Circadian Rhythms in *Limulus* Photoreceptors

II. Quantum Bumps

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ABSTRACT The light response of the lateral eye of the horseshoe crab, *Limulus polyphemus*, increases at night, while the frequency of spontaneous discrete fluctuations of its photoreceptor membrane potential (quantum bumps) decreases. These changes are controlled by a circadian clock in the brain, which transmits activity to the eye via efferent optic nerve fibers (Barlow, R. B., S. J. Bolanski, and M. L Brachman. 1977. *Science*. 197:86–89). Here we report the results of experiments in which we recorded from single *Limulus* photoeceptors in vivo for several days and studied in detail changes in their physiological and membrane properties. We found that: (a) The shape of (voltage) quantum bumps changes with the time of day. At night, spontaneous bumps and bumps evoked by dim light are prolonged. The return of the membrane potential to its resting level is delayed, but the rise time of the bump is unaffected. On average, the area under a bump is 2.4 times greater at night than during the day. (b) The rate of spontaneous bumps decreases at night by roughly a factor of 3, but their amplitude distribution remains unchanged. (c) The resting potential and resistance of the photoreceptor membrane do not change with the time of day. (d) the relationship between injected current and impulse rate of the second order neuron, the eccentric cell, also remains unchanged with the time of day. Thus the efferent input from the brain to the retina modulates some of the membrane properties of photoreceptor cells. Our findings suggest that the efferent input acts on ionic channels in the membrane to increase the sensitivity of the photoreceptor to light.

INTRODUCTION

The compound lateral eyes of the horseshoe crab, *Limulus polyphemus*, have been studied for decades as a model visual system (Hartline, 1967), and many of the fundamental processes elucidated by this research were later established in the visual systems of higher animals, including man. Each eye undergoes dramatic changes at night: efferent activity transmitted from the animal's brain increases the light

response of the eye by increasing its photon catch and gain (response per absorbed photon), and decreases the noise in the eye by reducing the rate of spontaneous discrete waves of membrane potential, the well-known quantum bumps (Barlow et al., 1977, 1987; Kaplan and Barlow, 1980). The increase in photon catch results from structural changes, which include pigment migration and changes in cell position and shape (Barlow et al., 1980; Chamberlain and Fiacco, 1985). The reduction of the rate of spontaneous quantum bumps by efferent input is remarkable, because the production of quantum bumps by light is not hindered. Although the mechanism of bump suppression is not yet understood, it is clear that the efferent input to the photoreceptor cells acts close to the site of transduction of light into an electrical signal.

In this study we focused our attention on the circadian changes in gain and noise. The increase in gain could, in principle, result from an increase in the input resistance of the photoreceptor membrane, from a change in its resting potential, or from a change in the amplitude or time course of the elementary quantum bumps, which summate to produce the receptor potential (Dodge et al., 1968). The gain increase of an ommatidium, measured as an increased discharge of the eccentric cell fiber (Barlow et al., 1977) could result from a change in the spike initiation mechanism of the eccentric cell. The decrease in noise appears to reflect a decrease in the rate of production of spontaneous quantum bumps. However, it could also result from a reduction in bump amplitude, which could make spontaneous bumps undetectable.

The experiments we describe here address these possibilities, and examine the cellular and biophysical mechanisms that underlie the dramatic circadian changes in the visual performance of this much-studied eye. Our results show a remarkable capacity of the brain to reach out to the most distal part of the visual system, the photoreceptor, and modulate its fundamental properties.

METHODS

Preparation and Recording

The experimental animals were adult male horseshoe crabs collected in the wild by the Marine Resources Department of The Marine Biological Laboratory, Woods Hole, MA. They were kept outdoors in open wooden troughs with running seawater, fed regularly, and exposed to natural day–night light cycles. At Woods Hole in July and August sunrise is at ~5:30 a.m. and sunset at ~7 p.m.

The methods for long-term recording of the membrane potential from *Limulus* photoreceptors in vivo are described elsewhere (Barlow et al., 1987). Briefly, a horseshoe crab was secured to a rigid platform and submerged in circulating seawater in a tank placed in a light-tight box with the room lights off. The room and seawater temperatures were maintained at 20° C throughout the experiment. A small segment of cornea (1 x 2 mm) was removed from the dorsal half of one lateral eye with a thin razor blade. A glass microelectrode (filled with 3 M KCl; resistance: 20–70 MΩ) was lowered into the eye with an Inchworm (Burleigh Instruments Inc., Fishers, NY) until a photoreceptor was impaled. In several experiments light was delivered to a single ommatidium from a tungsten source by a 70-μm light pipe. The intensity incident on an ommatidium at log I = 0 was 10^{12} photons/s (400–700 nm). In some experiments the electroretinogram (ERG) was recorded with a wick electrode resting on the

cornea. The stimulus for the ERG measurements was a 10-ms flash from a green light–emitting diode (LED). Spontaneous bumps were always analyzed from recordings made after the eye had been in complete darkness for at least 1 h. All the bumps analyzed in this paper were recorded from retinular cells, as judged by the large receptor potential and small action potentials in response to light.

Membrane resistance and potential were measured with a differential, negative capacitance bridge amplifier with a band width of 0–10 KHz (The Rockefeller University Electronics Laboratory, New York). The membrane resistance was measured using the bridge circuit of the amplifier. The membrane potential was recorded on a Hewlett-Packard instrumentation FM tape recorder (3964A) for later analysis on an IBM PC/AT computer using the ASYST software package (ASYST Software Technology, Rochester, NY).

Bump Analysis

The data from the tape recorder were digitized at a rate of 1,000 samples/s. Bump parameters (amplitude, area, rise and decay times, and intervals between bumps) were extracted either from the digitized records or from analogue records of a Gould chart recorder (band width: 0–100 Hz).

In the analysis of the digitized records the recorded potential was averaged for 50 ms before the rising phase of each bump, and the average was used as a reference baseline to determine the various parameters of the bumps. The digitized records were displayed on the computer's screen and magnified so that each bump filled the whole screen, and the beginning and end of each bump were determined by eye. The bump was then "excised" electronically for analysis. It was particularly easy to determine the beginning of each bump, since the rise time was rather short. Repeated measurements of the same bump showed that the variability in determining the start and end of a bump did not exceed 5%.

RESULTS

Fig. 1 shows traces of membrane potential recorded in darkness from a single photoreceptor during the day and at night. It illustrates two of the most intriguing effects of the efferent input to the *Limulus* lateral eye: the nighttime suppression of spontaneous quantum bumps, and a change in the shape of the bumps with the time of day. These observations have prompted us to study the effects of the nighttime efferent input on the properties of the quantum bumps, and on the electrical characteristics of the retinal cells. We first describe several features of the bumps: their shape, rate, and amplitude distribution.

