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

Intracellular Recordings

ROBERT B. BARLOW, JR. and EHUD KAPLAN

From the Institute for Sensory Research, Syracuse University, Syracuse, New York 13210, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543. Dr. Kaplan's present address is The Rockefeller University, New York 10021.

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ABSTRACT Two types of potential fluctuations, large and small, recorded intracellularly from photoreceptors in the dark-adapted *Limulus* eye *in situ* underlie the dual properties of the impulse discharge of the optic nerve fibers. The small potential fluctuations (SPFs)—the well-known quantum bumps—were normally less than 20 mV in amplitude. The large potential fluctuations (LPFs) were up to 80 mV in amplitude. LPFs appear to be regenerative events triggered by SPFs that enable single photon absorptions in retinular cells to fire off nerve impulses in the eccentric cell. In the dark, SPFs and LPFs occur spontaneously. At low light intensities, LPFs are the major components of the receptor potential. At high intensities, LPFs are suppressed and SPFs become the major components. SPFs and LPFs together enable single photoreceptor cells to encode approximately a 9-log unit range of light intensity. Excising the eye from the animal or cutting off its blood supply generally abolishes LPFs and thereby reduces the range of light intensity coded in the optic nerve discharge.

INTRODUCTION

Two receptor mechanisms appear to underlie the wide-range intensity coding in optic nerve fibers of *Limulus in situ* (Barlow and Kaplan, 1971). One mechanism functions at low light intensities (threshold $\simeq 1$ absorbed photon) and the other functions at high intensities (threshold $\simeq 10^5$ absorbed photons.) In a preceding paper we showed that the two-mechanism hypothesis is supported by several properties of the discharge of single optic nerve fibers recorded *in situ* (Kaplan and Barlow, 1975).

In this paper we present results of intracellular recordings *in situ* which indicate that the dual properties of the optic nerve responses originate in the primary photoreceptor, the retinular cell. The receptor potential of the retinular cell is comprised of two components: small potential fluctuations, SPFs ($\leq$20 mV), and large potential fluctuation, LPFs ($\leq$80 mV). The SPFs[1] resemble the

[1] Adolph (1964) used the notation SPF for the slow potential fluctuations he recorded from retinular cells in excised lateral eyes. Here we apply the notation to all potential fluctuations of less than 20

well-known quantum bumps recorded from excised eyes (Yeandle, 1975; Fuortes and Yeandle, 1964; Adolph, 1964; for review see Wolbarsht and Yeandle, 1967). Fuortes and Yeandle (1964) demonstrated that quantum bumps can be elicited by single photons. Dodge et al. (1968) showed that quantum bumps summate to produce the generator potential and that a reduction in the size of the bumps by ambient illumination is the major mechanism of light adaptation. The LPFs we record from lateral eyes *in situ* appear similar to the potentials Dowling (1968) recorded under optimal conditions from excised eyes of small *Limuli*.

Even though an SPF (quantum bump) amplifies appreciably a single photon absorption in a retinular cell, it alone cannot trigger a nerve impulse in the next order cell, the eccentric cell. To do so, either it must summate with other SPFs or the retinular cell must fire off an LPF. Together, SPFs and LPFs lead to wide-range intensity coding in single optic nerve fibers *in situ*. The properties of SPFs and LPFs account for most of the characteristics of the optic nerve responses.

MATERIALS AND METHODS

Experiments were carried out at the Marine Biological Laboratory, Woods Hole, Mass., where freshly caught horseshoe crabs were available. The technique we developed for recording transmembrane potentials from visual cells of the lateral eye *in situ* is similar in some respects to that for recording optic nerve activity (Kaplan and Barlow, 1975).

The animal was securely attached to a rigid platform in a small tank that was filled with seawater to the level of the lateral eyes. During the experiment the tank was placed in a light-proof, shielded cage and flushed continuously with fresh seawater from Great Harbor, Woods Hole (20°–22°C). In these experiments, as in the optic nerve experiments (Kaplan and Barlow, 1975), no anesthesia was used. The animal was therefore free to move its legs and exert pressure on the platform and carapace. Such movements often terminated successful microelectrode penetrations. To minimize the effects of animal movement we sandwiched the crab between the mounting platform and a rigid Lucite plate which pressed against the dorsal surface of the carapace. The micropipette manipulator was then attached to the Lucite plate.

