thermistor attached to the tail. Another thermistor was attached to the supporting platform beneath the gill books. A small current was passed across each thermistor, and the resulting potentials were monitored on a chart recorder. Movements of the tail and gills cooled the thermistors, changing the voltage drop across each device.

Electrical shock, the unconditioned stimulus (US), was applied to the muscle between the prosoma and opisthosoma of the carapace. Shocks, 1 ms in duration, were delivered via a Stimulus Isolation Unit driven by a Grass Stimulator (S48) at the rate of 10 pulses per s for 1 s. The voltage required to produce reliable changes in response (see *Definition of response,* below) varied from animal to animal, and sometimes from

session to session; it was adjusted accordingly, but was generally between 1 V and 10 V. Figure 3A shows examples of unconditioned telson and gill responses to the presentation of shock.

Light, the conditioned stimulus (CS), was transmitted to the lateral eye by a light pipe (1.2 cm dia) placed 1 cm from the cornea. The light stimuli were 10 s in duration and their wavelength and intensity were controlled by interference and neutral density filters. The unattenuated output of the light pipe, as measured with a calibrated photodiode (PIN 10UV, United Detector Technology, Inc., Santa Monica, California) was 7.5×10^{12} photons/cm^2/s at the surface of the cornea for experiments in which an interference filter was used ($\lambda = 520$ nm), and 1.3×10^{15} photons/cm^2/s at the cornea from 400 to 650 nm for experiments using white light.

Definition of response. Following each trial, the experimenter compared the pattern and amplitude of tail and gill movements observed during the stimulus period with movements during a baseline interval prior to stimulus presentation. During habituation, training, testing, and extinction, the baseline interval was 20 s pre-CS. For trials with shock only, the baseline interval was 10 s preceding shock and the interval which was examined for response was 5 s, beginning with US presentation. We defined any observable change in either gill ventilation or tail movement or both as a response.

In an attempt to reduce the probability of being deceived by using a subjective criterion for response, each author independently scored the data from the original chart records. One of us scored responses while the experiment was in progress, and the other after its completion. We alternated in the two roles, and the person who scored the data while the experiment was in progress later coded the chart records so that the other person could rescore them without knowledge of the stage of the experiment (light intensity, training *vs.* testing, etc.).

Training and testing. Animals were positioned in the apparatus, placed in a lightproof cage, and left undisturbed in darkness for 12 to 24 hours. At the end of this period, shock amplitude was adjusted to produce reliable responses in both tail and gill movements, and training was begun. During training, the CS remained unchanged in intensity for a given animal, with a CS-US interval of 10 s and an intertrial interval of at least 15 min. For the experiments reported here, training intensities were log I $= -4.9$ for L9, log I $= -3.7$ for L10, and log I $= -5.9$ for L16. Training continued until 60% or greater response occurred in two successive 20-trial blocks. Training sessions consisted of 2–6 blocks, and were timed to coincide with the animal's estimated subjective night. The animal remained in the apparatus throughout training. Figure 3B shows conditioned responses to light in an animal that had received 39 previous pairings.

Once an animal was trained, its visual performance was tested in the same apparatus without removing or otherwise disturbing the animal. The test stimuli had the same chromatic composition as the training stimulus; animals trained with white light were tested with white light, and those trained with 520 nm light were tested with 520 nm light. The parameters of the US were kept constant during tests. All tests began with the intensity at which the animal had been trained and proceeded in a descending staircase as follows: if a response was detected by the experimenter, the stimulus intensity was decreased by 0.5 log unit; if there was no respone by either gills or tail, stimulus intensity was increased by 0.5 log unit. This procedure resulted in a final oscillation between stimulus values to which the animal responded regularly and those to which it did not respond. It also produced a concentration of trials at the midpoint of the psychometric function relating stimulus intensity and probability of response. Tests consisted of 25-trial blocks, with one to five additional blank trials randomly inserted during each test block. On blank trials the light source was turned off, but all