Quantum Bumps Decay More Slowly at Night

Fig. 2 compares in detail spontaneous bumps recorded during the day with those recorded at night. Fig. 2 *A* shows 20 bumps recorded during the day, and Fig. 2 *B* shows the same bumps normalized to their peak amplitudes. Fig. 2, *C* and *D* show comparable data for 20 bumps recorded during the night. All bumps in Fig. 2 were recorded from the same dark-adapted photoreceptor cell.

There are two types of quantum bumps in Fig. 2: large and small. Both types occur day and night. The large bumps decay rapidly and have a stereotypical shape reminiscent of action potentials. There are seven in the daytime sample (Fig. 2 *A* and *B*) and three in the nighttime sample (Fig. 2 *C* and *D*). In a previous study we termed these bumps large potential fluctuations (LPFs). Their frequency is usually consider-

ably lower than that of the smaller and slower bumps, which we termed small potential fluctuations (SPFs) (Adolph, 1964; Dowling, 1968; Barlow and Kaplan, 1977). We found that the circadian clock affects the shape of the SPFs without changing the shape of the LPFs. Therefore, we will analyze here only SPFs (spontaneous or light evoked), which we refer to simply as quantum bumps.

Fig. 2 shows that the shape of the quantum bump changes significantly from day to night. The falling phase of the bump is prolonged at night, but its rising phase is not affected. Although most daytime bumps decay faster than most nighttime bumps, there is some overlap between the two populations.

Fig. 3 compares bumps recorded during the day and night from a single photoreceptor. Shown are the average of 20 spontaneous bumps recorded during the first night of an experiment (thick curve), the average of 20 bumps recorded
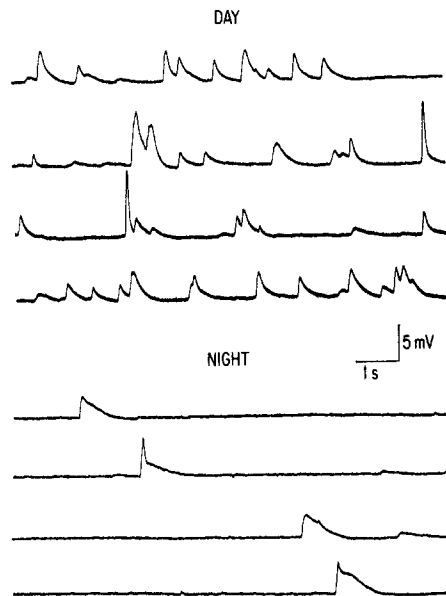

FIGURE 1. Sample recordings during daytime (7:30 a.m.) and nightime (10:15 p.m.) from a single photoreceptor. The membrane potential was 54 mV during the day and 50 mV during the night. The preparation was in complete darkness for at least 1 h before these data were recorded. The bumps shown here are typical of those recorded at other times during the day and at night. Each row represents 10 s.

during the following day (thin line), and the average of 20 bumps recorded during the second night (thick curve). We included every bump in each sequence, except for a few compound waves that were composed of several discrete bumps. The bumps were normalized to their peak amplitude before averaging. The resting potential and resistance were both stable throughout the period during which these bumps were recorded.

The change in the time course of the quantum bumps between the first night and the first day was not due to a deterioration of the recording or the preparation, because on the second night the bump changed back into its characteristic, slower nighttime shape. The daily changes in bump shape are therefore endogenous: they are controlled by the efferent input from a circadian clock located in the brain.

The Properties of Quantum Bumps

Rise and decay times. To study the circadian changes in the bump properties more closely, we extracted four parameters from each bump: its amplitude, the area under the bump, the time from the beginning to the peak of the bump (rise time), and the time from the peak to the end of the bump (decay time). Fig. 4, *A* and *B*, shows the distributions of the rise and decay times, respectively, for 100 daytime (open bars) and nighttime (solid bars) bumps. These data underscore what is seen in the smaller samples shown in Figs. 1 and 2: the rise time of the bumps is unaffected by the efferent input to the eye, but the decay time is prolonged. The average rise time is 80.5 ± 33.5 ms for daytime bumps, and 75.7 ± 34.9 ms for nighttime bumps. The mean decay time is 384 ± 141 ms for daytime bumps and 714.9 ± 266.3 ms for nighttime bumps.

Amplitude. In Fig. 4 *C* we show the distributions of the amplitudes of 100 spontaneous daytime and nighttime bumps. No significant difference can be detected between the two amplitude distributions. The average amplitude in this experiment was 3.6 mV for the daytime bumps (range: 0.76–19.9) and 3.8 mV for the nighttime bumps (range: 0.84–22.01).

In another experiment we compared 300 daytime bumps with 300 nighttime bumps. In this long-term recording, the bump amplitude ranged from 1 to 20 mV during the day (mean: 6.6 mV) and from 1 to 23 mV during the night (mean: 6.7 mV). The daytime and nighttime amplitude distributions were again similar. This similarity confirms the fact that the circadian clock does not change the average bump amplitude or the distribution of bump amplitudes.

Area. In Fig. 4 *D* we plotted the area under a single quantum bump vs. its peak amplitude to determine whether the area under a nighttime bump was larger than that under a daytime bump, regardless of amplitude. We used the area of the bumps as a sensitive measure for the rate of decay of the bump, since the decay cannot be adequately described by a single time constant (see Discussion). For this analysis we used the same bumps that were analyzed for Fig. 4 *C*. To reduce the overlap of the symbols, the results are shown on logarithmic coordinates. Daytime data are shown as open squares and nighttime data as solid circles.

Fig. 4 *D* shows that the area of the average nighttime bump is considerably greater than that of the average daytime bump, regardless of amplitude. The area of the average nighttime bump was 2.4 times as large as that of the average daytime bump after normalization of amplitude, due to the prolonged decay of the bump. If the conductance change during a bump were not affected by the efferent input, on average more than twice as much current would flow across the photoreceptor membrane during each nighttime bump as during a daytime bump.