To gain access to ommatidia, we removed a triangular-shaped section of cornea (0.6 mm base and 1.0 mm height) from the dorsal edge of the lateral eye. The section was kept as small as possible to minimize bleeding and reduce tissue pulsations caused by heart beat. A small stream of seawater flowed over the wound to promote blood coagulation (Levin and Bang, 1964) and moisten the exposed retinal tissue. A glass micropipette filled with 3 M KCl (20–70 MΩ) was lowered along a dorsoventral line through the opening in the cornea and advanced toward the rows of ommatidia located ventral to the cut. No attempt was made to record from ommatidia in the area exposed by the cut. Most cells were impaled either by gently tapping the micromanipulator or by causing the amplifier to oscillate with the capacitance neutralization circuit.

With this technique, stable transmembrane potentials were recorded from both retinular and eccentric cells *in situ* for periods of typically about 3 h with some recordings lasting up to 11 h. The cell potentials were amplified with a high impedance DC bridge amplifier (Electronics Laboratory, The Rockefeller University) and recorded on tape (model 3960,

mV, which includes all those recorded by Adolph. In view of the fact that retinular cells *in situ* generate potential fluctuations much larger than 20 mV, we shall refer to SPFs as "small potential fluctuations." The relationship between results recorded from cells in excised and unexcised eyes is discussed later in this paper.

Hewlett-Packard Co., Palo Alto, Calif.). The results were also displayed on a storage oscilloscope and on a pen recorder (model 220, Gould Brush, Gould, Inc., Instrument Systems Div., Cleveland, Ohio).

Light stimuli were delivered to the impaled visual cells via a single glass fiber (Barlow, 1969; Kaplan and Barlow, 1975). The output of the light pipe as measured with a calibrated photodiode (PIN 10D, United Detector Technology Inc., Santa Monica, Calif.) was 10^{12} photons/s between 400 and 700 nm at the arbitrary zero setting of the optical system (indicated by log I = 0 in the figures of this paper). The light pipe was aligned along the optic axis of the ommatidium under study and adjusted until it was in contact with the cornea directly in front of the ommatidium. During alignment the eye was illuminated with IR light and viewed through an image converter. Impaled cells were allowed to dark adapt for at least 30 min before testing.

RESULTS

Fluctuations in Membrane Potential

RETINULAR CELLS Fig. 1 shows a typical intracellular recording from a retinular cell in a dark-adapted lateral eye *in situ*. The cell elicited spontaneous fluctuations in membrane potential in the dark. Two types of potential fluctuations were distinguishable on the basis of amplitude: small potential fluctuations (SPFs) of less than 10 mV, and large potential fluctuations (LPFs) of about 30 mV. Recordings from other retinular cells *in situ* show that LPFs can reach 80 mV in amplitude whereas SPFs are normally less than 20 mV. SPF and LPF amplitudes were maximal for cells having large resting potentials (~80 mV) and high membrane resistances (50 MΩ or more). Typically, retinular cells *in situ* had resting potentials between −45 and −65 mV and membrane resistances from 10 to 30 MΩ. Variations in the values of these properties and in the amplitudes of SPFs and LPFs were observed within a single ommatidium and occasionally within a single retinular cell.

Small shifts in the position of the electrode tip within a retinular cell occasionally changed the amplitude of LPFs and SPFs without changing the cell's resting potential. Maximum amplitudes appeared to be restricted to a cellular region of high membrane resistance. Such a region may be the rhabdom as suggested by Dowling (1968). When conditions were optimal for recording LPFs and SPFs of maximal amplitude, the record contained no trace of nerve impulses fired by the eccentric cell (Fig. 1). It appears that rectifying junctions couple the retinular cells to the eccentric cell, a result Smith and Baumann (1969) found for excised eyes.

The amplitude histogram in Fig. 2 shows clearly the two types of potential fluctuations recorded from the retinular cell of Fig. 1. The mode at 30 mV represents LPFs and that below 10 mV is SPFs. Note that the SPFs outnumber the LPFs by about three to one. This was not always the case: LPFs often dominated the recordings. In every instance, however, the amplitude histogram was bimodal.

LPFs are often preceded by slow depolarizing potentials. The membrane potential of the LPF on the left in Fig. 1 depolarized slowly to about −50 mV and then rapidly to −25 mV. A prepotential is less apparent for the LPF on the right, nevertheless a slight notch is visible on the rising phase at about −50 mV. In both

cases the amplitude of the notch on the leading edge of the prepotential corresponds approximately to the peak of the distribution of SPF amplitudes in Fig. 2. These and other similar results suggest two possibilities: (*a*) SPFs trigger LPFs; or (*b*) SPFs and LPFs occur independently of one another.