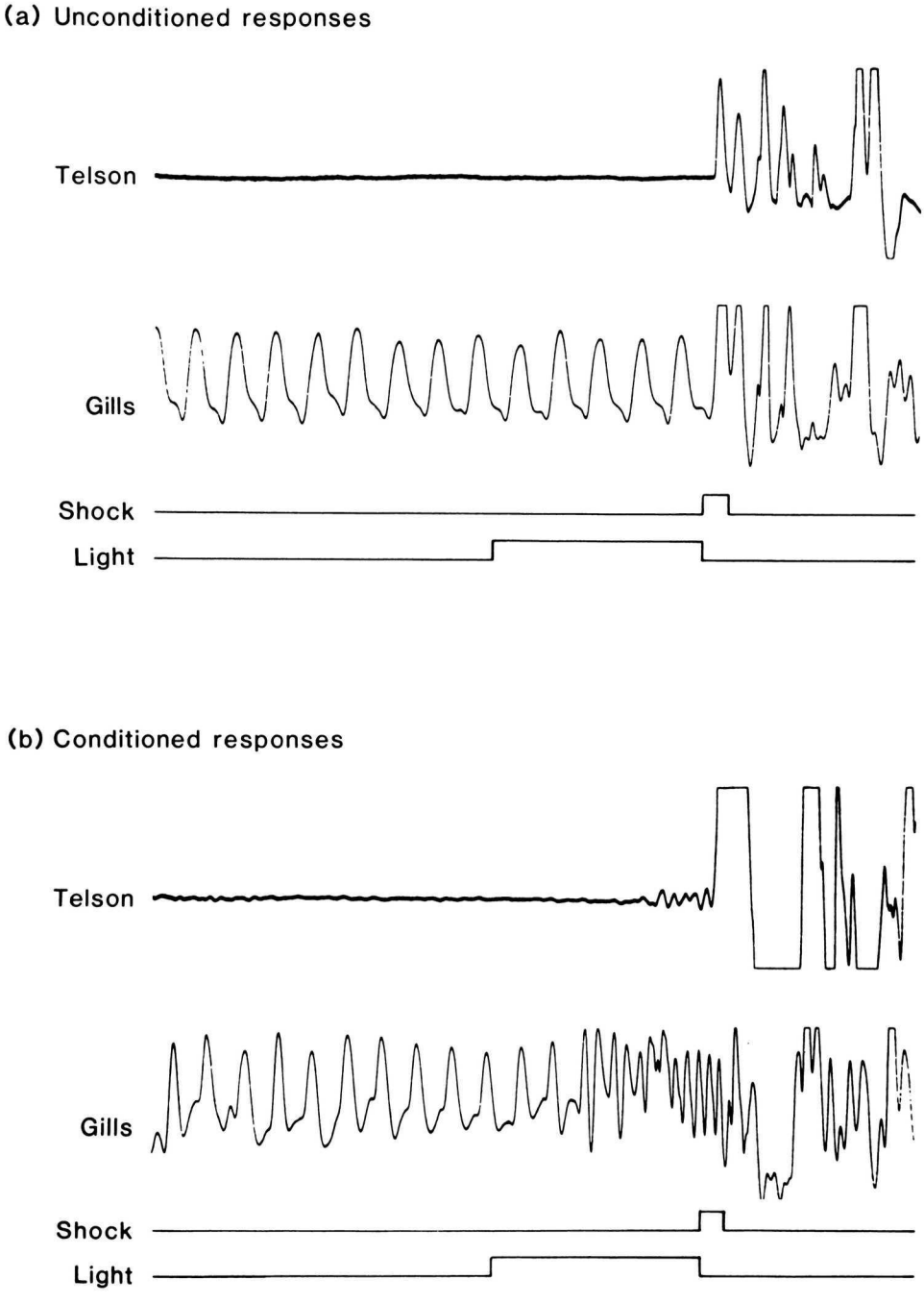

FIGURE 3. (a) Unconditioned responses of tail and gill to a 1-s series of current pulses applied near the muscle between the prosoma and opisthosoma. Neither the tail nor the gill responded to the 10-s flash of dim light (10^6 photons/cm^2/s) delivered to the lateral eye. (b) Conditioned responses of tail and gill to illumination of the lateral eye. Note responses occurred after light onset but before shock onset. This was the fourth trial of the third training session. The first session (1200–1340 h) contained 21 trials, the second (1900–2145 h) 15 trials, and the third (2345–0130 h) 14 trials.

other parameters (shutter, shock, etc.) remained the same as for a regular trial. Intertrial intervals were at least 5 min in order to maintain the lateral eye in a dark-adapted state. Animals were tested during times when retinal sensitivity is known to be maximal (subjective night) and minimal (subjective day). Most animals were tested twice during the day and twice at night, on two sequential days; some had only one test. The values reported here are the total percent response over both tests. Data from animals with >20% response to blanks were discarded.