Spontaneous Bumps Are Less Frequent at Night

As Fig. 1 illustrates, spontaneous bumps occur less frequently at night. Fig. 5 compares the interval distributions of 300 quantum bumps recorded during the night (*top*) and during the day (*bottom*). The average interval between the bumps was 1.8 s during the day and 5.5 s during the night. The figure shows that the interval distribution changed at night, primarily by the addition of long intervals. No
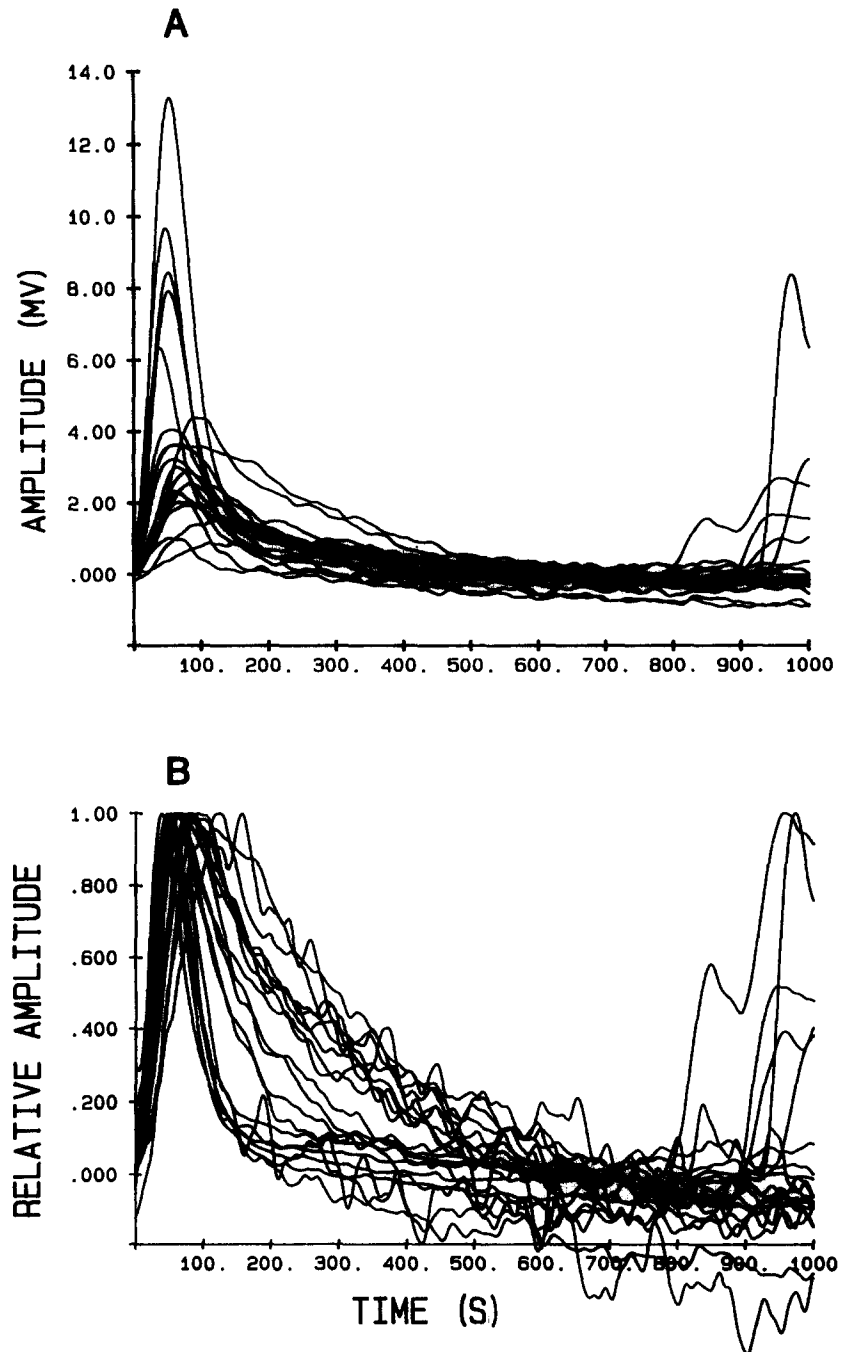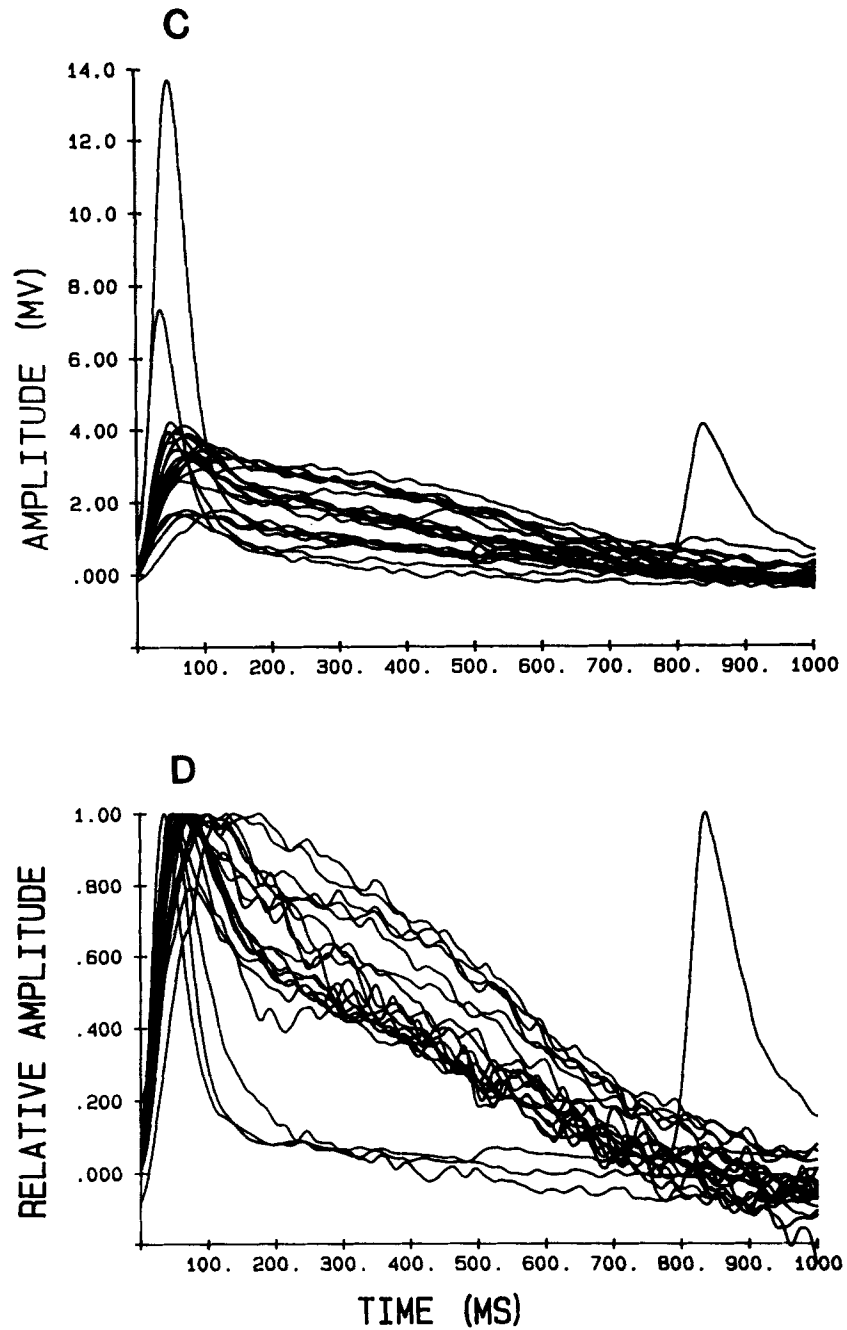
FIGURE 2

