Efferent Control of Temporal Response Properties of the *Limulus* Lateral Eye

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ABSTRACT The sensitivity of the *Limulus* lateral eye exhibits a pronounced circadian rhythm. At night a circadian oscillator in the brain activates efferent fibers in the optic nerve, inducing multiple changes in the physiological and anatomical characteristics of retinal cells. These changes increase the sensitivity of the retina by about five orders of magnitude. We investigated whether this increase in retinal sensitivity is accompanied by changes in the ability of the retina to process temporal information. We measured the frequency transfer characteristic (FTC) of single receptors (ommatidia) by recording the response of their optic nerve fibers to sinusoidally modulated light. We first measured the FTC in the less sensitive daytime state and then after converting the retina to the more sensitive nighttime state by electrical stimulation of the efferent fibers. The activation of these fibers shifted the peak of the FTC to lower frequencies and reduced the slope of the low-frequency limb. These changes reduce the eye's ability to detect rapid changes in light intensity but enhance its ability to detect dim flashes of light. Apparently *Limulus* sacrifices temporal resolution for increased visual sensitivity at night.

INTRODUCTION

The horseshoe crab, *Limulus polyphemus*, has evolved a remarkable adaptation to diurnal changes in environmental lighting. After the sun sets, the sensitivity of its visual system increases by $\sim 10^5$, nearly compensating for the changes in ambient light intensity (Barlow, 1988). This endogenous modulation of visual sensitivity is controlled by a circadian oscillator in the brain that is connected to the cells of the lateral compound eyes by efferent fibers in the optic nerve. The efferents are activated at night, decreasing the spontaneous activity of visual cells and increasing both the number of photons absorbed and the number of nerve impulses triggered per absorbed photon (Barlow et al., 1977, 1980, 1987; Kaplan and Barlow, 1980; Barlow, 1983). In this way, *Limulus* adapts its retina to dim illumination by decreasing retinal noise and increasing photon catch and retinal gain.

Fig. 1 presents a scheme for how the multiple changes in the retina at night combine to change the dynamic range and responses of a photoreceptor (ommatidium). The functions give the steady-state discharge of a single optic nerve fiber across a wide range of intensities at different stages of efferent activation.

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The "day" function was recorded from a dark-adapted animal at 1500 h when the efferents were inactive. This function has a characteristic shape which appears to reflect two receptor mechanisms: one operating at low light intensities and another at high intensities (Kaplan and Barlow, 1975). A plateau in the function separates the intensity ranges over which the two mechanisms operate. Seconds after the activation of the efferent fibers, the "day" function shifts to an intermediate state (*dashed line*). The intermediate state is produced by rapid changes in the physiology of the photoreceptor cells: a rapid reduction in spontaneous activity and a rapid increase in gain (Renninger et al., 1984; Barlow et al., 1987).

As the efferent input to the retina continues, the intermediate curve slowly shifts to the left and within ~1.5 h it reaches the final "night" function. This shift is produced by anatomical changes in the retina that increase the number of photons caught by the photoreceptors. The "night" function in Fig. 1 remains stable from the early evening to the early morning hours (0300 h) at which time the clock's efferent activity begins to decrease. The intensity-response function then shifts back to the daytime state.

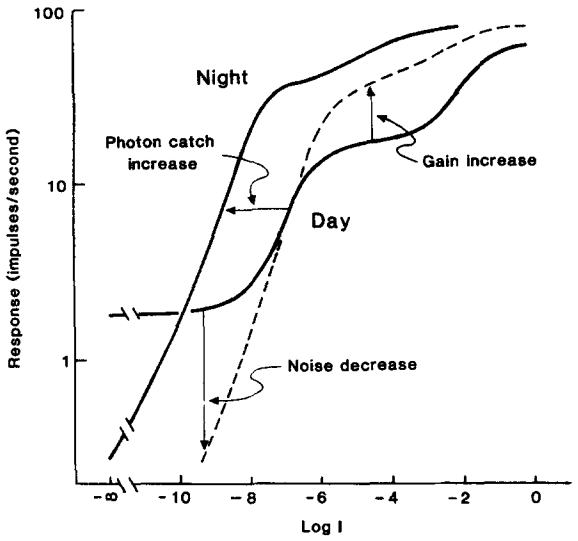

FIGURE 1. Scheme of how the circadian rhythm in *Limulus* alters the response of a single optic nerve fiber across intensities. The response is plotted on the ordinate as the mean response rate during the last 3 s of a 6-s flash delivered to the test ommatidium, while the remainder of the retina was in darkness. The log of the relative light intensity from an incandescent source (0 log units = 10^{12} photons/s) is plotted on the abscissa. The initial efferent input from the clock causes a rapid increase in gain and a rapid decrease in spontaneous activity, yielding an intermediate intensity-response function (*dashed line*). Continued efferent input produces slower anatomical changes that increase the photon catch, moving the intermediate function to the left. Filled and unfilled symbols indicate the firing rates at which FTCs were measured during the day and in the nighttime state, respectively. Adapted from Barlow et al., 1977, 1987.

