Properties of Visual Cells in the Lateral Eye of *Limulus In Situ*

Extracellular Recordings

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ABSTRACT Excitatory properties of visual cells in the lateral eye of *Limulus*, investigated by optic nerve recordings *in situ*, differ significantly from the properties of cells in the classical, excised eye preparation. The differences suggest the possibility that two receptor mechanisms function in the eye *in situ*: one mechanism encodes low light intensities and the other responds to high intensities. The two mechanisms enable each ommatidium to respond over an intensity range of approximately 10 log units. This hypothesis was tested by measuring the increment threshold and the spectral sensitivity, by studying light and dark adaptation, and by analyzing the variability of the impulse discharge. Although the results do not conclusively identify two receptor mechanisms, they indicate that a process or a part of a process that functions in the eye *in situ* is abolished by excising the eye or cutting off its blood supply.

INTRODUCTION

The lateral eye of the horseshoe crab, *Limulus polyphemus*, occupies a special place in visual and sensory physiology. Its apparent simplicity attracted many researchers, who discovered that the eye shares a number of characteristics with the visual systems of higher organisms, including man. Our knowledge of the functioning of the *Limulus* lateral eye comes almost entirely from experiments in which the eye had been excised from the animal (for reviews see Wolbarsht and Yeandle, 1967; and Hartline and Ratliff, 1972).[1] This technique is relatively easy and is well suited for many types of experiments; however, one drawback is that excision causes a gradual decline in the eye's sensitivity to light (Barlow and Kaplan, 1971). To overcome this problem, we recorded from single optic nerve fibers *in situ* without interrupting the blood supply to the eye.

[1] Biederman-Thorson and Thorson (1971) recorded from optic nerve fibers of the *Limulus* lateral eye *in situ*. However, their preparations were kept in the light-adapted state, a condition which causes many characteristics of the intact eye to resemble those of the excised eye.

We found that the eye *in situ* is indeed a stable preparation, but much to our surprise we discovered that its properties differ significantly from those of the excised eye. As shown in Fig. 1, ommatidia in the unexcised eye are extremely sensitive to dim illumination, responding to a few (perhaps one) absorbed photons. They discharge impulses in the dark and encode light intensity over a range of approximately 10 log units. In addition, the intensity function for the steady-state response of an ommatidium in the unexcised eye has a distinct plateau at intermediate light intensities. No plateau has

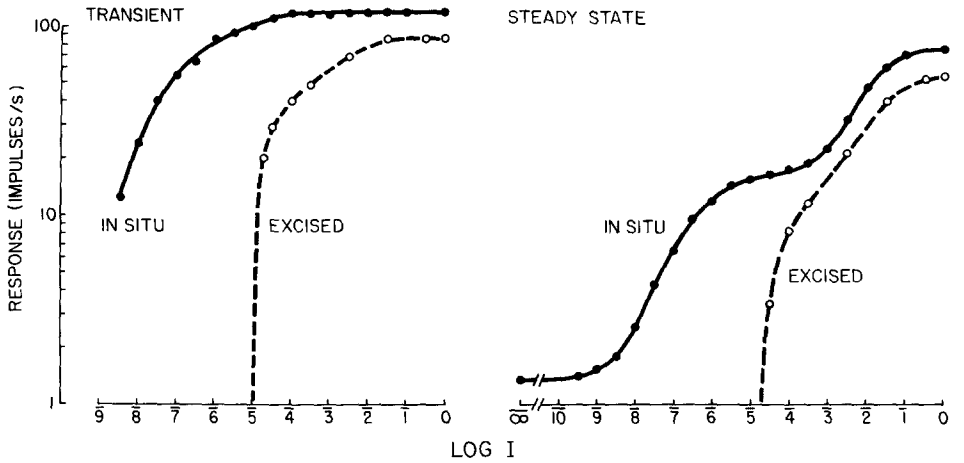

FIGURE 1. Intensity functions of ommatidia in the *in situ* and excised lateral eye of *Limulus*. Shown on the left are the intensity functions for the transient response, which is the initial peak of the impulse discharge at the onset of illumination. On the right are the intensity functions for the steady-state response, defined as the mean firing rate in the last 5 s of a 10-s flash. Each function is plotted as log of the impulse discharge on the ordinate versus log of the relative light intensity on the abscissa. At log $I = 0$ the quantal flux at the cornea was approximately 10^{12} photons/s between 400 and 700 nm. Darkness is indicated by log $I = -\infty$. All of the "*in situ*" data were recorded from a single optic nerve of an ommatidium in an unexcised lateral eye. The ommatidium was allowed to dark adapt completely between test flashes. To obtain the "excised" data, the same lateral eye was removed from the animal and the response measurements were repeated.

been found for ommatidia in the excised eye preparation. We ascribe these differences to the interruption of the blood supply to the eye and not to other trauma of excision (Barlow and Kaplan, 1971).

The 10-log unit range of the response of ommatidia in the intact eye is particularly intriguing. No known sensory receptor spans a similar range, and most operate over less than half this range on a logarithmic scale (Loewenstein, 1961; Baylor and Fuortes, 1970; Adams, 1971; and Werblin, 1971). It is attractive, then, to assume that two receptor mechanisms function in the eye *in situ*: one at low light intensities (threshold $\simeq$1 absorbed photon) and the other at high intensities (threshold $\simeq 10^5$ absorbed photons). The more

sensitive mechanism would saturate at intermediate intensities, and the less sensitive mechanism would saturate at high intensities. This hypothesis is supported by the shape of the intensity-response function, particularly the plateau region. According to this view, only the less sensitive mechanism survives excision of the eye. Such mechanisms could conceivably be two types of cells within an ommatidium, two different processes within a single cell, or two kinds of visual pigments. These and other possibilities will be considered.

We report here a series of experiments designed to test the two-mechanism hypothesis, and to elucidate some excitatory properties of ommatidia in the unexcised eye. The hypothesis was examined using some of the standard procedures applied to vertebrate retinas suspected of containing two types of receptors. The data are consistent with the view that two processes function inside the ommatidium. Having established the dual nature of the ommatidial response, we will show in a following paper, based on intracellular recordings from the eye *in situ*, that this duality may well originate in the primary photoreceptor itself, the retinular cell.

METHOD

Material and Biological Preparation

We experimented mainly on large male horseshoe crab (8–10 inches across the carapace) procured from the Harborton Marine Laboratory, Virginia, and the Marine Biological Laboratory, Woods Hole, Mass. In our laboratory the animals were kept in artificial seawater (ASW) (Instant Ocean, Aquarium Systems, Inc., Eastlake, Ohio) and fed regularly.

To record from single optic nerve fibers *in situ*, we first secured the crab to a rigid platform and attached to the dorsal surface of the carapace a support for the optical stimulator and recording electrode. The platform carrying the animal was then placed in a small tank that was filled with ASW to the level of the lateral eyes. The gill structure was continuously washed with aerated ASW through slots in the platform. The temperature (18–20°C) and pH (7–7.5) of the ASW in the tank were approximately the same as those in the large aquaria that held the animals until the experiment. The tank was placed in a light-proof, shielded cage.