FIGURE 2.   Samples of 20 spontaneous bumps recorded during the day (*A* and *B*) and at night (*C* and *D*). The bumps in *B* and *D* were normalized to their peak amplitude to facilitate the comparison of their shape. Note that in *C* and *D* there are some fast nightime quantum bumps. The bumps shown in this figure occurred sequentially, and were not selected in any special way; only (rare) compound bumps were excluded.

regrouping or bursting of quantum bumps was detected. Because of the nighttime decrease in the bump frequency, we had to sample for 9 h (6 p.m. to 3 a.m.) to obtain a sufficient number of bumps for the data in Fig. 5. Note that the bump frequency is not constant during this period, and is minimal around 9–11 PM (Barlow et al., 1987, Fig. 3).

The solid circles in Fig. 5 are the expected interval distributions predicted from Poisson distributions with mean intervals equal to the experimentally measured ones. The interval distributions for the nighttime are not statistically different from the Poisson prediction (chi square = 41.9), but the daytime interval distributions do deviate significantly from the Poisson prediction (chi square = 74.96, $P \leq 0.05$).
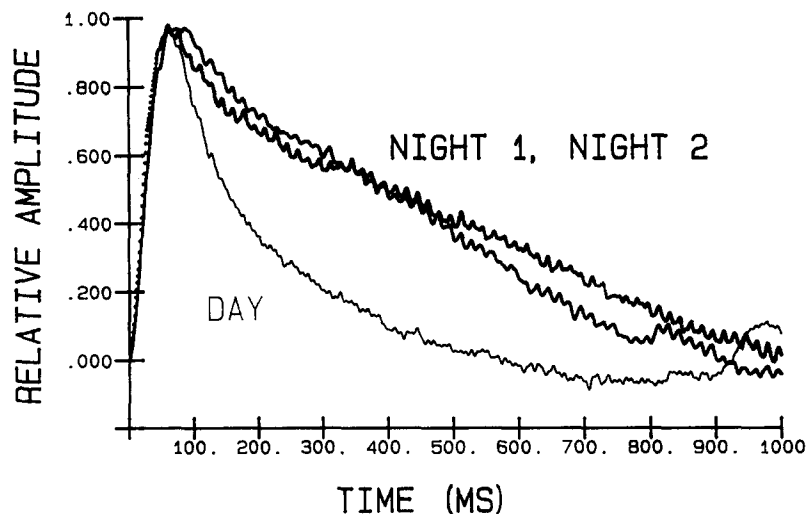

FIGURE 3.   The average daytime and nightime bump. Averages of 20 spontaneous quantum bumps, occurring during the first night, the following day (thin curve), and the second night of a long-term recording. The return of the nightime shape after the day proves that the change in shape was due to the circadian efferent input to the eye, and not to a drift or deterioration of the recording or the preparation.

Light-driven Bumps Are Also Affected by the Efferent Input

The data shown in Figs. 1–5 are from quantum bumps that occurred spontaneously in darkness. However, when we used dim flashes of light (log I = −8 to −9) to elicit single bumps during the day and at night, we observed that at night the decay phase of light-evoked bumps was prolonged as much as it was for spontaneous bumps. The light-evoked bumps appeared to share other features of spontaneous bumps, but we did not analyze their properties to the same extent.

Membrane Properties Do Not Change with Time of Day

A possible mechanism for the nighttime increase in the light response of photoreceptors (Kaplan and Barlow, 1980; Barlow et al., 1987) is an increase in the input

resistance of the photoreceptor membrane. Such an increase would produce a larger voltage response to the current produced by the adsorption of a photon. We tested this notion by measuring the membrane resistance during the day and during the night. During long-term impalements, we found that the microelectrode often clogged, preventing us from measuring accurately the membrane resistance of a given cell as a function of the time of day. We therefore chose instead to measure the membrane potential and resistance of populations of cells during the day and night.

Fig. 6 A shows the distribution of the membrane potentials of 57 cells recorded during the day (open bars) and 28 cells recorded at night (solid bars). The average resting membrane potential was 53.3 ± 8.4 mV during the day and 48.8 ± 9.8 mV during the night. In Fig. 6 B we show the resting potential plotted vs. membrane resistance for 33 cells. Open symbols represent data from the daytime and solid symbols show nighttime data. It is clear from these data that the membrane resistance and membrane potential of *Limulus* photoreceptors do not depend significantly on the time of day. The average membrane resistance of the photoreceptors was 11.3 ± 8.7 MΩ during the day and 11.8 ± 7.5 MΩ at night. There is no statistically significant difference between the daytime and nighttime values for either membrane resistance or membrane potential. Perhaps more compelling is the observation that in our longest and most stable recordings we did not observe circadian fluctuations of membrane potential or membrane resistance. In some of our most stable experiments the membrane potential did not change during the entire record period (>48 h) by more than 1 mV.

Fig. 6 C shows the voltage response to current pulses (≤1 nA) applied to the cell during the day and during the night. The figure demonstrates that the RC time constant of the membrane (which determines the rate of decay of the potential caused by a current pulse) is unchanged between day and night. Comparison of this figure with Fig. 3 also shows that the bump decay phase is considerably slower than the decay of the potential change caused by the depolarizing current steps. The time required to reach $1/e$ of the peak potential was ~520 ms during the night, 200 ms during the day, and only ~7 ms (both night and day) for the current pulse record. The time course of the bump is therefore governed by processes other than the passive electrical properties of the membrane.