Habituation. Most animals showed an unconditioned response to bright light (Fig. 1). To show that this response could be eliminated, several animals were exposed to the CS alone and were not exposed to the US. The CS was presented every 15 min, and responses were recorded as with conditioned animals. We measured the number of trials required for cessation of response.

Extinction. Following conditioning some animals were run in extinction sessions in which the CS alone was presented. Responses were recorded for each trial, and we counted the number of trials required for extinction.

Results

Of the 14 animals that underwent training, 9 eventually demonstrated conditioned behavior. The range of trials required to condition the nine animals was 20 to 160. The five that did not reach our criterion for training had all been collected more than seven days before training began. We attempted to measure behavioral thresholds during the day and at night for five of the conditioned animals. Below we report the threshold data for the three best cases. Data from the other two animals were similar.

Figure 4 shows representative acquisition and extinction data from one of the three animals reported below. A total of 80 CS-US paired trials were presented in four blocks of 20 trials each over a 24-h period. This animal (L16) responded on 50 to 70% of the first 40 trials, and on 80 to 90% of the last 40 trials. Testing was then carried out using the staircase method described above (see Fig. 7). Testing was followed by 40

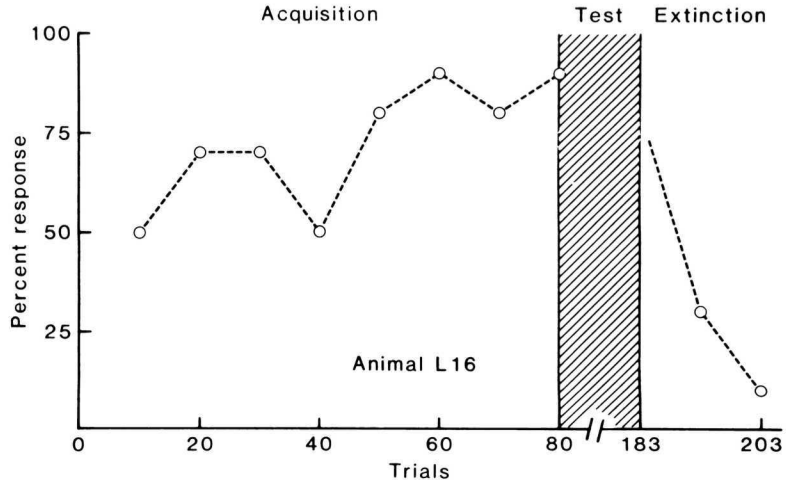

FIGURE 4. Acquisition and extinction data for animal L16. The ordinate is the percentage of trials during which a response was detected in either tail movement or gill ventilation in successive 10-trial blocks. The abscissa gives the cumulative number of trials. The conditioned stimulus delivered 7.5×10^5 photons/cm^2/s at the surface of the cornea (λ = 520 nm).

extinction trials. As Figure 4 shows, extinction of the conditioned response to light was rapid and complete after the shock was turned off.

Figure 5 shows psychometric functions for animal L9 tested with white light during the day (open circles) and at night (filled circles). It illustrates three characteristic features of *Limulus* performance during testing in the conditioning paradigm. First, the animals did not always respond to lights that were clearly above threshold (*e.g.*, from log I = −10.2 to −9.0 in Fig. 5); most conditioned animals responded to an average of only 60 to 70% of such stimuli during testing. This property did not differ between day and night conditions. Second, an intensity was nearly always found to which the animals would not respond (log I = −11.9 at night and log I = −11.5 during the day in Fig. 5). And third, intensities could be found that elicited a reliable response during the night but not during the day (*e.g.*, log I = −11.5 in Fig. 5).

The threshold of animal L9 was lower at night than during the day. We estimated the difference in sensitivity between day and night to be 0.7 log units by shifting the illustrated curves along the abscissa until the best fit was achieved by eye.