A hole, 1.9 cm in diameter, was cut in the carapace about 3 cm anterior to one of the lateral eyes. The optic nerve runs directly underneath the carapace at this point. The nerve trunk and the associated hepatic artery were cleared of surrounding tissue, cut, and pulled into a small recording chamber that was attached to the shell. The wall of the artery was opened to expose the optic nerve, which was then dissected with fine needles into small strands of fibers.[2] One strand containing a single active fiber was sucked into the glass tip (120-μm opening) of a suction electrode. The electrode

[2] The hepatic arteries carry blood toward the brain. Cutting either one of them does not interfere with the blood supply to the lateral eyes and does not reduce significantly the supply to the sinus surrounding the brain.

and the recording chamber were filled with ASW, and in most experiments the ASW contained 100 U/ml of penicillin and 100 μg/ml of streptomycin. The antibiotics slow down the deterioration of the cut optic nerve, making it possible to record from the same fiber for more than 100 h, about five times longer than is possible without antibiotics.

Recording and Stimulation

Nerve impulses were amplified and displayed on an oscilloscope. A level discriminator converted the amplified impulses from the CRT into uniform voltage pulses that were fed to a Linc-8 computer (Kletsky, 1971). The nerve impulses were also monitored by a loudspeaker and recorded on magnetic tape for further analysis.

Light was "piped" into the recorded ommatidium by a single glass fiber (diameter 76 μm, American Optical Corp., Buffalo, N. Y.; for details see Barlow, 1969). Once aligned on the optical axis of the ommatidium, the light pipe was glued to the cornea with a transparent, nontoxic adhesive (Eastman 910, Eastman Kodak Co., Rochester, N. Y.). This technique eliminated movement of the light pipe with respect to the ommatidium under study, and minimized light scatter into neighboring ommatidia. Typically, the intensity of the light falling on an adjacent unit was less than 10^{-5} of that illuminating the test ommatidium through the light pipe. The light source was a tungsten filament focused on one end of the light pipe with a × 45 microscope objective. The output of the light pipe as measured with a calibrated photodiode (PIN 10D, United Detector Technology Inc., Santa Monica, Calif.) was 10^{12} photos/s between 400 and 700 nm at the arbitrary zero setting of the optical system. This setting is indicated in the figures of this paper by $\log I = 0$. The light intensity was set by neutral density filters and circular wedges (Kodak) in the beam. The wedge position could be controlled either by hand or by the Linc-8 computer through a stepping motor.

Computer control of the light intensity enabled us to automate a number of the experiments, many of which ran 100 h or more. The computer also performed preliminary data analyses on line.

RESULTS

We measured the increment threshold function, the effects of light and dark adaptation, the spectral sensitivity, and the variability of the impulse discharge for single ommatidia in the *Limulus* lateral eye *in situ*. The results are considered in light of the hypothesis that two receptor mechanisms function in this eye.

Increment Threshold

TRANSIENT RESPONSE Single ommatidia were illuminated with 10-s flashes. At the fifth second, a 50-ms test flash was added to the background flash. The stimuli were delivered to the unit via a single light pipe. No neighboring receptors were illuminated. Trains of impulses from the test unit were collected and averaged by the computer for each background intensity. We adjusted the intensity of the test flash until a response increment was de-

tected in the averaged data. The upper curve in Fig. 2 gives the increment thresholds (test flash intensities, ΔI) for the transient response of a single ommatidium over an 11-log unit range of background intensities (I). The threshold function is fairly smooth with no strong indication that two receptor

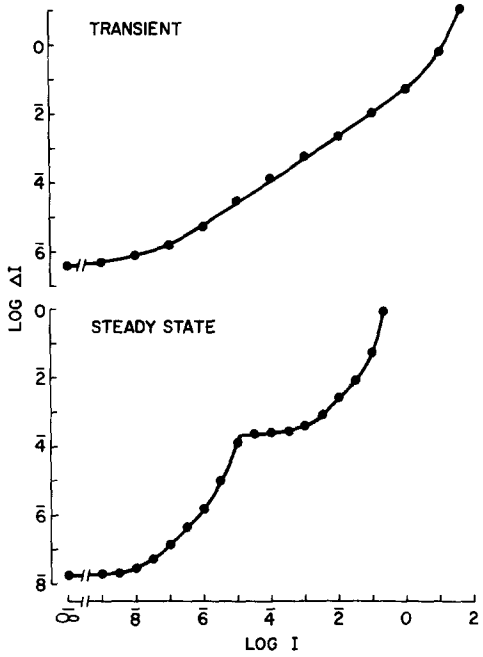

FIGURE 2. Increment threshold functions for the transient and steady-state responses of a single ommatidium in the lateral eye *in situ*. The upper curve gives the log of the intensity of a 50-ms flash (ΔI) that elicited a constant increment in firing rate of the response to the background (I). The responses were averaged by generating PST histograms containing a fixed number of impulses. The background intensities covered a range of nearly 11 log units with a quantal flux of about 10^{12} photons/s at log $I = 0$ (same calibration as in Fig. 1). The linear portion of the curve does not follow Weber's law: the slope is 0.7 rather than 1.0. The lower curve was computed directly from the steady-state curve in Fig. 1 as described in the text. Each point on the curve gives the log of the intensity increment (ΔI) that corresponds to an increase of 2 impulses/s in the steady-state response rate. The two linear portions of the curve obey Weber's law with slopes of 1.0.

mechanisms underlie the incremental responses. This result is not unexpected, since the intensity function in Fig. 1 for the transient response gives no indication of two mechanisms.

STEADY-STATE RESPONSE The existence of two receptor mechanisms is suggested by the shape of the intensity function for steady-state responses (Fig. 1). Is this also true for the shape of the increment threshold function for steady-state responses? All of the necessary information for answering

this question is contained in the intensity function. We can simply choose a criterion for the incremental response and read the value of the increment threshold (ΔI) from the intensity function for any background level (I). In the lower part of Fig. 2, we show the ΔI values read from the steady-state function in Fig. 1 for a criterion response of 2 impulses/s.

The shape of the $\Delta I/I$ function for steady-state responses in Fig. 2 is characteristic of dark-adapted ommatidia in unexcised *Limulus* eyes. It is also characteristic of rat ERG recordings (Green, 1971) and of human psychophysical measurements (Aguilar and Stiles, 1954). In this regard the *Limulus* eye behaves like visual systems that contain two receptor types.

Since the $\Delta I/I$ function is produced directly from the intensity function, the corresponding characteristics between the two curves are worth noting. First, a sigmoid-shaped function on log-log coordinates produces a characteristic $\Delta I/I$ function containing a quasilinear segment with positive slope. The function approaches asymptotically the abscissa on the left and the ordinate on the right. Two sigmoid functions cascaded as in Fig. 1 produce two characteristic $\Delta I/I$ functions which meet at a "knee." A linear segment in the intensity function (on log-linear coordinates) produces a linear segment of slope 1.0 in the $\Delta I/I$ function.