No Circadian Changes in the Excitatory Properties of the Second-order Neuron

The eccentric cell, a second-order neuron, converts the graded receptor potential of the retinular cells into nerve impulses which are transmitted to the brain through the optic nerve fibers. Fahrenbach (1971, 1985) observed that efferent fibers terminate on the eccentric cell body and its collaterals in the neural plexus. Barlow et al. (1977) found that the responses of single optic nerve fibers exhibit a circadian rhythm. We therefore asked whether such rhythms reflect, in part, circadian changes in the current–voltage relationship of the eccentric cell and/or changes in the encoding of the receptor potential into impulses by the spike-initiating zone of the eccentric cell, the axon hillock. To address these issues we injected depolarizing current into the eccentric cell body during the day and night and measured the rate of the resulting impulse discharge (Fig. 7). Our data are similar to those reported for the excised lateral eye (Purple, 1964), and show that the current-to-spike encoding properties of
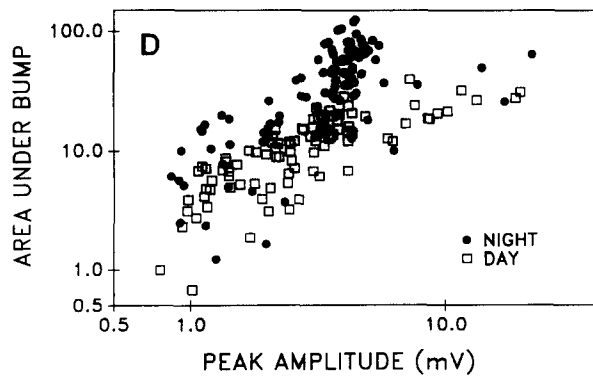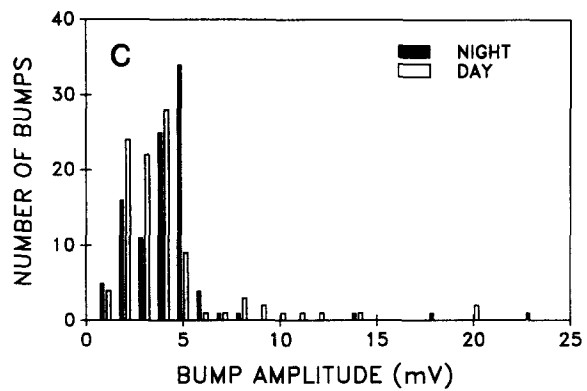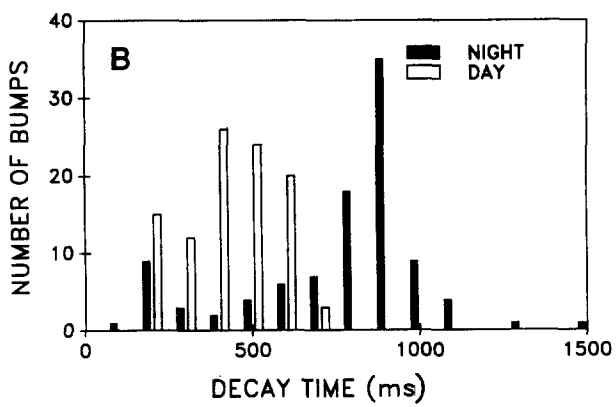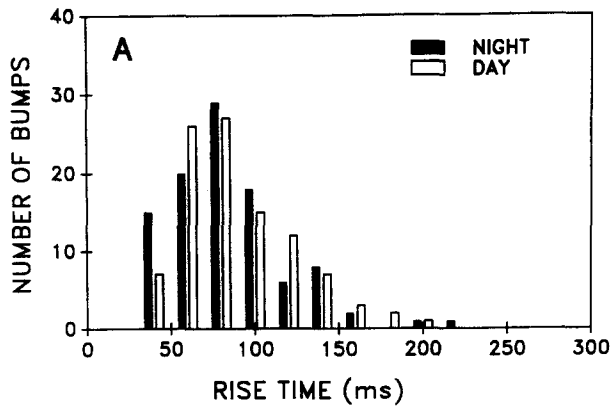
the eccentric cell are not significantly altered by the nighttime efferent input to the eye. We conclude that the changes in the excitatory properties of the *Limulus* lateral eye induced by the efferent input do not involve the eccentric cell. The function of the efferent input to the eccentric cell is not completely understood, but preliminary studies indicate a possible role in the modulation of lateral inhibitory interaction (Batra and Barlow, 1982; Renninger et al., manuscript in preparation).

DISCUSSION

One of the main findings of this study was that the nighttime efferent input to the lateral eyes changes the shape of quantum bumps: the decay of the bump is prolonged. What mechanisms underlie this process, and what is their functional significance to the animal's vision?. We will consider these questions, and explore other aspects of the nighttime increase in the eye's response to light.

Shape of the Quantum Bump

The quantum bump is the building block from which the photoreceptor generates its receptor potential by temporal summation (Dodge et al., 1968). It is, therefore, interesting to study the bump shape in some detail, and investigate the factors that determine this shape during the day and at night. The time course of quantum bumps recorded from photoreceptors in excised *Limulus* eyes has been described by the gamma function, which describes the impulse response of a cascade of several RC stages, all with the same time constant (Fuortes and Hodgkin, 1964; Levinson, 1966; Wong, 1978). Other authors have used different formulations, which included a separate time scale for the rise time and the decay time (Schnakenberg, 1988). Our own data from the *Limulus* eye in vivo cannot be satisfactorily fitted with a function with a single time constant. In the case of daytime quantum bumps a gamma function with parameters that can match the rise time and peak of the average bump fails to match the slower decay time. Nighttime bumps deviate from the gamma function even more, since their rise time is identical to that of the daytime bumps, but their decay is considerably slower (Fig. 3). Matching the shapes of both daytime and nighttime bumps requires more elaborate forms of multi-stage models, such as those discussed by Baylor et al. (1974, Eq. 43).

FIGURE 4. (*opposite*) The distributions of the parameters of the bump shape: distribution of the rise time (time to peak) (*A*) and decay time (time from peak to end of the bump) (*B*) for 100 spontaneous quantum bumps recorded during the day and during the night. The rise time distribution is not affected by the circadian clock. The decay time distribution, on the other hand, is significantly altered. The mean rise time was 80.5 ± 33.5 ms during the day and 75.7 ± 34.9 ms during the night. The mean decay time was 384 ± 141.2 ms during the day and 714.9 ± 266.3 ms during the night. *C*, The distribution of peak amplitude of spontaneous bumps during the day and during the night. The mean amplitude during the day was 3.6 ± 3.2 mV, and during the night, 3.8 ± 2.9 mV. In the recording from which these bumps were sampled there were only a few large, regenerative bumps (LPFs). *D*, The area under each bump plotted vs. its peak height on double logarithmic coordinates. Daytime bumps are shown as open squares, and nightime bumps as solid circles. Note that for any given peak amplitude, the nightime bumps have, on the average, a larger area.