Fig. 1 shows that the physiology of the retinal cells is modulated by activation of the efferent innervation, and that this modulation is reflected in the steady-state responses of the optic nerve. Do comparable changes occur in the dynamic responses? To answer this question, we measured the frequency transfer characteristic (FTC) of the responses of single fibers in the optic nerve with the retina in the daytime state and after activating the efferents by electrical stimulation. We found

that the FTC is indeed controlled by efferent input to the retina. For the same mean level of response, the FTC is smaller in amplitude and its peak shifts to lower frequencies when efferents are activated. This suggests that at night the horseshoe crab sacrifices temporal resolution for increased sensitivity.

METHODS

Biological Preparation

Experiments were performed on the lateral eyes of adult horseshoe crabs that measured 15–20 cm across the breadth of the carapace. Specimens were flown from the east coast of the United States to Syracuse, New York, placed in circulating artificial seawater (ASW; Instant Ocean, Aquarium Systems, Inc., Eastlake, OH), and fed fresh clams on a regular schedule. Animals were usually used within 2 mo of their arrival in Syracuse.

Our technique of recording from single optic nerve fibers in situ was similar to that described earlier (Barlow and Kaplan, 1971; Kaplan and Barlow, 1975). Surgery was started during the daytime, when efferents are inactive. An animal was fastened to an elevated wooden platform by stainless steel screws. A section of shell anterior to the eye was then removed. The optic nerve was separated from underlying tissue, transected, and drawn into a Lucite recording chamber which was securely fastened to the carapace. The chamber was filled with ASW that had been Millipore filtered and to which penicillin was sometimes added. The blood vessel surrounding the nerve was cut away. A small strand of nerve containing one active fiber, or one with an action potential that could be discriminated electronically, was teased from the main trunk and drawn into the small tip of a glass suction electrode. The remainder of the nerve was drawn into the tip of a larger suction electrode so that it could later be electrically stimulated to activate efferent fibers.

The animal and platform were placed in a small aquarium which was located in an electrically shielded lightproof box. The gills of the animal were submerged and continuously irrigated with aerated ASW.

Optical Stimulation

The light source for these experiments was a Tektronix 608 display monitor with a green P32 phosphor. Light from a single illuminated point on the face of the monitor was transmitted to the test ommatidium by a system of optics that reduced scattered light to neighboring photoreceptors. Any stray illumination was below the level needed to produce lateral inhibitory interactions in the network behind the retina (Barlow, 1969). Apart from the illumination of the test ommatidium, the retina remained in total darkness.

The light was first focused onto one end of a fiber optic light pipe (70 μm diameter) using a microscope objective. Then, with a specially designed manipulator (Barlow, 1967), the other end of the light pipe was aligned with the optic axis of the ommatidium and positioned so that the tip of the light pipe was in contact with the cornea directly in front of the ommatidium. Immersion oil was used to enhance optical coupling and reduce scatter between the light pipe and the cornea.

The intensity of illumination was controlled by a computer (PDP 11/34; Digital Equipment Corp., Marlboro, MA), via two digital to analog converters which fed into a summing amplifier. One converter incremented intensity in coarse steps and the other in fine steps. A coarse step was a hundred times the voltage of a fine step.

The output of the light pipe was calibrated using a PIN10-DF photodiode (United Detector Technology, Culver City, CA). An intensity of 0.0 log units corresponded to 0.27 fW (~680 photons/s) at the tip of the light pipe. The light intensity was either controlled directly by the

computer or was attenuated with neutral density filters placed between the monitor and the microscope objective. Modulation of the average intensity was performed by varying the output of the digital to analog converters. A table stored in the computer was used to compensate for the nonlinear relationship between the voltage applied to the input of the oscilloscope and the intensity of illumination.

Recording Procedure and Data Collection

After surgery had been completed and the ommatidium had been optically isolated, the shielded lightproof box was closed. The preparation was allowed to dark adapt for a period of several hours before recordings were made.

Action potentials from the nerve were amplified, filtered (300 Hz to 1 kHz), and electronically discriminated (model 702; World Precision Instruments, New Haven, CT). The output of the discriminator was transmitted to the computer which recorded the times of arrival of impulses to within 100 μs, and stored these times on disk.

Measuring the FTC

The intensity-response function was measured using 10-s flashes and the plateau was located (see Fig. 1). FTCs were measured at response rates above and below the plateau both during the day and at similar rates in a simulated nighttime state. The approximate response rates are indicated by the circles and triangles in Fig. 1.

The stimuli for measuring FTCs consisted of 10-s flashes of light that were modulated by a sum of sinusoids (Victor et al., 1977). The flashes were presented several minutes apart to maintain the retina in a dark-adapted condition. The two sets of interleaved modulation frequencies used are specified in Table I. They were selected using the formulae $0.5 \times 20^{2n/14}$ and $0.5 \times 20^{(2n-1)/14}$ Hz. This choice provided a finer grid of frequencies than that used by Victor et al. (1977) or later by Brodie et al. (1978*a*), but precluded measurement of response amplitudes at harmonic frequencies. Amplitudes of modulation for each frequency were chosen to be approximately inverse to the response amplitudes expected at that frequency (Brodie et al., 1978*a*).