Light Adaptation

Can the existence of two receptor mechanisms be demonstrated by light adaptation? To answer this question we adapted a single ommatidium to an intermediate intensity of $\log I = -4$ for several minutes, and then changed the intensity to a different level for 10 s. The steady-state discharge was measured during the last 5 s of the 10-s exposure. After the 10-s test the incident intensity was reset to the adapting level of $\log I = -4$. This procedure was repeated for a range of test intensities.

Fig. 3 gives the light-adapted (open circles) and dark-adapted (filled circles) response rates as a function of intensity for one such experiment. Each light-adapted rate was measured after the unit's response to the adapting light had stabilized. The dark-adapted rates were determined after the unit had reached its most sensitive dark-adapted state.

In this experiment, light adaptation reduced the range of the intensity function from 10 to 5 log units with no substantial changes in the shape of the function at the higher intensities. These results are consistent with the view that each ommatidium contains two receptor mechanisms having different thresholds for excitation: one mechanism encodes the lower range of intensities ($\log I = -10$ to -5) and the other encodes the upper range ($\log I = -5$ to 0). Under such conditions, certain intensities could selectively light adapt the lower mechanism.

Note that the light-adapted intensity function of the intact eye (Fig. 3) is

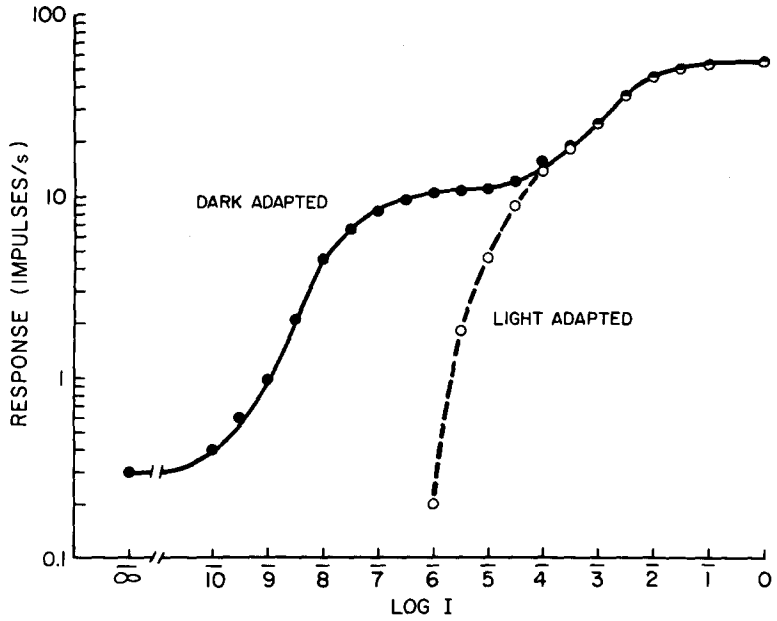

FIGURE 3. The effect of light adaptation on the intensity function of the steady-state response of a single ommatidium *in situ*. To generate the dark-adapted curve, the eye was allowed to fully dark adapt between test flashes. The light-adapted curve was obtained by presenting 10-s test flashes during brief interruptions in a steady, adapting light of $\log I = -4$.

similar to the dark-adapted function of the excised eye (Fig. 1). The reduction in sensitivity by light adaptation in this experiment is nearly equal to that caused by excision. It appears that light adaptation and excision desensitize one receptor mechanism more than the other.

Fig. 4 provides further evidence for two mechanisms. In this experiment we illuminated a dark-adapted ommatidium for 35-s periods and measured intensity functions of the response from 5 to 10 s and from 30 to 35 s.[3] Note that the response to some intensities declined after 10 s. However, the response to very low or intermediate intensities did not change after 10 s. The lowest intensities may weakly stimulate the "lower" mechanism and thus not adapt it. Intermediate intensities may completely adapt the "lower" mechanism but only weakly stimulate the "upper" one. The highest intensities would then adapt both mechanisms. The differences between the "5–10-s" and "30–35-s" intensity functions are shown in Fig. 4 by thin dashed curves.

[3] Earlier we defined the response from 5 to 10 s as the "steady-state" response. This definition of steady state is in part a matter of convenience. The initial transient response is complete by 3 s and the response rate is nearly constant from 5 to 10 s.

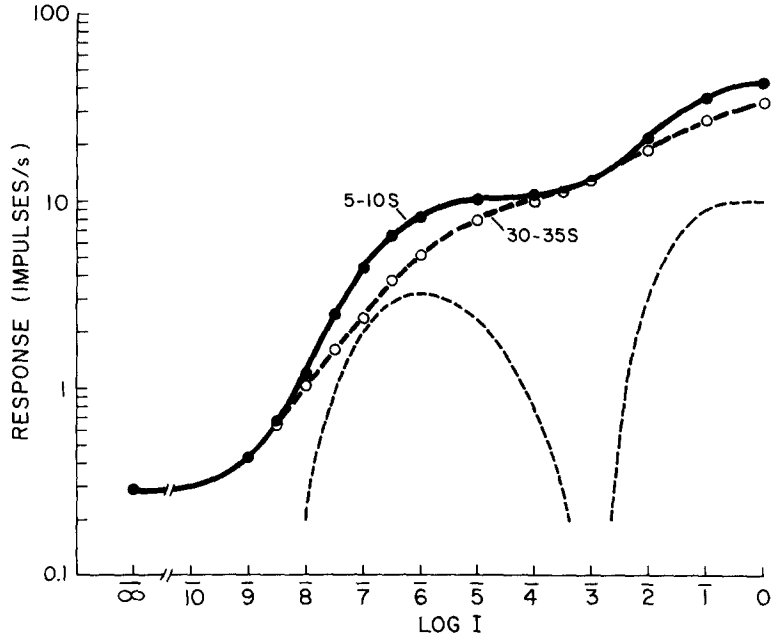

FIGURE 4. Intensity functions of an ommatidium at two different times during a 40-s flash. The filled circles are the mean firing rates from 5–10 s and the empty circles are the mean rates between 30–35 s. The eye was fully dark adapted before each test flash. The thin dashed curves give the response differences between the 5–10-s and 30–35-s intensity functions.

Dark Adaptation

To test whether the process of dark adaptation reveals the existence of two mechanisms we exposed single ommatidia to bright adapting flashes, and tracked the recovery of their transient and steady-state responses.

TRANSIENT RESPONSE The adapting flashes were 10–60 s in duration and delivered 10^{11} photons/s to a single ommatidium at the cornea. The recovery of sensitivity was tracked after each adapting flash. We define sensitivity as the inverse of the log of the relative light intensity of a 0.2-s flash sufficient to evoke a fixed number of impulses.