The repolarizing (decay) phase of the average quantum bump recorded during the day appears smooth and nearly exponential. But in many individual quantum bumps one can clearly see two phases, an initial fast decay followed by a slower return to the resting potential. These two components are similar to the ones observed by Baylor et al. (1974) in the hyperpolarizing light responses of turtle cones. Their time course
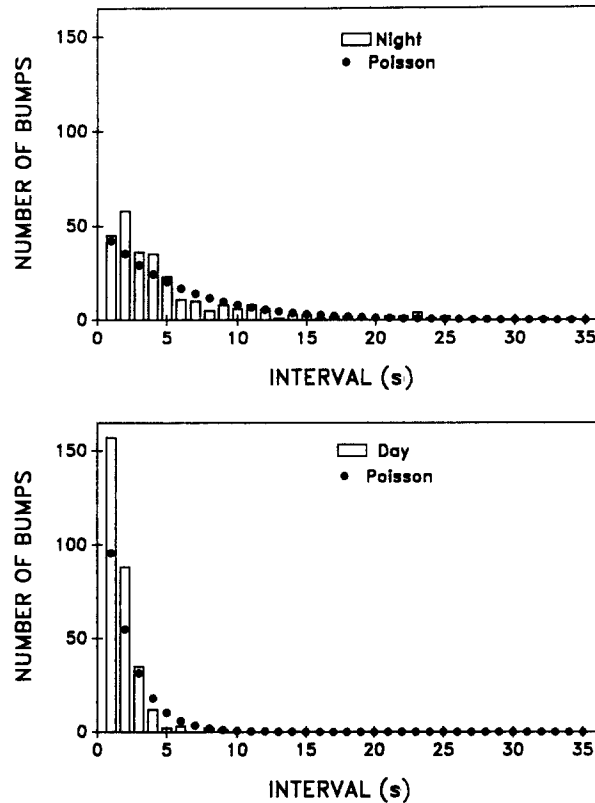

FIGURE 5. The distribution of intervals between 300 spontaneous quantum bumps during the night (*top*) and during the day (*bottom*). The mean interbump interval was 1.8 s during the day and 5.5 s during the night. Note that in order to collect 300 quantum bumps during the night, we had to include bumps that occurred between 6 p.m. and 3 a.m. The frequency of bumps is not constant during this period: it is minimal between 9 and 11 p.m., when the rate approaches 0 bumps/s (Barlow et al., 1987). The solid circles show the expected frequency of intervals if the bumps were produced by a Poisson process with the mean of the experimentally measured distributions.

is similar to that shown by these authors (their Fig. 17), who assumed that the two components of the turtle cone response react differently to increasing light intensity, and reflect a sequence of linked events that determines the shape of the falling phase of the flash response. We could not apply their analysis to our data because the regenerative "spike" (LPF) of *Limulus* photoreceptors precludes the study of the
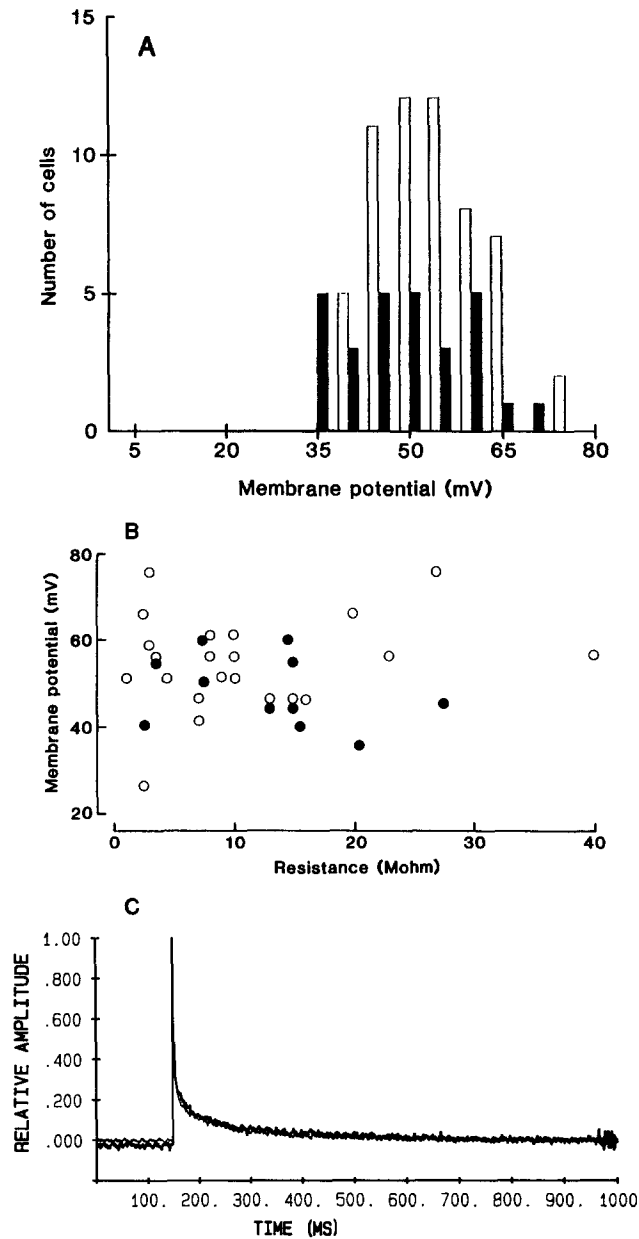
FIGURE 6.   *A*, The distribution of the resting potential of retinular cells during the day (open bars) and during the night (solid bars). No significant difference can be found between the daytime and nighttime data. *B*, The resting potential of retinular cells plotted vs. the membrane resistance for daytime (open symbols) and nighttime (solid symbols). The two parameters depend neither on the time of day nor on each other. *C*, Voltage responses to depolarizing current steps (<1 nA) obtained during the day and during the night. The daytime and nighttime traces are virtually indistinguishable. Note that the voltage responses are considerably shorter than the time course of even the daytime bump.

dependence of the response amplitude and time course on light intensity. Single photons can elicit LPFs (Bayer, 1976; Kaplan and Barlow, 1976). Unfortunately photoreceptors in the lateral eye of *Limulus* in situ cannot be voltage-clamped properly to eliminate regenerative events. The ionic origin of the two components of the decay phase of quantum bumps is not known.

The decay phases of both daytime and nighttime average quantum bumps shown in Fig. 3 can be adequately described by an exponential decay for 500 ms after the peak of the bump. The time constant of the exponential decay is almost three times faster for daytime than for nighttime bumps. After the first 500 ms a single exponential fails to describe the decay of the bump, primarily because of a slight hyperpolarization that follows the bumps. We have observed this hyperpolarization in both daytime and nighttime bumps. Its origin might be an overshoot of the repolarizing outward current that brings the receptor potential back to its resting level. An exponential decay phase was also observed in current bumps recorded from *Limulus* ventral photoreceptors by Wong (1978) and Keiper et al. (1984). Our
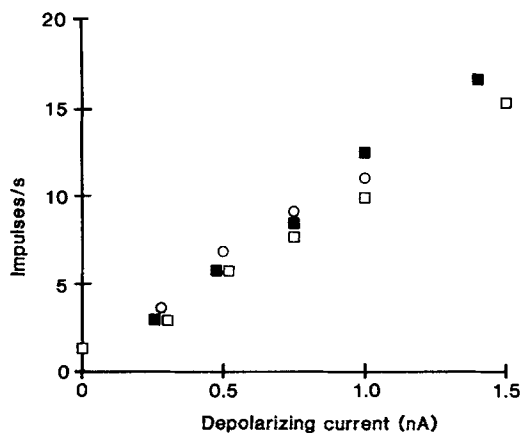
FIGURE 7. The excitatory properties of the eccentric cell are unchanged by the circadian rhythm. The firing rate of an eccentric cell is plotted vs. the amplitude of the injected current. Open symbols are daytime data from two eccentric cells, solid symbols are nightime data from another eccentric cell.

data are not directly comparable since their data were collected using a voltage clamp recording, and we recorded voltage bumps from photoreceptors in the lateral eye.