The response from 3 s after the onset of the stimulus to its end was fit with a mean level, a linear ramp, sines and cosines at the seven frequencies of modulation and their second harmonics. Over this interval, the response rate is relatively constant. The linear ramp accounts for any drift of the response rate (Knight et al., 1970), and will not be considered further. The fitting procedure used was identical to that of Brodie et al. (1978*a*).

The cumulative SEM of the amplitude and phase of every component was also computed after each stimulus presentation. These values were used to determine when sufficient data had been collected. Typically, data collection was terminated if the SEM of the amplitude was $<30\%$ of the mean amplitude for frequencies $<\sim 5$ Hz.

Linearity was tested by comparing the response to the sum of sinusoids with the response to the frequencies presented individually. Fig. 2 shows the results of such a test. The amplitude of the FTC is plotted against the frequency of modulation in Hertz. The amplitude of the response is presented as percent modulation of the impulse rate per percent modulation of the incident light at each frequency. The vertical error bars beside each symbol give the SEM of response amplitude. Triangles denote responses measured by the sum of sinusoids method, while squares denote responses measured by presenting sine waves at each frequency. Differences between the two are at most 30%. Although in this experiment the SEMs were less than these differences, in most experiments the SEMs were ~30%. Thus, the responses were usually linear within the precision of the measurements, although nonlinearities may have contributed to some of the irregularities in the measured FTCs. Lower levels of

TABLE I

Table of Frequencies

Frequency	Percent modulation	
	Low response rates	High response rates
Hz		
0.77	15	11
1.18	12	9
1.81	11	8
2.77	8	6
4.25	9	7
6.52	10	8
10.00	15	11
	80	60
0.62	15	11
0.95	12	9
1.46	11	8
2.24	8	6
3.44	9	7
5.24	10	8
8.09	15	11
	80	60

Amplitudes of modulation at each frequency when the sum of sinusoids method was used. At low response rates 80% modulation was used, while at higher response rates 60% modulation was used. At each response rate two sets of interleaved frequencies were presented.

modulation would have reduced these effects, but would have necessitated recording from an optic nerve fiber for considerably longer periods of time.

After data were collected for the daytime condition the optic nerve was electrically stimulated. The regime of stimulation varied somewhat from experiment to experiment, but generally imitated the endogenous efferent activity in the optic nerve at night. Such a regime

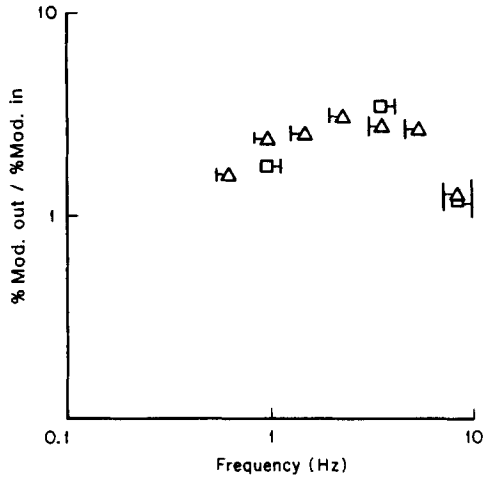

FIGURE 2. Linearity of the FTC. Measurements were conducted in the daytime state at a mean response rate below the plateau in the intensity-response function of Fig. 1. Triangles and squares give the results using a sum of sinusoids (80% modulation) and single frequencies, respectively. The vertical error bars beside each symbol give the SEM of response amplitude. Amplitudes of modulation are as listed in Table I. The incident light intensity was 3 log units and the mean response rate was 9 impulses/s. 40 repetitions averaged.

induces changes in the response of the eye similar to those that occur naturally (Barlow et al., 1977, 1980, 1987; Kaplan and Barlow, 1980).

Stimulation typically consisted of a train of 2-ms pulses delivered at a rate of 4 s^{-1}. Trains were 24 s long, and presented at a rate of 1 train/min. After every sixth train, stimulation was suspended for 2 min, the sensitivity of the ommatidium was tested, and stimulation was resumed.

The cycle of electrical stimulation and testing was repeated for ~2 h, the time required for the effects of efferent input to reach a steady state. Thereafter, data were collected 2 min after every sixth train of electrical pulses.

At various times during the experiment, the lightproof box was opened under dim illumination. The fluid in the chamber was replaced with fresh ASW or an organ culture medium which was a modified version of that developed by Bayer and Barlow (1978). The culture medium omitted glutamine, retinol, and tocopherol from the Bayer and Barlow recipe. In earlier experiments the chamber was continuously superfused with either ASW or the organ culture medium. This procedure was discarded because it did not appear to increase the longevity of nerve fibers.