Fig. 5 shows the dark-adaptation curve for a single ommatidium. Note that after a few minutes in the dark, the threshold leveled off, and after about 10 min dropped to a new level. This secondary drop in threshold was not observed in all experiments. When found it usually coincided with the reappearance of spontaneous activity (indicated by the arrow marked SA). Since impulses that occur spontaneously cannot be distinguished from light-evoked responses, the data are difficult to interpret. Therefore, dark-adaptation curves like the one shown in Fig. 5 cannot be used to support the notion that

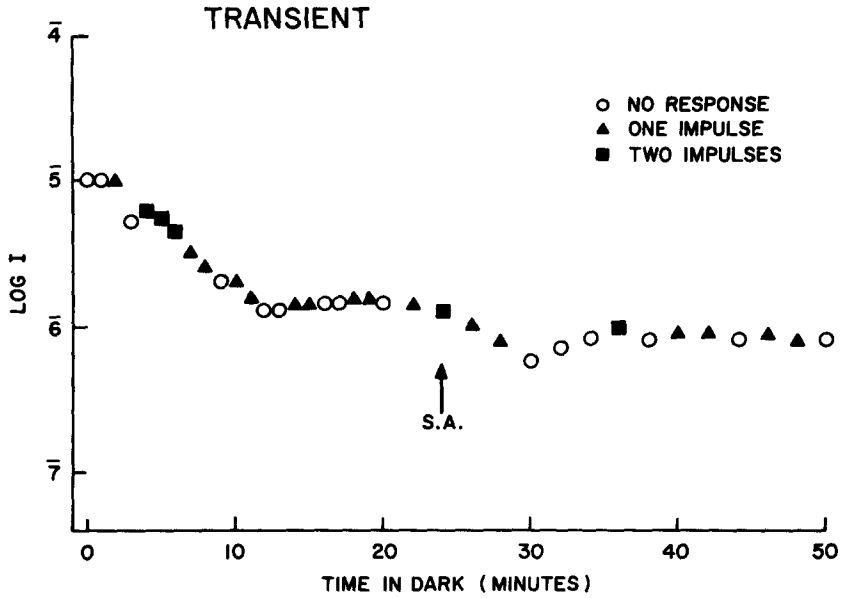

FIGURE 5. Dark adaptation of the transient response of a single ommatidium *in situ*. A 10-s adapting flash of log $I = -1$ immediately preceded the zero point on the abscissa. Test flashes (50 ms) were presented every 30 s for the first 15 min in the dark, and then every 60 s. Different testing rates did not seem to influence the result. The filled symbols represent responses. The empty circles indicate failures to elicit a response, and therefore thee estimate the lower bound for the threshold. The arrow marked "SA" indicates the reappearance of spontaneous activity in the discharge of the single optic nerve fiber.

an ommatidium contains two receptor mechanisms which recover their sensitivity at different rates.

STEADY-STATE RESPONSE A single ommatidium was adapted for 15 min to an intermediate intensity (10^8 photons/s at the cornea). At some point in time after the adapting light was turned off, a 10-s test flash was presented and the mean firing rate during the last 5 s of the test flash was measured. Only one test flash could be presented after each adapting flash since the intensity of the test flash was well over threshold and adapted the ommatidium. After each test, the unit was allowed to dark adapt completely, and the experiment was repeated for different time intervals between the adapting and test flashes (the extended recording time and the stability of the *in situ* preparation made these experiments possible). The test-flash intensities were dim (10^6 photons/s), intermediate ($10^{7.5}$ photons/s), and bright (10^{10} photons/s), corresponding to responses below, on, and above the plateau of the intensity function.

Fig. 6 gives the time-course of recovery of the steady-state response for the three test-flash intensities. Note that the responses to the bright test flashes recovered almost immediately, whereas those to the intermediate and dim

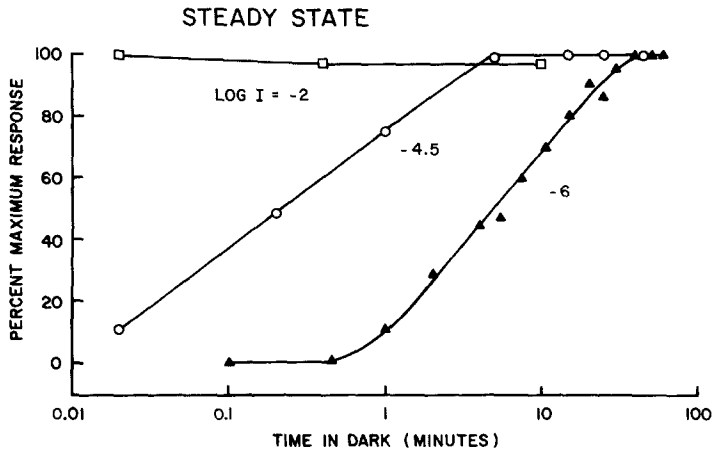

FIGURE 6. Dark adaptation of the steady-state response of a single ommatidium *in situ* after 15 min of illumination to log $I = -4$. The three curves give the normalized steady-state responses to 10-s test flashes at three different intensities (log $I = -2$, -4.5, and -6). Only one test flash was presented after each adapting period. The ommatidium was allowed to completely dark adapt before the next adapting flash was presented. Adapting lights brighter than log $I = -4$ gave similar results.

flashes recovered more slowly. With regard to the two-mechanism hypothesis, these results suggest that the less sensitive mechanism dark adapts rapidly and the more sensitive mechanism adapts slowly.

Spectral Sensitivity

The *Limulus* lateral eye is generally viewed as containing a single visual pigment with maximal sensitivity at 520 nm (Hubbard and Wald, 1960); however, other pigments may be involved (Graham and Hartline, 1935; Anderson et al., 1973). We considered the possibility that the two hypothesized mechanisms depend on separate visual pigments. To test this possibility, we measured first the response of a dark-adapted ommatidum to brief flashes whose wavelength was varied between 400 and 700 nm. Since light adaptation appears to reduce the sensitivity of just one of the mechanisms (Fig. 3), we light adapted the unit and repeated the measurements.

Fig. 7 shows the spectral sensitivity of a single ommatidium under light- and dark-adapted conditions. The light-adapted curve was shifted upwards by 1.5 log units for comparison with the other curves. Note that the spectral sensitivity is the same for both states of adaptation and is similar to that of units in the excised eye (Graham and Hartline, 1935). The results are also in excellent agreement with the density spectrum ($\lambda_{max} = 520$ nm) measured by Hubbard and Wald (1960) for visual pigment extracted from the lateral eye of *Limulus*.

To further test for the existence of more than one visual pigment, we mea-

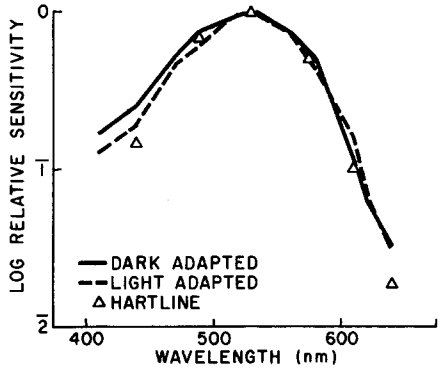

FIGURE 7. Spectral sensitivity of an ommatidium *in situ*. Dark-adapted results are given by the solid line and light-adapted data by the dashed line. The sensitivity is the inverse of the log relative light intensity of a brief test flash that elicited a constant response. The light-adapted curve was shifted upwards by 1.5 log units. The triangles are data of Graham and Hartline (1935) from the excised eye.

sured the steady-state intensity functions of a single dark-adapted ommatidium for white (450–700 nm), blue (470 nm), and red (640 nm) lights. The resulting functions shown in Fig. 8 have been shifted horizontally to compensate for the different sensitivities to the colored stimuli. Other wavelengths produced similar results. The shape of the intensity function is independent of the wavelength of incident light. Therefore, if two receptor mechanisms generate the steady-state response, both mechanisms utilize the same visual pigment.