The data in Fig. 3 support the notion that SPFs trigger LPFs. The retinular cell responses in this figure were elicited by dim, brief flashes. 7 of the 10 flashes elicited responses, of which six were SPFs. Two SPFs were followed closely by LPFs. In both cases the LPF fired on the repolarizing phase of the SPF, not on the peak. Several of the larger SPFs were not followed by LPFs. If SPFs trigger LPFs, then these data indicate that the triggering threshold fluctuates.

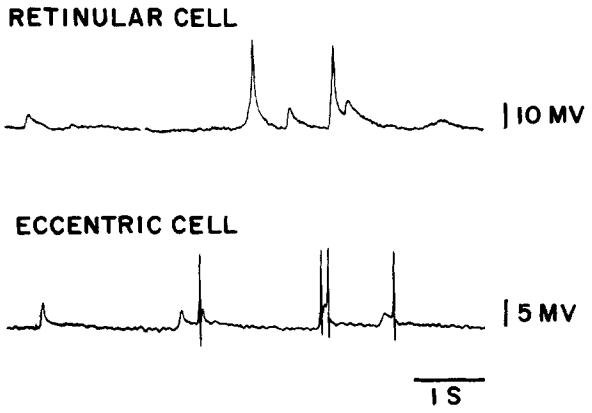

FIGURE 1. Intracellular recordings from dark-adapted cells *in situ* in the dark. The retinular cell spontaneously generated two types of potential fluctuations: quantum bumps (SPFs) and regenerative-like potentials (LPFs). Small depolarizing potentials in the eccentric cell often triggered one or more impulses in the optic nerve fiber. Amplitude of nerve impulses was attenuated 50% (to ~17 mV) by the bandpass characteristic of the chart recorder. All other potentials in both cells are faithfully reproduced. Recordings were taken from different eyes.

The LPF appears to be a regenerative event since it can be triggered as an all-or-none potential by small depolarizing currents delivered to the recording micropipette (data not shown). In no case did we find the peak amplitude of an LPF to overshoot zero membrane potential. Also, we found no evidence indicating that LPFs propagate as regenerative events within the body of the retinular cell or down its axon.

The LPFs in Fig. 3 are among the largest we recorded from retinular cells *in situ*. Each LPF reached a peak amplitude of 80 mV. The peak of the depolarizing potential was followed first by a rapid phase of repolarization of about 8 mV and then a slow phase of repolarization. Such wave forms are characteristic of regenerative potentials in other tissue (Fozzard and Gibbons, 1973).

The effects of light adaptation further suggest that LPFs are regenerative events. Fig. 4 shows the response and partial recovery of a retinular cell to an intense 10-s adapting flash. The dark-adapted cell spontaneously generated SPFs and LPFs before the flash but not immediately afterward. Small fluctua-

tions in membrane potential appear about 2 min after the adapting flash, and at 4 min SPFs are clearly visible. The first LPF occurred at 5.3 min. The fact that the first LPF after the flash had the same amplitude as those occurring before the flash suggests that LPFs are all-or-none potentials. The data show that LPFs

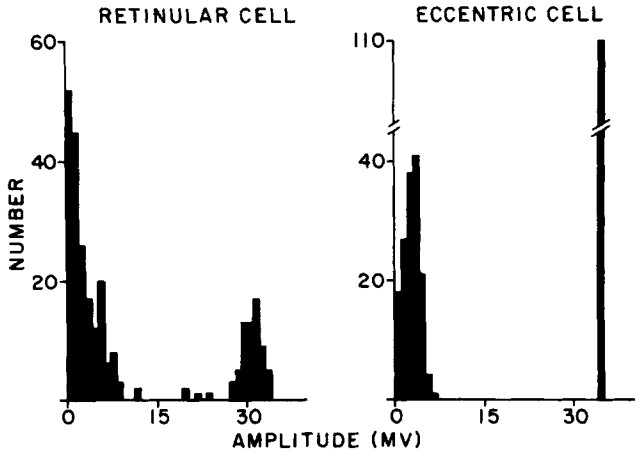

FIGURE 2. Amplitude histograms of the potential fluctuations recorded in the dark from the cells in Fig. 1. 260 events were measured for each cell. Lower and upper modes correspond to SPFs and LPFs for the retinular cell and to depolarizing potentials and nerve impulses for the eccentric cell. Sharp mode at 34 mV represents amplitude of nerve impulses before attenuation by chart recorder.