Analysis

Features of the FTC were objectively assessed by fitting appropriate functions to the physiological data, with each data point weighted according to its SEM. The peak frequency was found by fitting the logarithm of the measured FTC with a third degree polynomial of $\log f$, $G_p(f) = c_0 + c_1 \log f + c_2(\log f)^2 + c_3(\log f)^3$ using the method of least squares (Bevington, 1969). Only the lowest thirteen frequency points were fitted, because the polynomial could not adequately fit the steep high-frequency slope. The peak of the fitted function $G_p(f)$ was used as an estimate of the peak frequency and amplitude of the FTC. The slope of the low frequency limb was estimated by similarly fitting the low frequency part of the logarithm of the measured FTC with a straight line: $G_s(f) = c_0' + c_1' \log f$. The fit included all data points below the peak frequency as estimated from the polynomial fit. The slope of the straight line was taken as the slope of the low-frequency limb.

RESULTS

Daytime State

The dynamic responses of single optic nerve fibers are shaped by light intensity as well as by the circadian clock. The FTCs measured at high intensities during the day peak at higher frequencies and have low-frequency limbs with steeper slopes than those measured at low intensities.

Fig. 3 shows the FTCs measured at mean response rates above (*circles*) and below (*triangles*) the plateau in the intensity-response function (see Fig. 1). Fig. 3 *A* plots the amplitude of the FTC on the ordinate vs. frequency of modulation on the abscissa. The amplitude is plotted as percent modulation of the response rate per percent modulation of the incident light intensity. The fits were calculated as described in Methods. Fig. 3 *B* plots the corresponding phase data in radians vs. frequency in Hertz.

Maximum modulation of the response occurred at 1.8 and 2.8 Hz, respectively for the low (*triangles*, 10 impulses/s, 2.0 log units) and high (*circles*, 31 impulses/s, 5.5 log units) response rates, while the slopes of the low-frequency limbs were 1.9 and 2.7 dB/octave. Thus, both the frequency at which the modulation of the

response is at maximum and the slope of the low-frequency limb increase with light intensity.

The amplitude of the FTC also increases with light intensity as does the phase. Moreover, the decline of phase with frequency is slower at the higher intensity. All of these changes were observed in the three experiments in which FTCs were measured at both the response rates above and below the plateau in the intensity-response function.

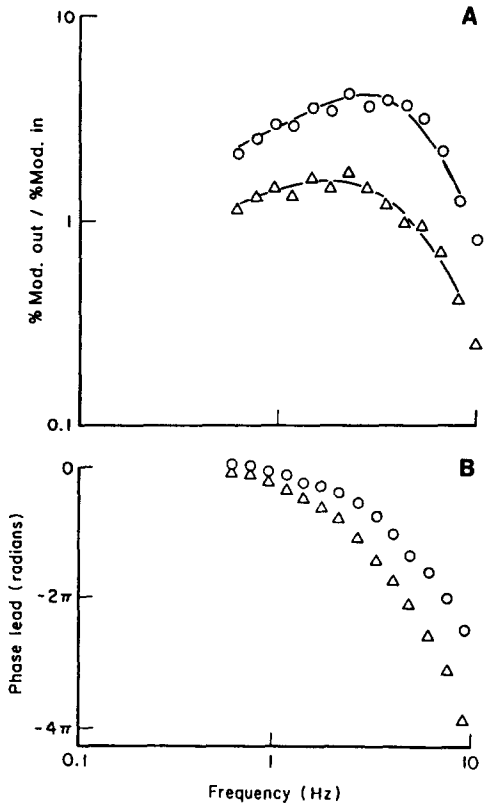

FIGURE 3. FTCs from a single ommatidium measured at two response rates during the day. The response rates were chosen below (*triangles*) and above (*circles*) the plateau in the intensity-response function of Fig. 1. The two mean response rates were 10 and 31 impulses/s, and the mean intensities 2.0 and 5.5 log units, respectively. (*A*) Amplitude of the FTC. The ordinate is the ratio of the percent modulation of the response rate to the percent modulation of the intensity at a given frequency. The curves are fits to the data which were generated as described in Methods. (*B*) Phase of the FTC. The phase lead in radians is plotted here. Phase was measured *modulo* 2π and the values were adjusted to give the smoothest curve. Responses averaged: 67 at the lower and 2 at the higher response rate. Experiment 12/1/81.

Nighttime State

Light intensity shapes the dynamic responses at night much as it does during the day. Fig. 4 shows FTCs measured during the simulated nighttime state from the same optic nerve fiber as in Fig. 3. The incident light intensities were reduced to yield the same mean response rates as in Fig. 3. Coordinates are the same in both figures. Note that both the frequency at which modulation of the response is maximal and the slope of the low-frequency limb increase with light intensity as they did in the daytime state (Fig. 3). Maximum modulation of the response occurs at 1.4 and 1.7 Hz for the low and high intensities, respectively, while the slopes of the low-frequency limbs are 1.2 and 3.5 dB/octave. At the higher intensity the ampli-

tude of the FTC is larger and the phase declines more slowly with increasing frequency of modulation.