Variability of the Impulse Discharge

In the preceding experiments the emphasis has been on finding a dual behavior, some measure that would characterize or identify two receptor mechanisms for encoding light intensity. The impulse discharge exhibits one type of dual behavior.

UNEXCISED EYE Fig. 9 shows segments of the discharge of a single dark-adapted ommatidium for a large range of light intensities. For each intensity the impulse discharge is plotted as a sequence of instantaneous firing rates (the inverse of the interspike intervals) for a 2-s period. In darkness and under dim illumination, the firing rate is irregular, consisting mainly of bursts of several impulses. As the intensity is increased, the bursts become more frequent and the number of impulses within each burst increases. At higher intensities a striking change occurs: the bursts disappear and the discharge becomes regular.

Fig. 10 presents histograms of the instantaneous firing rates in Fig. 9. Note that the histograms change systematically with increasing light intensity

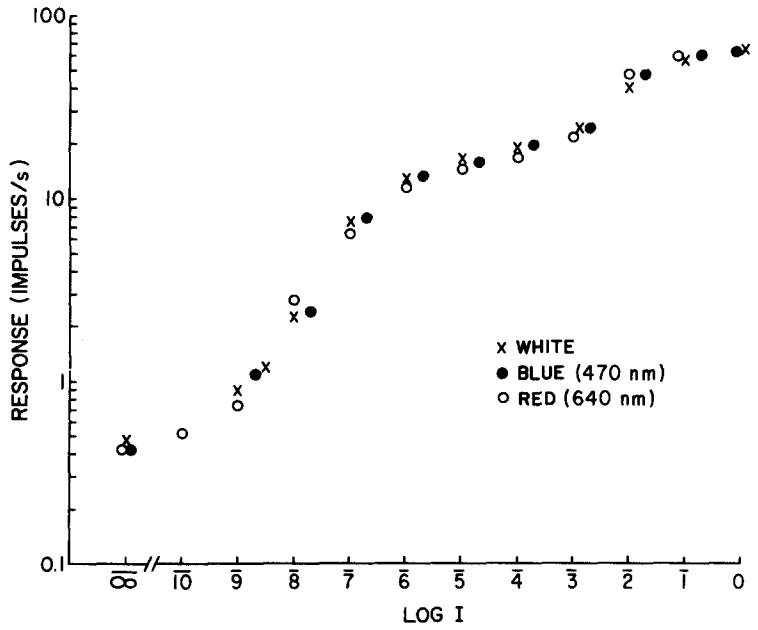

FIGURE 8. Wavelength independence of the steady-state intensity function. The three symbols represent responses of a single ommatidium to illumination by white, blue, and red lights. The responses are the mean discharge rates of an optic nerve fiber during the last 5 s of 10-s flashes. The curves are shifted on the log I axis to compensate for the different spectral sensitivities and to facilitate comparison among the curves.

(from bottom to top). Two peaks are detectable at low light levels and in darkness. The lower peak is due to the *inter*burst interval, and the upper peak represents the *intra*burst frequency. At intermediate intensities the frequency within the bursts increases and the bimodal characteristic of the impulse discharge becomes clearer. Further increases in intensity reduce the intervals between bursts, and the left mode becomes less pronounced. At high intensities the bursting disappears completely, and a single peak remains in the histogram.

Fig. 11 (upper half) gives the relative variability of the segments of the impulse discharge shown in Fig. 9. The steady-state response rates for each intensity are replotted from Fig. 1. Note that the plateau in the intensity function coincides with a sharp reduction in relative variability (Fig. 11), with a decrease in bursting in the impulse discharge (Fig. 9), and with a loss of the bimodal distribution in the histogram (Fig. 10). Apparently, the plateau is a transition zone between two types of firing patterns: regular and irregular. Such a dual behavior may be evidence for two receptors mechanisms.

EXCISED EYE In addition to reducing the sensitivity of the eye (Fig. 1), excision also affects the variability of the impulse discharge. The lower half

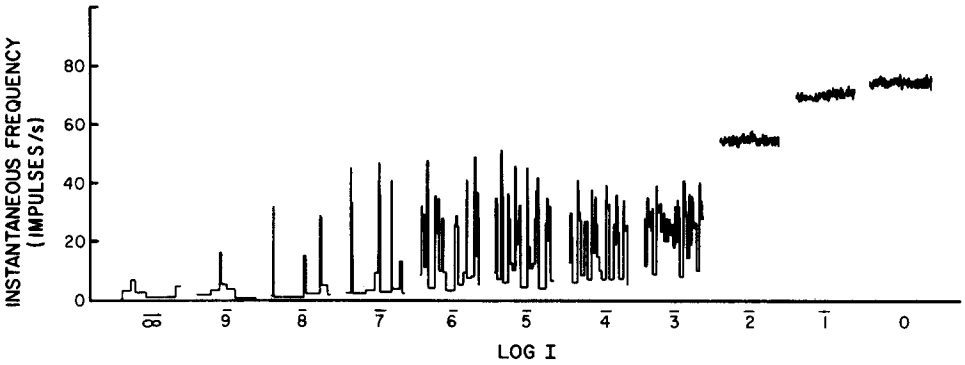

FIGURE 9. The effect of light intensity on the firing pattern of a single ommatidium *in situ*. Shown are 11 2-s segments of the steady-state instantaneous firing rate (inverse of interspike interval) in the dark and at the 10 intensities indicated on the abscissa. Each response was elicited only after the unit was fully dark adapted. The average firing rate for this unit is plotted for each test intensity in Fig. 1 (*in situ* curve on right). Compare with Fig. 10.

of Fig. 11 gives the relative variability and the mean firing rate of the response from a single optic nerve fiber of an excised eye, the same eye that yielded the *in situ* data in Figs. 1, 9, 10, and 11 (upper half). Note that in the excised eye the variability is smaller than in the intact eye. These results for the excised eye agree well with those of Shapley (1971). Regarding the two-mechanism hypothesis, our data suggest that excision reduces the functioning of the more sensitive mechanism without affecting the less sensitive one.

EFFECTS OF LIGHT ADAPTATION The state of adaptation of an ommatidium strongly influences its firing pattern. Fig. 12 plots for one ommatidium the instantaneous firing rates at three different states of adaptation: dark-adapted (left), mildly light adapted (middle), and strongly light adapted (right). These three records were selected for comparison because they have the same firing rate (10.2 impulses/s). The irregular firing pattern on the left is characteristic of the response from a dark-adapted ommatidium. The other two patterns show that light adaptation causes a more regular discharge.