Figure 6 illustrates the inter-observer reliability of our scoring technique. The chart records for the data in Figure 5 were separately scored during the experiment by MP who knew the intensity of the stimulus and after the experiment by RB who did not. The two sets of scorings were similar with a high statistical correlation ($r = .79$, $P < .0001$ for daytime data and $r = .64$, $P < .005$ for nighttime data). The average of our two scorings was used to construct Figure 5. All other figures were constructed from scorings of one observer.

Figure 7 shows a decrease in threshold at night when conditioned animals were tested with monochromatic light ($\lambda = 520$ nm). This figure combines results from two animals. Note that the data from one animal resemble those from the other on an

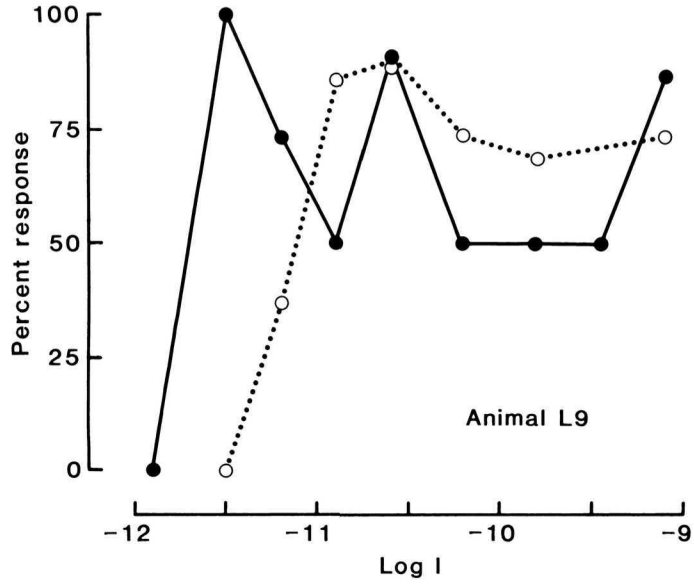

FIGURE 5. Psychometric functions for animal L9 conditioned to respond to flashes of white light. Percentage of trials containing detectable responses of either the tail or gill during the day (open circles) and night (filled circles) is plotted on the ordinate as a function of the relative intensity of the conditioned stimulus plotted on the abscissa. Each point is the average of the two values plotted in Figure 6.

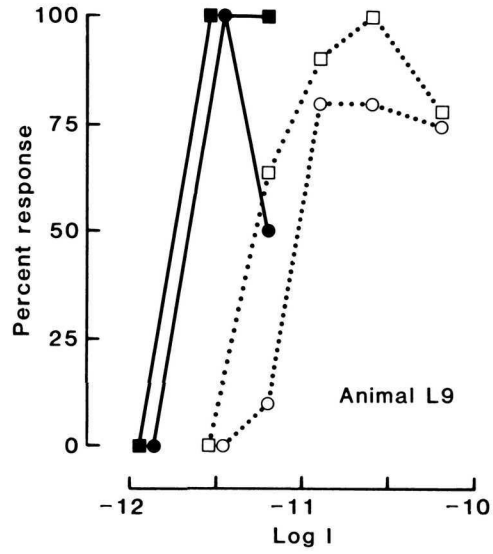

FIGURE 6. Individual scoring by the two authors of part of the data in Figure 5. MP (squares) judged whether responses had occurred during each trial while the experiment was in progress. RB (circles) scored the same data after completion of the experiment with no information about stimulus intensity. Open symbols are daytime data and filled symbols are nighttime data. Axes are the same as in Figure 5.