At the lower intensity the FTC has a low-pass shape: the slope of the low-frequency limb is so shallow that a peak is barely discernible. In this instance, the peak frequency obtained by our fitting procedure is of questionable value. Consequently, the shift of the FTC to higher frequencies with brighter illumination is possibly greater than our analysis indicates.

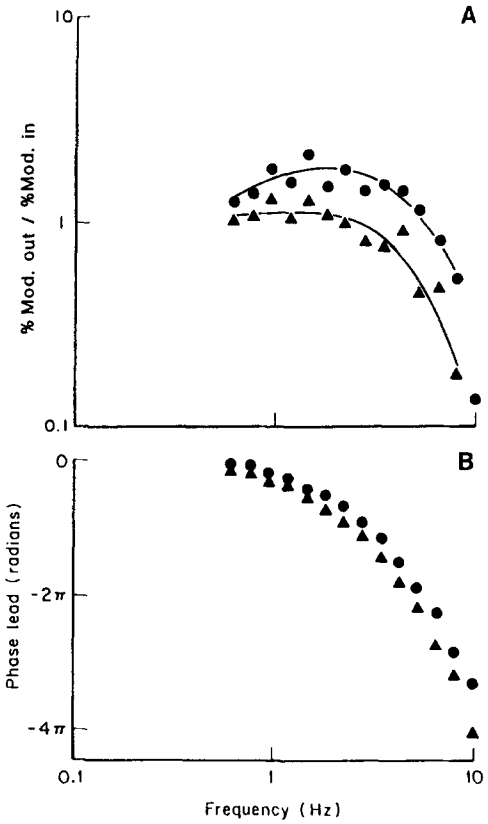

FIGURE 4. FTCs measured at two response rates in the nighttime state from the same ommatidium as in Fig. 3. Response rates and intensities were 11 impulses/s, 0.0 log units (*filled triangles*) and 28 impulses/s, 1.0 log unit (*filled circles*). (*A*) Amplitude of the FTC. The curves are fits to the data which were generated as described in Methods. (*B*) Phase of the FTC. Responses averaged: 144 at the lower and 45 at the higher response rate.

Daytime Vs. Nighttime Responses

A comparison of the temporal properties of the two states of the retina must take into account the dependence of these properties on the level of photoreceptor illumination. The circadian efferent input that shifts the retina to the nighttime state does so in part by changing the structure of the retina to increase the light intensity reaching the photoreceptors (Barlow et al., 1980). Simply measuring the temporal characteristics of the photoreceptor in both states at the same level of illumination is insufficient: any changes observed might merely be the result of the increase in the light reaching the photoreceptor in the nighttime state. We therefore chose instead to compare FTCs at the same average response rates in the two states. This compar-

ison does not maintain the same light intensity at the photoreceptor, but it does insure that any differences observed in the dynamics must involve factors besides the change in effective intensity.

We found that FTCs measured in the nighttime state of the retina have smaller amplitudes and lower peak frequencies than those measured during the day at the same mean response rate. In Fig. 5 the fits to the FTCs measured in the nighttime state (*long dashes*), have been superposed on the fits to the FTCs of Fig. 3 which were measured during the day (*short dashes*). The average response rates in impulses per second under each condition are shown in parentheses. Maximum modulation of the response occurs at a lower frequency in the nighttime state than at a similar response rate during the day, and at all frequencies the amplitude of the FTC is smaller. At the higher response rate, the low-frequency slope is greater at night in this preparation, but this was not observed in other experiments.

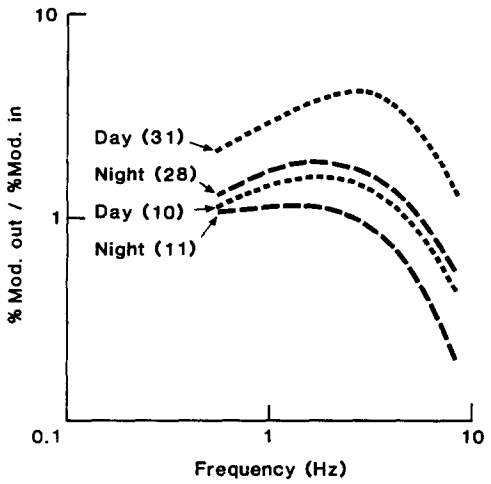

FIGURE 5. Comparison of daytime FTCs with nighttime FTCs. The fits to the daytime (*short dashes*) and nighttime (*long dashes*) FTCs of Figs. 3 and 4 are plotted on the same axes for comparison. Numbers in parentheses are mean response rates in impulses per second. At similar response rates the peak frequency and amplitude are lower in the nighttime state than during the day.