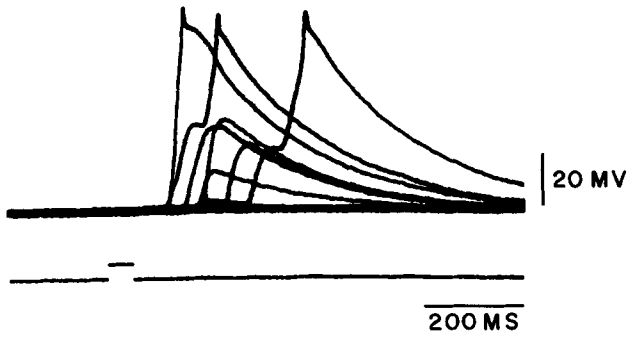

FIGURE 3. Intracellular responses from a dark-adapted retinular cell *in situ* to 10 50-ms flashes of constant intensity. Flashes were repeated one per minute at log I = −5. The regenerative-like potentials (LPFs) reached a peak amplitude of about 80 mV but did not overshoot zero membrane potential.

are more sensitive than SPFs to the effects of light adaptation, an observation also made by Dowling (1968).

ECCENTRIC CELLS Fig. 1 shows that the membrane potential of an eccentric cell fluctuates in the dark and that the fluctuations can trigger impulses in the associated optic nerve fiber. In the right half of the record two impulses

(~100 ms apart) fired near the peak of a depolarizing potential. The two other impulses in the record fired on the repolarizing phases of potential fluctuations. Note that the largest potential fluctuation in the record did not trigger a nerve impulse. The eccentric cell data are similar in some respects to the retinular cell data (upper trace), i.e. depolarizing potentials trigger regenerative events and the triggering threshold fluctuates.

The histogram in Fig. 2 shows that the potential fluctuations recorded from the eccentric cell (Fig. 1) were less than 5 mV in amplitude and the nerve impulses were 34 mV peak-to-peak (see legend of Fig. 1). In view of the fact that the eccentric cell receives its input from the retinular cells, how is it possible for the potential fluctuations in the eccentric cell to fall within a single mode when those in the retinular cell are divided into two modes (LPFs and SPFs)? Our data

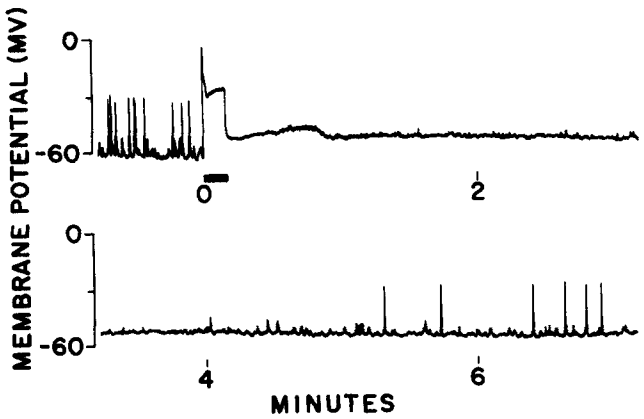

FIGURE 4. Effect of an intense adapting flash on the membrane potential of a dark-adapted retinular cell *in situ*. The 10-s flash presented at 0 min delivered a total of 10^{13} photons (10 s of log I = 0) to the ommatidium at the cornea. Bottom trace is a continuation of top trace. Note that SPFs and LPFs were generated spontaneously before the flash but not immediately afterward and that SPFs recovered earlier than LPFs.

do not provide a clear answer. One possible explanation is that a nerve impulse in the eccentric cell masks the amplitude of the depolarizing potential which generated the impulse. If this were the case, then the application of tetrodotoxin would reveal a second mode, since LPFs are insensitive to tetrodotoxin (Dowling, 1968). No experiments were done with tetrodotoxin.

Typically, eccentric cells *in situ* had resting potentials between −50 and −70 mV and membrane resistances from 4 to 15 MΩ. Eccentric cells were infrequently encountered; however, when impaled they often yielded stable recordings for periods of up to 11 h.