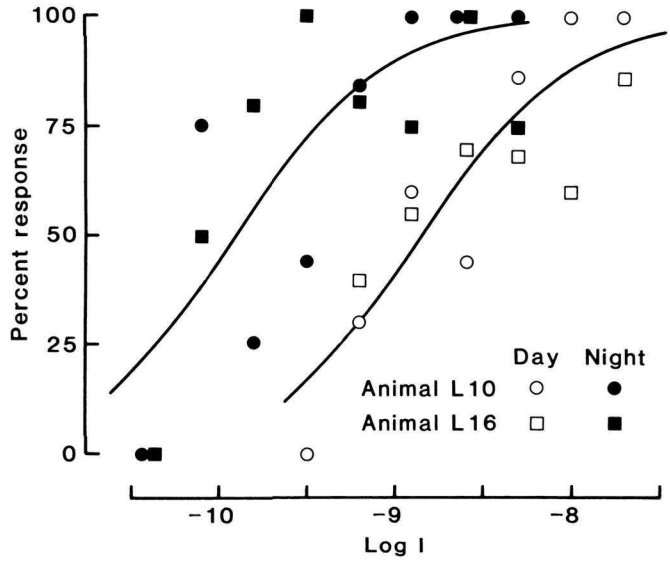

FIGURE 7. Percent response as a function of light intensity for two animals conditioned to respond to flashes of monochromatic light (λ = 520 nm). Daytime (open symbols) and nighttime (filled symbols) results are given for male animals L10 (circles) and L16 (squares). Axes are the same as in Figure 5. The intensity at the surface of the cornea was 750 photons/cm^2/s at log I = −10. Curves are hyperbolic tangent functions fitted to the average data for both animals by least-squares regression analysis. The difference between the curves at 60% response is 1.1 log units, showing that on average animals were 13 times more sensitive at night than during the day.

absolute scale, and that the features present in Figure 5 are also present here: some intensities elicited no responses and some that elicited responses at night elicited no response during the day.

The average decrease in threshold at night was determined by fitting hyperbolic tangent functions (solid lines) to the combined sets of data. The fitted curves are separated by 1.1 log units, indicating that on the average the animals were about 13 times more sensitive at night than during the day. Fitting hyperbolic tangent functions to the individual data (curves not shown) yielded nighttime increases in visual sensitivity of 0.92 log units for animal L10 and 1.27 log units for animal L16. The mean day-night difference in sensitivity for all three animals (L9, L10, and L16) was 1.0 log unit.

Discussion

The visual sensitivity of *Limulus* measured behaviorally changes with time of day. Animals kept in constant darkness exhibited higher sensitivity at night than during the day. The changes in sensitivity are similar in time course (Experiment 1) and in magnitude (Experiment 2) to the circadian rhythms in retinal sensitivity recorded physiologically (Barlow, 1983, see below). Thus it seems reasonable to conclude that the changes in visual sensitivity measured behaviorally result from rhythmic changes in retinal sensitivity generated by a circadian clock. Although the clock is located in the protocerebrum of the central nervous system (Eisele *et al.*, 1982), its known influence on the lateral eye is sufficient to account for the changes in behavior reported here.

Endogenous changes in visual sensitivity were detected for both conditioned and unconditioned responses; however, the latter required substantially higher levels of retinal illumination. For example, to elicit unconditioned tail movements with high probability (>.60) at night required light flashes delivering 10^8 photons/cm^2/s at the surface of the cornea (Fig. 2). On the other hand, conditioned tail responses were evoked at the same rate at night by light flashes containing 2.5×10^3 photons/cm^2/s (Fig. 5). The difference probably reflects the increased arousal level of the animal in the conditioning experiment due to the association of shock with the visual stimulus.

Comparison of behavioral and physiological results

Physiological recordings indicate that less than 10 photons/s incident on a single ommatidium are sufficient to elicit an optic nerve discharge at night (Barlow *et al.*, 1977). As mentioned above, flashes containing 2.5×10^3 photons/cm^2/s ($\log I = -11.7$ in Fig. 5) were required at night to elicit reliable conditioned tail or gill movements. The adult retina is about 0.8 cm^2 in area and contains about 900 ommatidia, giving a receptor density of approximately 1100 ommatidia/cm^2. Thus the nighttime threshold in Figure 5 is equivalent to about 2.3 photons/s incident on a single ommatidium. This threshold flux corresponds well to that measured physiologically at night.

The daytime threshold in Figure 5 is 1.3×10^4 photons/cm^2/s ($\log I = -11.0$ at 60% response) which is equivalent to 12 photons/s incident in a single ommatidium. This threshold flux is much lower than the physiologically measured value of 1000 photons/s/ommatidium (Barlow *et al.*, 1977). One possible explanation for the discrepancy is that the physiological threshold is based on the response of a single optic nerve fiber, whereas the behavioral threshold results from the combined activities of all optic nerve fibers. During the day the high level of spontaneous activity of a single optic nerve fiber in the dark (1–5 impulses/s) can mask responses of a single receptor