Variability of Response Rate

The response rate of a single optic nerve fiber is more variable at night than during the day (Barlow, R. B., and G. H. Renninger, personal observation; Batra, 1983) and at low rather than high light levels regardless of the time of day (Kaplan and Barlow, 1975; Batra, 1983). As a consequence, we were able to measure the FTC precisely with just one stimulus presentation at higher light levels during the day; but we had to average the responses to 40 stimulus presentations when the retina was in the nighttime state. About 40 presentations were also required at the lower response rates when the retina was in the daytime state. At these rates in the nighttime state, averaging the responses to over 100 presentations was not entirely satisfactory. Furthermore, response rates tended to be more stable when the retina was in the daytime state. In this state, the average response rate during the first and last presentations were within ~10% of one another. In the nighttime state, the average response rate often declined by as much as 30%. It appears that the nighttime state may be more sensitive to the effects of light adaptation.

The effects of variability in response rate on our measurements of the peak of the FTC and on the slope of the low-frequency limb were estimated using the techniques outlined by Bevington (1969). The relative standard error of the peak frequency typically ranged from 20% at high response rates during the day to 40% or more at low response rates in the nighttime state. The relative standard error of the slope of the low-frequency limb typically ranged from 5 to 40% or more for the same two conditions. The large relative errors in estimating features of the FTC at low response rates in the nighttime state were due to the low-pass shape of this function, as discussed above.

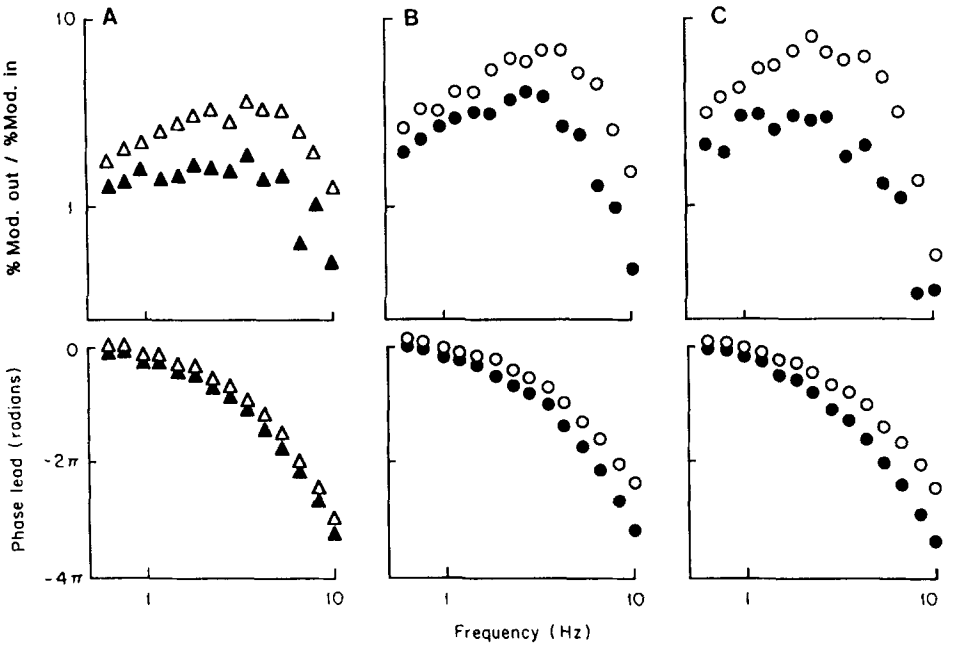

FIGURE 6. FTCs during the day (*unfilled symbols*) and in the nighttime state (*filled symbols*) for three ommatidia in as many eyes. Upper panel in each pair shows the amplitude of the FTC and lower panel shows the phase. The FTCs in *A* were measured at response rates below the plateau in the intensity-response function and those in *B* and *C* were measured at rates above the plateau. Data for each FTC:

	Intensity	Response rate	Peak frequency	Low-frequency slope	Responses averaged	Experiment
	log units	*impulses/s*	*Hz*	*dB/octave*		
A Day	3.0	9.3	3.1	2.8	42	26/1/81
Night	1.0	9.5	2.5	1.2	43	
B Day	5.5	16.2	3.2	3.4	2	2/3/81
Night	2.5	18.9	2.4	2.9	24	
C Day	5.5	19.5	2.5	4.2	2	4/3/81
Night	2.0	18.0	1.7	2.9	37	

Summary

A decrease in amplitude of the FTC and a shift to lower frequencies appears to be characteristic of the nighttime state of the retina. This is shown in Fig. 6 which depicts FTCs measured from three ommatidia in daytime and nighttime states at roughly the same response rates. The FTCs in Fig. 6 *A* were measured at rates below the plateau in the daytime intensity-response function while those in Fig. 6, *B* and *C* were measured above the plateau. Unfilled symbols denote responses measured in the daytime state, and filled symbols denote responses measured in the nighttime state.

In sum, transforming the retina to the nighttime state produces four characteristic changes in the FTC. In the nighttime state, FTCs have (*a*) smaller amplitudes, (*b*) maximum modulation of the response at lower frequencies, (*c*) low-frequency limbs with shallower slopes, and (*d*) phases that decline more rapidly with frequency.