The Bump Shape Is Not Determined by the Membrane RC or Electrical Coupling between Receptors

At night, the area of the membrane that contains rhodopsin (rhabdom) is enlarged (Chamberlain and Barlow, 1984). One might therefore expect the RC time constant of the membrane to be increased and, as a consequence, the time course of quantum bumps to be prolonged. However, the prolongation of the nighttime quantum bumps cannot be ascribed to a change in the membrane capacitance, since the rise time of the nighttime quantum bumps is identical to that measured during the day. For the same reason, a change in the electrical coupling between the photoreceptors in the ommatidium (Smith et al., 1965) would also fail to account for the change in bump shape. If a coupling change occurred, it would influence the amplitude

distribution of the quantum bumps and the resistance of the cell membrane, but we have not detected any significant circadian change in these parameters (see Figs. 4 and 6 B).

In addition, the voltage responses to current steps applied to the cell produce transients that are much shorter than the bump duration (compare Fig. 6 C with Fig. 3). They are similar to the voltage responses reported by Purple (1964) for the excised *Limulus* lateral eye. The cell's time constant is ~5–10 ms, which is also similar to what Baylor et al. (1974) measured in turtle cones. It seems, therefore, that the change in the time course of quantum bumps is due to a specific effect of the efferent input on ionic channels in the photoreceptor's membrane, and not to a change in the membrane capacitance, the membrane resistance (Fig. 6 B), or the coupling among photoreceptors in an ommatidium.

Does Photoreceptor Gain Increase at All Light Intensities?

The nighttime structural changes in an ommatidium increase its photon catch (Barlow et al., 1987) and thus increase its light response. However, the responses we recorded when we used moderate and bright light intensities increased at night by more than one would have expected from an increase in photon catch alone. This additional increase is consistent with the notion that, above a certain membrane potential, voltage-sensitive K^+ channels are affected by the efferent input. If such channels were totally or partially blocked at night, they would be less effective in opposing the depolarization of the membrane caused by the inward sodium and calcium currents, and the receptor potential would remain high.

In addition to the gain increase revealed by bright light stimulation (see Barlow et al., 1987), the prolongation of the quantum bumps we have described in this paper is an additional form of gain increase, which appears to be independent of light intensity (Barlow et al., manuscript in preparation). We expect that this increase in response per photon is opposed (and eventually canceled) at high light levels by the shortening of quantum responses due to light adaptation (Dodge et al., 1968). The increase in gain at night described in our recent paper (Barlow et al., 1987) must function by other means to increase the photoreceptor response at high light levels.

What Is the Mechanism for the Change in Bump Shape?

The *Limulus* receptor potential is a complicated phenomenon. The membrane is depolarized by two inward currents, carried by Na^+ and Ca^{2+} ions, and then repolarized by several kinds of outward currents: the A current (a fast-inactivating, large K^+ current blocked by 4-amino-pyridine, 4-AP), a delayed-rectifying current (a smaller K^+ current, blocked by tetraethylammonium, TEA) (Lisman et al., 1982), and perhaps a Ca^{2+}-activated K^+ current (Schmidt and Fein, 1979).

Octopamine, which has been shown to be synthesized and transported to the *Limulus* eye by efferent fibers emanating from the brain (Battelle et al., 1982), is known to increase the intracellular levels of cyclic AMP in various systems (for example, Nathanson and Greengard, 1973; Axelrod and Saavedra, 1977; Kaupp et al., 1982). Cyclic AMP has been shown in other systems to block voltage-sensitive K^+ channels (Siegelbaum et al., 1982; Strong and Kaczmarek, 1986). In *Limulus* such a blockage would reduce and slow down the repolarization of the photoreceptor's membrane after the rising phase of a bump, and thus enable more current to flow

across the membrane. This is one of the mechanisms by which the circadian clock in the *Limulus* brain might increase the response to light. A potential difficulty with this explanation is the observation that in some systems the voltage-sensitive K^+ channels have a relatively high threshold (around -40 mV), and thus may not be activated by small (2–4 mV) quantum bumps. However, our recordings were made in the lateral eye of *Limulus*, and it is possible that the smaller bumps we recorded were actually larger bumps conducted electrotonically from other retinular cells (see, for example, Stieve, 1965). Large bumps may trigger voltage-sensitive K^+ channels.

Another possibility is that the photoreceptors in the lateral eye contain Ca^{2+}-sensitive K^+ channels. It has been shown that light raises the free Ca^{2+} inside the ventral photoreceptor of *Limulus* (Levy and Fein, 1985; Payne and Fein, 1986). The rise caused by a single bump is too small to be detected by current experimental techniques, but could be large enough to trigger K^+ channels in the vicinity of the membrane patch that gave rise to the quantum bump. We note, however, that although Ca^{2+}-sensitive K^+ channels have been reported in several other systems, their existence in the lateral eye of *Limulus* has not been demonstrated, and there is some controversy regarding their existence in the ventral photoreceptor of *Limulus* (Schmidt and Fein, 1979; Lisman et al., 1982).

Keiper et al. (1984) have proposed a model of bump initiation in the ventral photoreceptors of *Limulus* in which the absorption of a photon causes the release or activation of an internal transmitter that opens ionic channels in the membrane. They consider the decay phase of bumps to reflect either the inactivation of this internal transmitter or the inactivation of the ionic channels themselves. In their model, the diffusion coefficient of the internal transmitter is assumed to influence the rise time of the bump. Our observation that the rise time of bumps is not affected by the circadian clock indicates that the putative transmitter is probably not affected by circadian changes in the eye, and that the locus of the effect is closer to the ionic channels themselves.

We note that the prolongation of the bumps at night is reminiscent of the increased latency and duration that O'Day and Lisman (1985) reported in the flash response of ventral photoreceptors after octopamine perfusion. Their result is consistent with our observations, and with our interpretation that it is the efferent-induced release of octopamine in the lateral eye that is responsible for the change in the time course of the quantum bumps.