Photon Sensitivity

LINEARITY OF RESPONSE RATE NEAR THRESHOLD Single-photon events elicit quantum bumps (SPFs) from retinular cells in the excised *Limulus* eye (Fuortes and Yeandle, 1964). If SPFs elicit LPFs in the dark-adapted eye *in situ* (Fig. 3),

then the threshold for eliciting an LPF is also a single-photon event. This appears to be the case. The data in Fig. 5 show that near threshold the frequency of occurrence of LPFs is directly proportional to the incident light intensity. Direct proportionality is indicated on the log-log coordinates by the straight line with a slope of 1.0. A closer fit to the line at the dim test intensities is achieved by subtracting the spontaneous rate in darkness (log I = $-\infty$) from the mean rate at each intensity. The range of direct proportionality between incident photons and LPFs in retinular cells is approximately 2 log units, which is about equal to the range of direct proportionality between photons and nerve impulses in

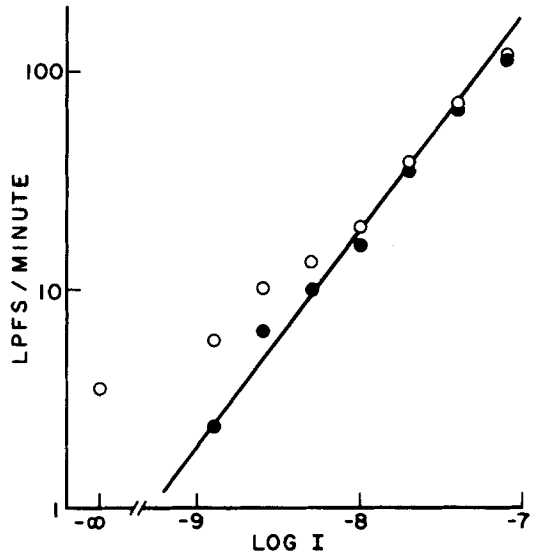

FIGURE 5. Linearity of the intensity-response function for LPFs at low light levels. Ordinate plots on a logarithmic scale the mean number of LPFs elicited from a dark-adapted retinular cell by 1-min flashes; abscissa is log of relative light intensity incident on the ommatidium at the cornea. 10^3 photons/s were incident on the unit at log I = -9. Filled circles give the response rates after substracting the mean spontaneous rate (log I = $-\infty$). The close fit of the data to the line of slope 1.0 is consistent with the result of Fig. 6: single photons elicit single LPFs.

eccentric cells (Kaplan and Barlow, 1976). Within this range a single photon absorption can elicit an LPF in a retinular cell which in turn can elicit nerve impulses in the eccentric cell.

FREQUENCY-OF-RESPONSE CURVES Information on the energy requirements at threshold can often be derived from frequency-of-response measurements of the type shown in Fig. 6. The triangles give for each test intensity the proportion of flashes that elicited one or more LPFs from a single retinular cell *in situ*. The abscissa gives an estimate of the number of photons absorbed per flash by the visual pigment of the ommatidium under study. We describe elsewhere (Kaplan and Barlow, 1976) the factors involved in estimating the abscissa values. The curve is the Poisson sum, $P_{(n,a)}$ for $n = 1$ computed from the equation

$$P_{(n,a)} = \sum_{x=n}^{\infty} \frac{e^{-a}a^x}{x!} = 1 - \sum_{x=0}^{n-1} \frac{e^{-a}a^x}{x!}. \quad (1)$$

This equation gives the probability that at least n photons are absorbed from a flash that delivers a absorbed photons on the average. Increasing the value of n increases the slope of the sigmoid curve for the Poisson sum, $P_{(n,a)}$. The shape of the curve is characteristic of the threshold n for low values of n. This analysis gives a lower limit to the estimation of threshold (Pirenne, 1967). The good fit in Fig. 6 between the theoretical curve for $n = 1$ and the experimental data for a retinular cell suggests that one absorbed photon elicited an LPF. On the other