Table II summarizes the data from Figs. 3–6. Both the frequency at which maximum modulation of the response occurs and the slopes of the low-frequency limbs show a progressive increase from FTCs measured at low response rates in the nighttime state to those measured at high response rates in the daytime state.

TABLE II

Peak Frequencies and Slopes of Low-Frequency Limbs

	Peak frequency	Low-frequency slope	*N*
	Hz	*dB/octave*	
Nighttime, low response rate	2.0 ± 0.8	1.2 ± 0.0	2
Nighttime, high response rate	1.9 ± 0.4	3.1 ± 0.4	3
Daytime, low response rate	2.6 ± 0.5	2.6 ± 0.7	5
Daytime, high response rate	3.3 ± 0.7	3.5 ± 0.6	5

Peak frequencies and slopes of low-frequency limbs of FTCs during the day and in the nighttime state. Pairs of numbers represent mean and standard deviation of the measurements made. The last column lists the number of measurements under a particular condition.

DISCUSSION

Efferent Input Changes the Dynamic Response of the Retina

Efferent optic nerve activity reduces the amplitude of the FTC and shifts its maximum amplitude to lower frequencies. In the face of a millionfold reduction in ambient lighting *Limulus* sacrifices temporal resolution at night for increased sensitivity.

Limulus is not unique in this respect. The temporal resolution of the visual systems of many animals changes with the level of ambient illumination (Brown, 1965). Visual systems generally respond to reduced illumination with an increase in temporal summation that results in reduced temporal resolution. However, *Limulus* not only responds to changes in ambient illumination but anticipates the changes, adjusting the properties of its visual system with an internal circadian clock. To our knowledge, such a dual control of temporal processing in the retina has not yet been demonstrated in any other animal. In vertebrates, the increase in temporal summation under reduced illumination is associated with a shift from cone photoreceptors

to rod photoreceptors. *Limulus,* on the other hand, adapts its retina to diurnal changes in illumination using only a single type of photoreceptor.

In addition to adapting the dynamics of its visual response, *Limulus* also adjusts the gain of its retina. The amplitude of the FTC is larger during the day than at night. This means that the same percent modulation of the light intensity produces a larger modulation of the response rate during the day, indicating a larger dynamic gain. However, at night there is a larger absolute gain, since more impulses are generated for a given number of photons captured. In other words, *Limulus* trades a larger dynamic gain during the day for a greater absolute gain at night.

Mechanisms Underlying Changes in the FTC

The shift of the FTC to lower frequencies implies that some or all of the time constants of transduction are longer at night. Possible changes could involve the duration of quantum bumps, their shape, the time constant for adaptation and the time constant for self-inhibition. Kaplan et al. (1986) have in fact found that the duration of both spontaneous and light-evoked quantum bumps increases at night. Do changes in any or all of these temporal properties contribute to the shift of the FTC?

We explored this question by fitting the daytime FTCs with theoretical functions[1] generated with various values for the parameters associated with individual temporal properties. We then varied the parameters to obtain fits to the FTCs of the nighttime state. We found that several combinations of changes in temporal properties produce theoretical functions that adequately fit both the amplitude and the phase of the nighttime FTCs. Parameters associated with the shape and duration of bumps, as well as their dispersion, tended to affect the peak of the FTC and the slope of the high-frequency limb. Parameters for adaptation and self-inhibition controlled the slope of the low-frequency limb, and also affected the peak frequency. Thus it is difficult to say which temporal properties are, in fact, being modulated. However, we did find that simply increasing the duration of quantum bumps does not account for the nighttime change in the shape of the FTC.

The present experiments are inconclusive on the question of whether the lengthening of the time constants are a direct consequence of efferent input to retinal cells, or an indirect consequence of the nighttime increase in retinal sensitivity. We recorded FTCs at the same response rates during the nighttime and daytime states. Since efferent input increases the number of impulses per absorbed photon (Barlow et al., 1977), it is likely that a given response rate required the absorption of fewer photons in the nighttime state. The shape of the FTC is influenced by the average light intensity (Dodge et al., 1968; Wong and Knight, 1980; Figs. 3–5). Consequently, it is possible that the longer time constants inferred from the FTCs result from a combination of two factors: an increase in the duration of time constants at lower levels of illumination and an increase in absolute gain as a result of efferent input.

[1] The forms of the theoretical functions used were the same as those used by Brodie et al. (1978*b*), except that the term for lateral inhibition was omitted.

The Effect of Intensity on the Dynamic Response

An increase in the average light intensity raises the dynamic gain, the slope of the low-frequency limb of the FTC, and the frequency at which maximum modulation of the response occurs. These changes occur in both the daytime and nighttime states of the retina. These changes are analogous to those found in the vertebrate retina (Maffei et al., 1971; Kaplan et al., 1979). The FTCs of ganglion cells of the vertebrate retina shift to higher frequencies under brighter illumination (Kaplan et al., 1979). Raising the level of illumination also raises the critical fusion frequency of the a-wave of the vertebrate electroretinogram, which is thought to reflect the activity of the photoreceptors (van de Grind et al., 1973). Changes in the dynamics of the visual response may well occur at the earliest stages of visual processing.