to low levels of illumination (Barlow *et al.*, 1977). If the spontaneous activity of each optic nerve fiber is independent of that of the others, then summing across an ensemble of n nerve fibers can reduce threshold by a factor of $n^{-1/2}$ for large field stimuli. In the behavioral experiments, light stimuli illuminated the entire array of approximately 900 ommatidia in the adult eye. Since each ommatidium transmits nerve impulses to the brain over a single nerve fiber, the intensities required to elicit detectable responses in the behavioral experiments may be about 30 times lower than those required for single fiber responses. Thus the physiological threshold of 1000 photons/s/ommatidium for single receptor illumination would be reduced to about 30 photons/s/ommatidium for whole eye illumination. This lower value is within range of the behavioral threshold of 12 photons/s/ommatidium in Figure 5.

Visual sensitivity measured behaviorally during the day therefore agrees reasonably well with that measured physiologically if the intrinsic noise of the retina is considered. At night behavioral and physiological measures of sensitivity agree well without consideration of optic nerve noise, possibly because such noise is suppressed by a circadian clock located in the brain of *Limulus*. We measured the nighttime behavioral thresholds during the period (9 p.m. to midnight) when efferent optic nerve activity generated by the clock is known to nearly abolish the spontaneous afferent optic nerve activity generated by the retina (Barlow *et al.*, 1977).

Laboratory vs. *sea conditions*

All of our behavioral measurements were carried out in the laboratory with animals maintained under dark adapted conditions so we could investigate the endogenous changes in visual sensitivity. In the animal's natural habitat, the sea, daily fluctuations in ambient illumination produce additional changes in visual sensitivity. Light adaptation reduces visual sensitivity and abolishes spontaneous activity in optic nerve fibers (Kaplan and Barlow, 1975). As a consequence, the masking effects of retinal noise discussed above need not be considered when the animal is light adapted. It appears likely that under natural conditions the visual performance of the animal is probably not impaired at any time by retinal noise which is reduced by light adaptation during the day and by a clock at night.

Behavioral manifestations of circadian rhythms in retinal sensitivity are rarely measured; instead, physiological studies have been the rule. One reason is the difficulty of reliably eliciting and observing behaviors for the long periods necessary to demonstrate all characteristics of circadian rhythms. Partly because of such limitations, the evidence for circadian changes in sensitivity reported here is indirect. Nonetheless, our findings that (1) the probability of eliciting a particular visually mediated behavior correlates highly with the circadian changes in the ERG, and (2) the changes in absolute sensitivity correspond with those measured physiologically, strongly suggest that these visually evoked behaviors are governed by the same mechanism (clock) that generates the circadian changes in retinal physiology. It is interesting to note that visual sensitivity of humans (Bassi and Powers, submitted) and rats (Rosenswasser *et al.*, 1979) measured behaviorally also appears to vary on a circadian cycle.

True conditioning vs. *pseudoconditioning*

Demonstrating true conditioned responses in *Limulus* has been problematic, and the results reported here are no exception. First we, like Cole (1924), observed that *Limulus* maintained in captivity for more than about a week, do not perform well in behavioral tasks. In our hands, only those animals that had been in the lab for less

than a week at the time the experiment began gave reliable responses. Second, previous experiments on conditioning in *Limulus* have been criticized for not including control procedures for pseudoconditioning (Makous, 1969). We also have not done so in this study, but preliminary experiments using similar procedures showed that pseudoconditioning did not occur (R. Barlow, unpub. data). Whether animals were truly conditioned in the present study seems to us to be of less importance, however, than the demonstration that changes in the relative and absolute visual sensitivity of the animal can be predicted by the endogenous physiological changes of its visual system.

Possible role of circadian changes in visual sensitivity for Limulus *in its natural habitat*

Animals in the population from which our sample was drawn mate during late spring and early summer, predominantly during the high tide at night. The relative phase of the lunar and solar days and the level of ambient illumination appear to play critical roles in the animal's mating behavior (Howard *et al.*, 1984). However, males in search of mates can visually detect a female-like object under water regardless of the time of day (Barlow *et al.*, 1984). Such excellent visual performance in the presence of large fluctuations in ambient illumination is remarkable in a species that possesses only one receptor type. We speculate that the circadian changes in retinal sensitivity help both male and female *Limulus* determine whether the environmental conditions are appropriate for mating and, if so, help males locate and approach females during day and night.

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