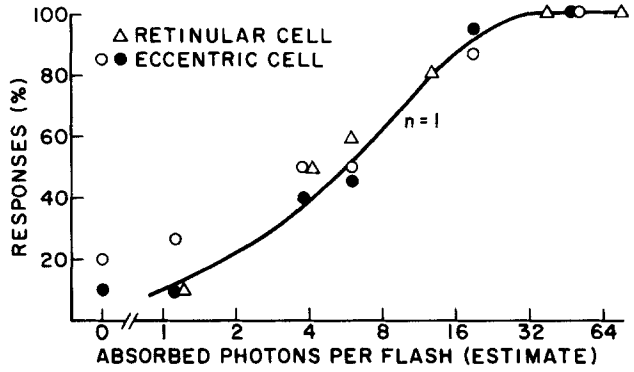

FIGURE 6. Frequency-of-response data from dark-adapted cells *in situ*. Triangles give the relative frequency of eliciting one or more LPFs from a retinular cell at the flash intensities plotted on the abscissa. Unfilled and filled circles give the relative frequencies of eliciting, respectively, one or more depolarizing potentials and nerve impulses from an eccentric cell. The curve is the theoretical Poisson sum for $n = 1$. Each experimental point gives the percentage response to 15–20 flashes, 50 ms in duration, delivered every 15 s. Points at 0 give spontaneous events in 1-s count windows. Method of estimating the number of absorbed photons is given elsewhere (Kaplan and Barlow, 1976). The agreement between theory and experiments supports the notion that near threshold each type of response can be elicited by a single absorbed photon. Retinular and eccentric cell data were taken from different eyes.

hand, the abscissa values in Fig. 6 indicate that eight absorbed photons were required to elicit an LPF. That is, the estimated number of photons absorbed at threshold (63% response) is eight rather than one. A similar or larger discrepancy was found for other cells.

The discrepancy in Fig. 6 between the threshold values derived from the Poisson analysis ($n = 1$) and from the absorption estimates ($n = 8$) may be a measure of the quantal efficiency of eliciting an LPF or it may reflect an error in estimating the number of absorbed photons. Another possibility is that the threshold for eliciting the LPFs fluctuated during the experiment. A noisy threshold flattens the frequency-of-response curves and thus may yield a low value for threshold (Kaplan and Barlow, 1976). For example, the theoretical curve for a noisy threshold with a mean value of 3 is similar in shape to the curve

in Fig. 6 for a fixed threshold of $n = 1$. Some degree of threshold fluctuation is suggested by the data in Figs. 1 and 2. However, we do not know whether the fluctuations are sufficient to account for the discrepancy in the threshold measurements.

The open circles in Fig. 6 give the proportions of flashes at each intensity that elicited one or more depolarizing potentials from a single dark-adapted eccentric cell *in situ*. The filled circles give the results for one or more nerve impulses from the same eccentric cell. The agreement between both sets of data and the Poisson curve for $n = 1$ suggests that single-photon events triggered depolarizing potentials and nerve impulses. These data are subject to the same interpretations given above for the retinular cell data. The result that one absorbed photon can elicit one or more nerve impulses has been reported elsewhere (Kaplan and Barlow, 1976). The result that single depolarizing potentials can fire off nerve impulses is consistent with the recordings presented in Fig. 1.

Intensity-Response Characteristics

Fig. 7 shows receptor potentials recorded from a dark-adapted retinular cell at various levels of illumination. The response to dim light (log I = −7) is characterized by a mixture of small and large potential fluctuations. The SPFs are less than 15 mV in amplitude and the LPFs range from 30 to 45 mV. At log I = −6 the LPFs and SPFs begin to merge together, producing a "steady-state" response of large fluctuating potentials superimposed on a small constant depolarization. This record represents a transition from responses composed of large discrete depolarizations which return to the cell resting potential (log I = −7) to those containing relatively small voltage fluctuations superimposed on a large steady potential (log I = −5 and −3). Large depolarizations, the LPFs, dominate the steady-state responses to dim illumination.

At log I = −6 the onset of illumination elicited a large transient depolarization (~55 mV) with a "spike" on the leading edge. The spike has a peak depolarization about equal in amplitude to the LPFs elicited by log I = −7. It seems reasonable to conclude that the spike is an LPF. A similar spike has been observed in recordings from the excised eye (see Discussion). The transient depolarization immediately after the spike is most likely to be composed not of LPFs but of SPFs summating on the tail of the initial spike. This possibility is suggested by Bayer's (1975) finding that an LPF generated by a ventral photoreceptor in *Limulus* produces a relative refractory period for eliciting a second LPF.