The firing pattern may convey information about the state of adaptation of the ommatidium. The mean discharge rate and the discharge pattern together may carry information about the past and present levels of illumination of an ommatidium. This possibility has been suggested by Ratliff et al. (1969) for the firing patterns of single ommatidia in the excised eye of *Limulus*. We have found that in the intact eye the reduction in the variability of the impulse discharge due to light adaptation is more than 10 times greater than in the excised eye. Indeed, the spike train may be a dual code. This type of coding may not be limited to *Limulus*: it has been suggested for frog dimming fibers (Chung et al., 1970; Lurie, 1973) and for cat optic nerve fibers (San-

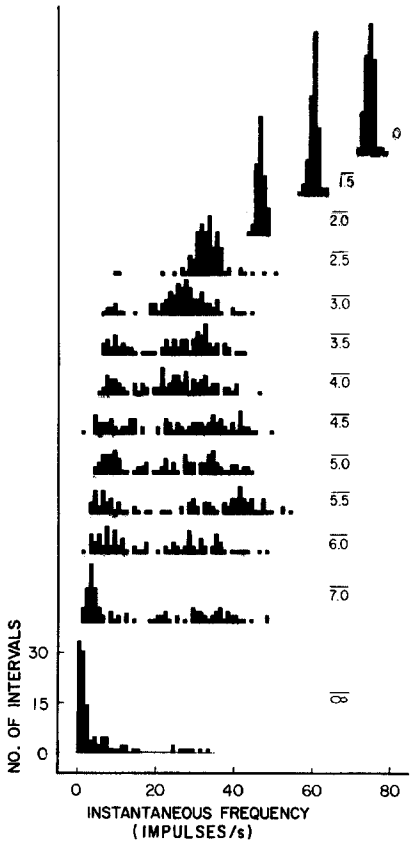

FIGURE 10. Histograms of the instantaneous firing rates shown in Fig. 9. Each histogram represents the distribution of the instantaneous firing rate (inverse of the interspike interval) at the light intensity indicated on the right. The symbol $\overline{\infty}$ indicates darkness. Not all of the data in Fig. 9 are included. Each histogram represents 100 impulses. At dim and intermediate intensities, the distributions are bimodal, indicating the bursting activity that characterizes the response at these light levels. At high intensities, the lower mode disappears, and only the high mode corresponding to the regular high firing rate remains.

derson et al., 1973). This phenomenon is consistent with the view that the mechanism responsible for the irregular discharge is vulnerable to bright adapting lights.

DISCUSSION

The following picture seems to emerge from our results. Two receptor mechanisms function in the eye *in situ*. Together the two mechanisms enable an ommatidium to operate over an intensity range of 10 log units. The properties of the two mechanisms, arbitrarily labeled A and B, can be summarized as follows (Table I).

We assume that the plateau in the intensity function for steady-state

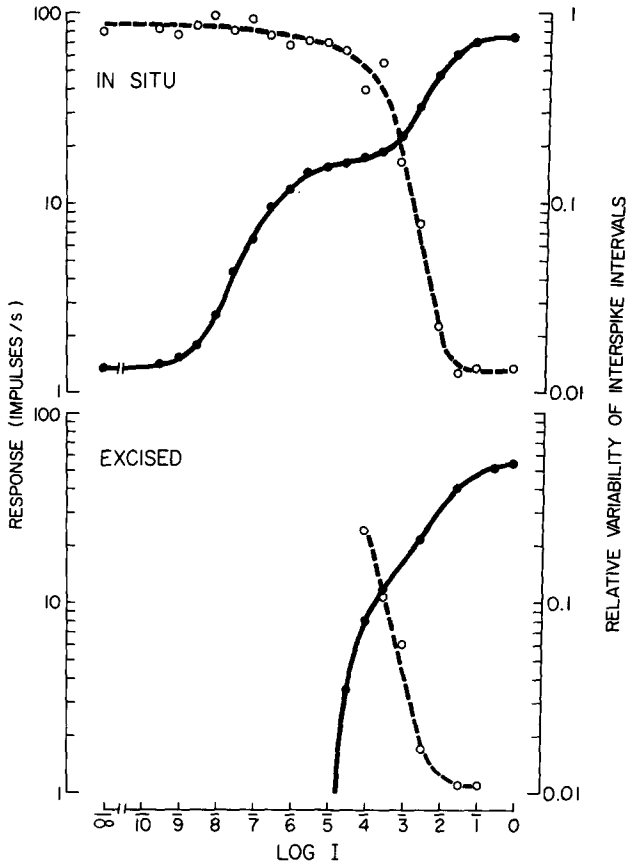

FIGURE 11. Relative variability of the firing pattern shown in Fig. 9. The filled circles and solid lines represent the steady-state responses for ommatidia in the *in situ* and excised eye (data are taken from Fig. 1). The empty circles and dashed lines give the relative variabilities of the interspike intervals (right ordinate), which is defined as the standard deviation divided by the mean of the intervals. Notice that the variability of the *in situ* data decreases sharply near the plateau region in the intensity function. Note also that the relative variability of the responses from the eye *in situ* exceeds that of the excised eye.

response (Fig. 1) is a transition region from mechanism A to B and represents the saturation level of the more sensitive mechanism. Thus the intensity function can be viewed as a composite of the functions of the two mechanisms. The two segments, above and below the plateau, have essentially the same shape. Zwislocki (1973) recently showed that an equation,[4] which describes the steady-state intensity functions of many sensory receptors, can fit each segment of the steady-state intensity function in Fig. 1. To describe the entire

[4] Zwislocki's equation has the form $R/R_m = (1 - e^{-F})\text{-}T$ where R is the receptor's firing rate, R_m is the response saturation level, and T is the firing threshold. The exponent F is a function of the intrinsic receptor noise, stimulus energy, spontaneous activity, and R_m.

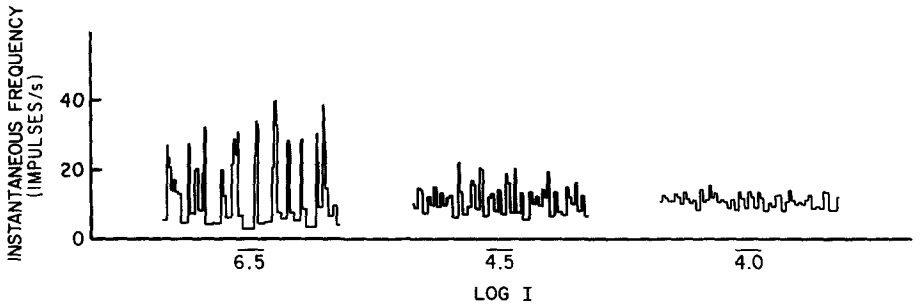

FIGURE 12. The effect of light adaptation on the firing pattern of a single ommatidium *in situ*. Shown are three 4-s segments of the instantaneous firing rate versus time for three intensities of illumination at different states of adaptation (increasing from left to right). The mean firing rate is the same for the three records, but the variability of the firing pattern decreases dramatically with light adaptation. Compare with Fig. 9.