Conductance changes of K^+ channels is not the only process that could account for the prolongation of the quantum bump. For example, the decay phase of the bump might be controlled by the mean open time of the sodium and/or calcium channels that give rise to the depolarization. Our experiments do not allow us to distinguish between these alternatives. However, our results do allow us to rule out another possibility, suggested by Keiper et al. (1984) and Dirnberger et al. (1985). These authors propose that the time course of bumps could be influenced by the rate of production of a transmitter that opens the ionic channels through which the depolarizing current flows. This seems unlikely, since the rise times of bumps during the day and at night are virtually identical.

The proposed action of octopamine on the *Limulus* photoreceptor is similar to that of other neuromodulators which increase the intracellular concentration of the

second messenger cyclic AMP. This messenger often activates protein kinases that phosphorylate proteins of ionic channels in the cell membrane. For example, in the fish retina dopamine has been shown to uncouple the syncytium of horizontal cells, as well as modulate the glutamate-gated conductances of these cells (Knapp and Dowling, 1987; Mangel and Dowling, 1987). Both involve the second messenger cAMP. We note that in the retina of the white perch interplexiform cells, spanning both plexiform layers, receive input from efferent fibers arriving from the brain and innervate horizontal cells (Zucker and Dowling, 1987). It is thus possible that the modulatory processes we propose for *Limulus* appear in other nervous systems.

The Circadian Clock and Bump Generation

The circadian clock in the animal's brain affects the generation of bumps, since the spontaneous bump rate is lowered at night. Lisman (1985) provided evidence for the production of spontaneous bumps from the conversion of metarhodopsin back to activated rhodopsin in UV-sensitive median photoreceptors of *Limulus*. He hypothesized that multiple phosphorylations of metarhodopsin decrease the back conversion and thus lower the rate of spontaneous bumps. Edwards and Battelle (1987) found that octopamine (presumably by activation of a cAMP phosphokinase) phosphorylates a 122-kD protein in the lateral eye of *Limulus*, but found no evidence that smaller proteins, such as rhodopsin, were phosphorylated. In addition, a preliminary test of Lisman's hypothesis by one of us (R. B. Barlow) provided no support for the idea that metarhodopsin plays a significant role in the generation of spontaneous bumps in the lateral eye.

The Distribution of Intervals between Bumps

As Fig. 5 shows, the interval distribution for the daytime bumps deviated significantly from the Poisson prediction. A possible explanation for the daytime deviation from a Poisson-like interval distribution might have been our inability to detect small bumps in recordings obtained during the day. Indeed, the interval distributions we measured are well described by a Poisson process whose rate is substantially higher than what we have measured. However, we feel we can reject this explanation since the amplitude distribution of the nighttime bumps was not significantly different from the distribution for the daytime bumps, and therefore we do not believe that a class of small bumps has disappeared during the day.

The result shown in Fig. 5 is different from what was reported by Yeandle and Spiegler (1973) for light-evoked bumps recorded from the ventral photoreceptor of *Limulus*, where the interval distribution was predicted by a Poisson process. It is not clear yet whether the difference between our results and theirs is due to the fact that we recorded spontaneous bumps from the lateral eye in vivo, whereas Yeandle and Spiegler recorded light-driven bumps from the excised ventral photoreceptor.

The Transient Response to Light Steps

The membrane potential at the peak of the transient response to a bright light flash was always positive at night, whereas during the day it was always <0 mV. This was true for every cell from which we were able to record during the transition period

from day to night. This small nighttime increase in peak amplitude of response is consistent with our hypothesis that the repolarizing outward ionic current is reduced at night. Another possible explanation is that either there are more Na^+ and Ca^{2+} channels available at night than during the day, or that their conductance is increased at night, although such an increase seems unlikely.

Efferent Effects on the Eccentric Cell

The eccentric cell is the site of the inhibitory interactions in the *Limulus* eye. Fahrenbach (1985) reported that efferent fibers make synaptic contacts on axon collaterals of eccentric cells, suggesting that the clock's output can influence the inhibitory network. Indeed, Batra and Barlow (1982) detected a circadian change in the inhibitory properties of the lateral eye, but showed that this change does not involve the encoding properties of the eccentric cell.

Renninger et al. (1988) have shown recently that simulating the efferent activity by electric shocks delivered to the optic nerve produced almost no change in the encoding properties of the eccentric cell. The range of depolarization we used in our experiments was more restricted than the range they used. Within that range our data (Fig. 7) agree well with their results. Therefore, we concluded that the efferent input to the eccentric cell does not influence its excitatory properties.

How Might the Nighttime Change in Bump Shape Affect Limulus Vision?

An obvious consequence of the prolongation of the decay phase of the quantum bumps which we have described in this paper is a significant change in the temporal frequency response of the photoreceptor. At night the temporal integration time of the photoreceptor would increase, and the eye would be unable to respond to temporal modulation at frequencies that are effective during the day. We have not measured the frequency response in our intracellular recordings, nor have we attempted to estimate the bump parameters from the power spectrum of the voltage fluctuations in response to dim light. This is because such calculations assume linear summation of quantum bumps, and the in vivo recordings contain enough large, regenerative quantum bumps (LPFs) to seriously contaminate spectral estimates. However, Batra and Barlow (1990) reported a nighttime change in the temporal frequency response of single optic nerve fibers of the lateral eye. They found a lowering of the high frequency cutoff, which is just what one would predict from our results. This slowing down of the response while increasing its size amounts to trading temporal resolution for maximal sensitivity. It is the temporal analogue of sacrificing spatial resolution for sensitivity, which is the result of the nighttime increase in the acceptance angle of the ommatidium (Barlow et al., 1980).

If the circadian clock modulated voltage-sensitive K^+ channels, the cell would lose some of its ability to adapt to the ambient illumination. Such channels have been implicated in the neural mechanisms of light adaptation (Pepose and Lisman, 1978; Lisman et al., 1982; O'Day et al., 1982). They appear to lower the plateau level of the receptor potential during bright illumination, and thereby allow the receptor to respond to additional increments in light intensity. This sacrifice of adaptation capability is another example of the overriding importance that this visual system seems to place on maximizing its nighttime sensitivity.

We do not yet fully understand why this ancient creature is so adamant about capturing every available photon at night, at the expense of preserving the exact time of arrival or precise location of the photons. Visual performance under such conditions is, after all, only marginal. Recent evidence indicates that the eyes are used in detecting mates at night (Barlow et al., 1982, 1986). Perhaps it is the preservation of the species that dictates the increase of visual sensitivity.

In conclusion, we have described here several significant changes which the circadian clock in the animal's brain affects in the *Limulus* lateral eye at night: the gain (response per photon) increases for dim and bright lights, and in the dim–light range the gain increase is achieved by sacrificing temporal resolution. The picture is still far from complete. How can the clock block spontaneous bumps without blocking light-driven ones? Do structural and physiological changes reflect independent processes or merely proceed at different rates? These and other questions remain to be resolved in this most-studied of eyes.

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