Moderate light intensity (log I = −5) elicited an initial transient depolarization followed by a steady-state response containing small voltage fluctuations. The peak amplitude of the steady-state fluctuations is smaller than the amplitude of an LPF. Apparently moderate intensities suppress LPFs (Fig. 4), leaving SPFs as the primary component of the steady-state response. At high intensities (log I = −3) the receptor potential is relatively smooth, presumably because of the large reduction in SPF amplitude caused by light adaptation (Dodge et al., 1968). Transition from the initial transient depolarization to the steady-state response appears to result from a delay in the onset of light adaptation (Dodge et al., 1968; Lisman and Brown, 1975).

Fig. 8 plots the amplitude of the transient and steady-state components of the receptor potential as a function of light intensity. The data were recorded from a single, dark-adapted retinular cell. The unfilled circles give the peak amplitudes of the initial transient components and filled circles give the time-averaged amplitude of the steady-state components. We note that integrating the receptor potential to produce the steady-state function does not show the potential fluctuations that are characteristic of the responses to dim light intensities. The same is true for the steady-state function of the spike discharge of a single optic nerve fiber (Kaplan and Barlow, 1975). A comparison of the steady-state inten-

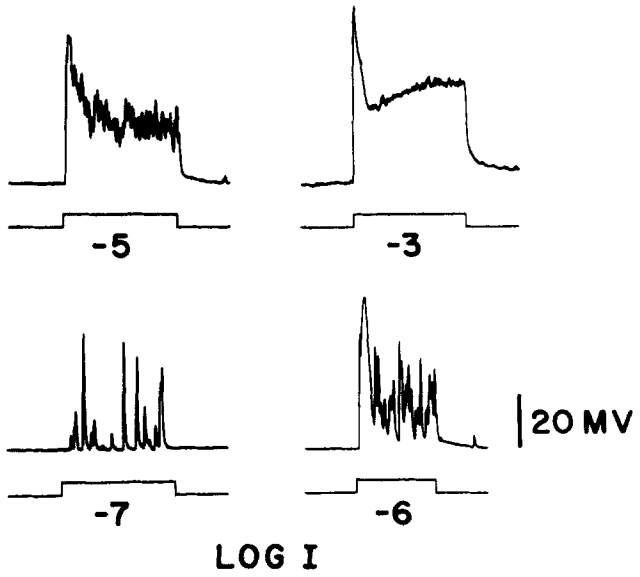

FIGURE 7. Effect of light intensity on the intracellular response of a dark-adapted retinular cell *in situ*. Shown are four responses at the intensities indicated under the stimulus bars. Flash duration was 4.5 s at log I = −6 and 6.5 s for the other intensities. Each response was elicited only after the receptor was fully dark adapted.

sity function of a retinular cell (Fig. 8) to that of an eccentric cell (Fig. 1 in Kaplan and Barlow, 1975) shows that both extend over a similar intensity range and both contain a plateau at the midrange of light intensities.

The plateau is characteristic of the optic nerve responses we recorded using a technique described elsewhere (Barlow and Kaplan, 1971). The plateau is also characteristic of the intensity-response functions recorded from retinular cells during the daytime; however, it was less pronounced in the functions obtained during the night. Similar effects of the time of day have been observed on the shape of the intensity-response function for optic nerve responses (Barlow et al., 1976). The results presented in this paper indicate that the response characteristics of single retinular cells can account for several characteristics of the discharge of single optic nerve fibers.

Spike Firing Mechanism

The similarity between the shapes of the intensity functions of the optic nerve discharge and the receptor potential implies that the firing rate of an eccentric cell is proportional to the membrane depolarization of a retinular cell. We did not attempt to record these data simultaneously from the two types of cells *in situ*. Instead we recorded both spike discharge and generator potential from an eccentric cell. Fig. 9 plots the steady-state firing rate against the mean level of