TABLE I

PROPERTIES OF THE TWO HYPOTHESIZED RECEPTOR MECHANISMS

	A	B
Threshold (absorbed photons)	~1	10^5
Operating range	10^5 for both	
Shape of intensity function	Similar for both (see below)	
Spontaneous activity	Yes	No
Firing pattern	Irregular (bursting)	Regular
Dark adaptation	Slow	Rapid
Increment threshold (slope of linear segment)	1.0 for both	
Spectral sensitivity	Same for both	
Survival after eye excision	Short	Long

function, Zwislocki's equation has to be applied to each segment separately with only minor changes in the parameters. This analysis strengthens the notion that the entire function is composed of two segments having similar shapes.

In the following sections of the Discussion we shall review other evidence for the existence of two receptor mechanisms in the *Limulus* lateral eye, and examine some suggestions for their identification. In addition, we shall consider alternative hypotheses that do not assume a dual receptor function.

Other Evidence for Two Receptor Mechanisms

The idea that more than one receptor mechanism functions in the *Limulus* eye is not new. Wulff (1950) found two components in the ERG of excised *Limulus* eyes, and proposed that the more sensitive component originates in the eccentric cell of the ommatidium and the less sensitive component comes from retinular cells. Lipetz (1958) suggested that the eccentric cell contained two

regions with different sensitivities to current flow: the cell body and the axon hillock. Adolph (1964) detected two types of quantum potentials: slow rise and fast rise. Two classes of the potential fluctuations were also apparent in Dowling's recordings (1968) from excised eyes of small *Limuli*. Fuortes and O'Bryan (1972) proposed that the intracellularly recorded smooth receptor potential and the large quantum bumps represent two separate components, perhaps of separate origins. Yeandle (1967) suggested that the transient component of the receptor potential originated in a different part of the retinular cell than either the initial "spike" or the steady component. This idea was extended by Wulff and Mueller (1973) who proposed that the transient component was produced by the nonrhabdomeric part of the retinular cell, and the initial spike and perhaps the steady component were contributed by the rhabdomeric part. The results of these studies may eventually prove helpful in identifying the two mechanisms characterized in this paper.

ECCENTRIC AND RETINULAR CELLS The retinular cells are known to be photosensitive (Fuortes, 1959). Is the eccentric cell photosensitive? The evidence from excised eyes indicates that it is not (Waterman and Wiersma, 1954; Borsellino et al., 1965). However, the eccentric cell dendrite is partially covered with microvilli that are presumed to contain photopigment (Lasansky, 1967; Fahrenbach, 1969). Thus the photosensitivity of the eccentric cell in the eye *in situ* remains a possibility.

Although hypoxia can abolish the light response of *Limulus* ommatidia (Baumann and Mauro, 1973), we do not know if it affects differentially certain regions within the cells of the ommatidium. Such information may allow us to identify elements that are responsible for the dual properties of ommatidia in the eye *in situ*.

Intact vs. Excised Eyes

All the experiments cited in the discussion above were performed on excised *Limulus* eyes. Since our results show that excision reduces substantially the dual response characteristics of ommatidia, it would appear that the excised-eye results have little bearing on the intact eye. However, excision might not completely abolish the dual properties of ommatidia. The shoulder on the intensity characteristic in Fig. 11 (lower half) suggests that at least part of the more sensitive mechanism functions in the excised eye. We know that the effects of excision are not instantaneous (Barlow and Kaplan, 1971). The sensitivity of an ommatidium declines slowly if the eye is excised under dim illumination and exposures to bright lights are avoided after excision. Such conditions did not generally exist in the experiments cited above.

Dowling's experiment may be an exception. The eyes of small *Limuli* used by Dowling may be more resistant to the effects of excision than the eyes of adult animals. This could explain the appearance in Dowling's recording

of large potential fluctuations (LPF's), the same type of potentials that we have recorded from cells in the unexcised eye (Kaplan et al., 1973). We agree with Dowling that the LPF's may enable the eye to signal the absorption of small numbers of photons, and we feel that the LPF's may be the basis of the more sensitive receptor mechanism.

The data presented thus far seem to indicate that (*a*) two receptor mechanisms function in the eye *in situ*, (*b*) one mechanism and a trace of the other generally survive after the eye is excised, (*c*) both mechanisms function in the excised eyes of small *Limuli*, and (*d*) under optimal conditions both mechanisms function for a short time in the excised eyes of adult animals.

Light Adaptation

Light adaptation of ommatidia in the eye *in situ* did not produce the expected result: it did not shift the entire intensity function to the right on the log I axis (Fig. 3). Parallel shifts of the intensity function caused by light adaptation have been reported for ommatidia in the excised eye of *Limulus* (Hartline and McDonald, 1947), for crayfish ommatidia (Glantz, 1968), for skate rods (Dowling and Ripps, 1972), and for *Necturus* cones (Normann and Werblin, 1974). A nonparallel shift of the type shown in Fig. 3 is not readily explainable from our present notions of light adaptation. We suggest that light adaptation with log $I = -4$ may have entirely adapted the lower mechanism without significantly affecting the upper mechanism. Thus the results of light adaptation may be evidence for the functioning of two mechanisms.

We note that adapting lights more intense than log $I = -4$ shifted the "light-adapted" function in Fig. 3 parallel to the right. These results are identical to those obtained by Hartline and McDonald (1947); however, we have not included them in Fig. 3. A complete description of a light-adaptation experiment for a large range of intensities is beyond the scope of this report and will be presented in a following paper.

A Possible Role for Calcium

Calcium ions have recently been implicated in the process of light adaptation in the ventral photoreceptor of *Limulus* (Lisman and Brown, 1972). Light increases the intracellular concentration of free Ca^{++} (Brown and Blinks, 1972) which adapts the cell. This observation may help explain the similarity between the light-adapted lateral eye *in situ* and the excised eye, if we assume that the results from the ventral photoreceptor apply to the lateral eye. Excision of the lateral eye may impair its ability to bind, pump out, or otherwise decrease the concentration of intracellular free Ca^{++}. Consequently, the cells would become partially loaded with free Ca^{++} and thus appear light adapted. Specifically, a cell in an excised eye would have an

elevated threshold and a restricted operating range (Fig. 1) just like light-adapted cells in the eye *in situ* (Fig. 3). We note that ommatidia in excised eyes dark adapt (Hartline and McDonald, 1947). In terms of the calcium hypothesis, excised eyes may retain some capacity to reduce the concentration of intracellular free Ca^{++}.

Alternative Hypotheses

Assuming two mechanisms at the receptor level is parsimonious and attractive, but alternative hypotheses should be considered. They are directed primarily toward explaining the shape of the intensity function (Fig. 1) without resorting to a duality of receptor mechanisms.

INHIBITION Ommatidia neighboring the test unit discharge impulses in darkness. This activity may inhibit the response of the test unit. The sensitivity of an ommatidium to lateral inhibition increases in proportion to its own firing rate, and in many ommatidia reaches a maximum at intermediate firing rates and declines at higher rates (Barlow and Lange, 1974). Thus any inhibition caused by the spontaneous activity of neighboring ommatidia would increase as the intensity on the test ommatidium increases and might be sufficient to produce the plateau in Fig. 1. A further increase of intensity may reduce the sensitivity of the test ommatidium to lateral inhibition, or may cause the test unit to disinhibit itself by inhibiting its neighbors, or both. This could increase the firing rate of the test unit and thus cause the rise in the intensity function in Fig. 1 after the plateau region.