The changes we have observed in the FTC both during the day and at night when the light intensity is increased are consistent with the adaptive changes seen in the excised eye. In the excised eye, adaptive changes are attributed to a decrease in the duration and amplitude of the underlying quantum bumps (Adolph, 1964; Dodge et al., 1968). They also appear to be due to changes in the degree to which earlier bumps reduce the amplitude of later bumps (Wong et al., 1980).

The interpretation of these changes in the in situ eye is complicated by the presence of two types of bumps, the small potential fluctuations (SPFs) and the large potential fluctuations (LPFs; Adolph, 1964; Dowling, 1968; Barlow and Kaplan, 1977). For example, the increase in the peak frequency at higher intensities can be interpreted in several ways.

Modulation of the incident light modulates the frequency of SPFs and LPFs, which in turn modulates the response of optic nerve fibers. At higher frequencies, where the period of the modulation is less than the duration of individual fluctuations, modulation of the potential fluctuations no longer occurs and the amplitude of the FTC declines. If the SPFs trigger LPFs, which are responsible for firing impulses at low light levels, then the frequency at which the decline occurs, and hence the peak frequency, should reflect the duration of the longer fluctuation.

On the one hand, if SPFs are longer, then the increase in this cutoff frequency at higher intensities should reflect a reduction in the duration of SPFs. On the other hand, if LPFs are longer, then the decrease may be a consequence of a transition from LPF-coded responses at low intensities to those coded solely by SPFs at higher intensities. Bayer and Barlow (1978) have shown that LPFs in the ventral photoreceptors of *Limulus* have a refractory period that may also contribute to the lower peak frequency in dim light. Similarly, changes in the slope of the low frequency limb may reflect changes in adaptation of both LPFs and SPFs. These issues could perhaps be clarified by repeating the experiments of Dodge et al. (1968) and Wong and Knight (1980) on the lateral eye in situ. Such experiments would also yield the parameters of the adapting-bump model for the eye in situ.

Comparison with Dynamic Response of the Excised Retina

FTCs of ommatidia measured when the eye is in situ extend over a wider range of frequencies than when the eye is excised. Knight et al. (1970) measured FTCs of ommatidia in the excised eye. We have averaged their measurements and renormal-

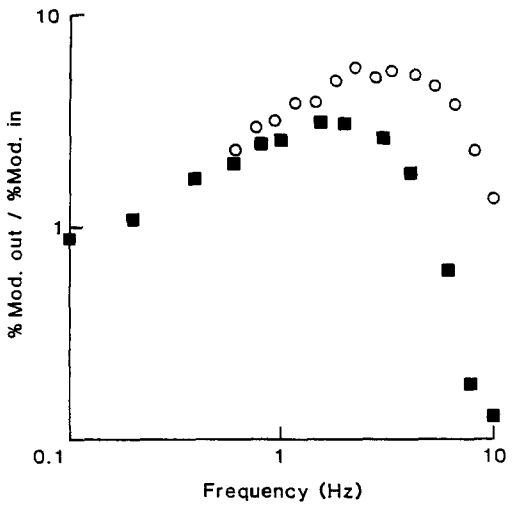

FIGURE 7. Comparison of average FTCs from in situ (*unfilled circles*) and excised eyes (*filled squares*). The FTCs of the in situ eye were measured during the day at response rates above the plateau of the intensity-response function. Excised eye data were averaged from the results of Knight et al. (1970). The average was performed by first calculating the sine and cosine components of the response at each modulation frequency for each of the five FTCs shown by Knight et al. (1970), averaging the corresponding components, and then calculating the amplitude of the average at each modulation frequency.

ized them so that the amplitude is presented as percent modulation of response per percent modulation of intensity. The amplitude portion of this average is shown by filled squares in Fig. 7. Because ommatidia of the excised retina only respond to light intensities above the plateau in the intensity-response function of Fig. 1 (Barlow and Kaplan, 1971), we compared the FTCs measured in the excised eye to our averaged measurements of the FTC at these intensities (*unfilled circles*). The FTC measured in the in situ retina extends to higher frequencies than that measured after excision. We also measured the peak frequency and the slope of the low-frequency limbs of the individual FTCs from the excised retina. The primary change is the peak frequency, which is 2.0 ± 0.5 Hz for the excised eye and 3.3 ± 0.7 Hz for the in situ preparation. The simplest explanation for this difference is that the duration of bumps in the photoreceptor cells is shorter in the in situ retina. We were able to mimic the effect of excision theoretically by using suitable values for the parameters of transduction in the in situ retina to generate a fit to the physiological data, and then increasing the bump duration. Shorter bump durations in the in situ eye have been hypothesized by others (Brodie et al., 1978*b*).

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