This explanation failed to withstand several experiments in which we light adapted ommatidia neighboring the test unit, thus eliminating their spontaneous activity and reducing their sensitivity to scattered light. In addition, calculations show that the amount of inhibition attributable to spontaneous activity and to responses to scattered light is not sufficient to account for the plateau region.

PIGMENT MIGRATION Screening pigment migrates in the *Limulus* eye after light adaptation, forming a restricting iris between the spokes of the rhabdom (Miller, 1958) and in the distal region of the ommatidium, near the cone cell layer (Behrens, 1974). If we assume that the pigment in *Limulus* begins to move at intensities just before the plateau region, that it moves as rapidly as it does in some other compound eyes (Bernard, 1973), and that it attenuates light by approximately 2 log units, then we would expect an intensity function like the one in Fig. 1. Although the time-course of the pigment migration is not known, it seems unlikely that enough pigment could move within 2 s to account for the plateau in the steady-state intensity function. In addition, a pigment "filter" should not affect the firing pattern, but Figs. 9–11 show that the pattern changes dramatically once the light intensity exceeds a certain level.

PHOTOMECHANICAL CHANGES Behrens (1974) has recently reported that pronounced anatomical changes take place in the ommatidium when the *in situ* eye is light adapted. Specifically, she found that in the dark-adapted unexcised eye the eccentric cell dendrite and the nearby rhabdom structure of the retinular cells migrate distally toward the base of the corneal lens. The convoluted appearance of the dendrite and rhabdom creates the impression that these structures have been pushed up against the cone cell region at the base of the lens. Light adaptation moves the dendrite and rhabdom proximally, away from the lens, decreases the width of the dendrite, reduces the thickness of the rhabdomeres, and straightens these structures. This light-adapted state of the ommatidium has been the standard picture of the anatomy of the ommatidium (Ratliff et al., 1963; Fahrenbach, 1969). The movement of the photosensitive regions within the ommatidium may change the sensitivity to incident illumination and may contribute to the dual properties of ommatidia in the eye *in situ*. However, we need to know more about the time-course and the physiological effects of these photomechanical changes before we can link them to the findings reported here.

ADAPTATION OF QUANTAL RESPONSES Dodge et al. (1968) have proposed that the major mechanism of light adaptation in the *Limulus* eye is a reduction in the size of the quantal responses. They showed that the quantal bumps summate to produce the generator potential. The production of the bumps is a shot process (Yeandle, 1957; Adolph, 1964). The generator potential is then the mean of a shot process, which is the product of bump amplitude, rate, and duration (Rice, 1954). A decrease in bump amplitude will thus reduce the generator potential. This analysis of adaptation is based on data from excised *Limulus* eyes. Can it be extended to the eye *in situ* which responds to intensities below the threshold of the excised eye?

If adaptation in the *Limulus* lateral eye is localized as it is in some invertebrate photoreceptors (Hagins et al., 1962; Fein, 1973), then at low light levels the bump amplitude should not depend on light intensity. Combining this idea with the adapting bump concept, we hypothesize that the average amplitude of the quantal responses will decrease with light intensity as indicated by the dashed line in Fig. 13. According to this view, dim lights have almost no effect on the bump amplitude. Moderate intensities have a strong effect and high intensities have a somewhat weaker effect. Based on the data of Dodge et al. (1968), we assume that bump rate is proportional to light intensity over a large range of intensities and that bump duration varies only slightly. The product of amplitude, rate, and duration will then give the intensity function for the generator potential (filled circles in Fig. 13).

The predicted intensity function for the generator potential in Fig. 13 has the same general shape as the steady-state function of the optic nerve discharge in Fig. 1. It may be possible, therefore, to ascribe that shape not to a

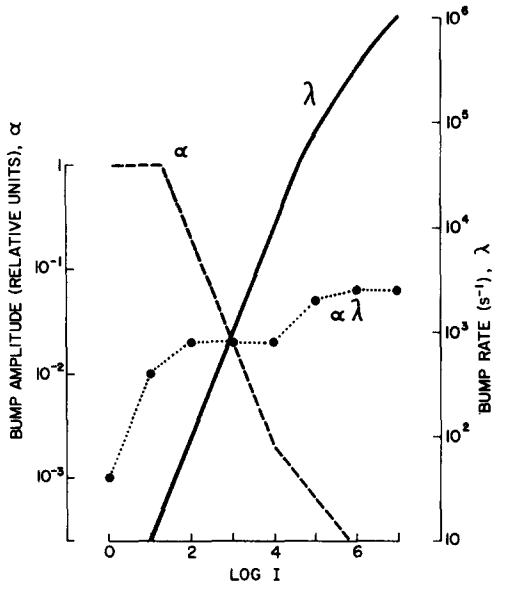

FIGURE 13. Extension of the adapting bump model to the eye *in situ*. The solid line is the rate of production of quantal responses (bump rate, λ), the dashed line is the bump amplitude (α), and the filled circles are the products of α and λ for integer values of log *I*. The filled circles connected by a dotted line give the predicted intensity function of the generator potential. Note that the predicted function has the same general shape as that for the optic nerve discharge shown in Fig. 1.

duality of receptor mechanisms, but rather to a nonlinear adaptation of the size of the quantal responses. This possibility can be checked experimentally. We should note, however, that this model predicts that, as light intensity is increased, the variance of the response will decrease more smoothly than it actually does (Figs. 9, 10) and will do so at lower light intensities.

This model for adaptation in the eye *in situ* explains the shape of the intensity characteristic by assuming one type of quantal bump, whose amplitude is adjusted according to the light level. The model is similar to the one proposed by Lisman (1971) for the ventral photoreceptor of *Limulus*. Lisman assumes two classes of bumps: large bumps originating from dark-adapted regions of the receptor and smaller bumps from light-adapted regions. The relative proportions of the two classes vary as light intensity is increased, producing an intensity function that is somewhat similar to the one in Fig. 1. A comparable characteristic for receptor current was, in fact, measured by Lisman and Brown (Lisman, 1971).

The alternative hypotheses suggested above to explain the response characteristics of ommatidia in the *in situ* eye concern just one aspect of the response: the plateau in the steady-state intensity function. They do not readily account for the effects of light adaptation or for the changes in the variability of the impulse discharge with light intensity.

CONCLUSIONS

A series of experiments, investigating the excitatory properties of the *Limulus* eye *in situ*, examined at the same time the possibility that single ommatidia in the eye use two mechanisms for light reception. The data do not allow us to conclude positively that two mechanisms do, indeed, exist. Some experiments (light adaptation, increment threshold, variability analysis) supported the hypothesis; others (dark adaptation, spectral sensitivity) were open to various interpretations. Alternative hypotheses could conceivably account for some of the results.

To gain further insight into the processes underlying the receptor properties described in this paper, we recorded intracellularly from visual cells in the lateral eye *in situ*. These experiments will be described in a following paper.

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