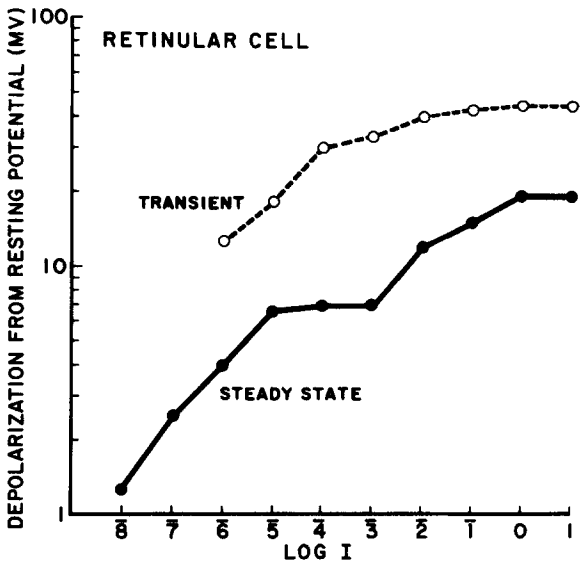

FIGURE 8. Intensity functions recorded intracellularly from a dark-adapted retinular cell *in situ*. The unfilled circles and broken line represent the peak of the initial transient depolarization, and the filled circles and solid line give the mean steady-state depolarization of the receptor potential. Steady-state response was defined as that occurring 3 s after the onset of illumination. All of the data were recorded from a single retinular cell *in situ*. Because the eye was allowed to fully dark adapt between test flashes, the experiment required about 8 h of recording time. We plot here the results of one of the few experiments in which this was achieved.

depolarization for five eccentric cells in as many eyes. The data points were determined over a wide range of intensities. Low and moderate intensities, including those that produced the plateau in the intensity function, elicited steady-state responses of up to about 15 mV mean depolarization. Within this range firing rate is nearly a linear function of membrane depolarization. At higher intensities, the data deviate from linearity; small increments in depolarization produced large increments in firing rate.

Within the range of linearity in Fig. 9, 1.0 mV of depolarization in an eccentric cell increased the firing rate in the optic nerve fiber by about 1.0 impulse per second. A similar voltage-to-spike conversion factor has been measured for excised eyes (MacNichol, 1956; Fuortes, 1958; Purple, 1964). The deviation from

linearity at high levels of depolarization in Fig. 9 was also found in excised eyes (Fuortes and Poggio, 1963). Such agreement between the intact and excised eye data indicates that excision does not damage the spike firing mechanism.

We should point out that Fig. 9 plots spike frequency against the mean level of depolarization, not the instantaneous level. A mean depolarization of 1 mV actually corresponded to membrane potentials fluctuating between 0 and 5 mV. Spikes were generated in an optic nerve fiber only when the amplitude of

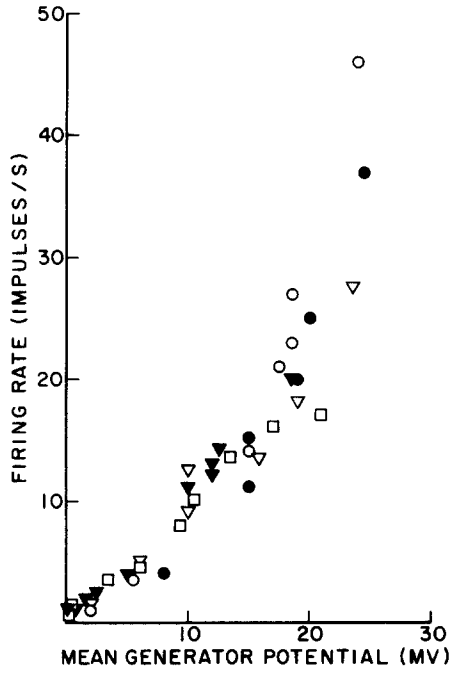

FIGURE 9. Frequency of firing as a function of amplitude of generator potential. Ordinate plots the steady-state firing rate, defined as the mean rate in the last 3 s of a 6-s flash. Abscissa gives the mean level of depolarization of the generator potential. Voltage-to-spike conversion factor is about 1.0 impulses/s/mV for generator potentials below 15 mV and increases sharply for potentials above 15–20 mV. Data were taken from five dark-adapted eccentric cells in as many eyes.

depolarization above resting potential exceeded about 3 mV, which is consistent with data from excised eyes (MacNichol, 1958; Purple, 1964).

Variability of the Spike Discharge

Fig. 10 shows that fluctuations in the generator potential of an eccentric cell produced fluctuations in the spike discharge. Note that discharges near the peaks of some potential fluctuations contained bursts of three to four impulses. Such variability is characteristic of optic nerve responses *in situ* (Kaplan and Barlow, 1975). The variability appears to reflect the summation of SPFs and LPFs in retinular cells.

DISCUSSION

Large and small potential fluctuations, LPFs and SPFs, characterize intracellular recordings from retinular cells in the *Limulus* eye *in situ*. The LPFs and SPFs are the basic components of the receptor potential. Together they provide a 9-log unit range of intensity coding and underlie the dual characteristics of optic nerve responses.

Two Receptor Mechanisms

Single optic nerve fibers *in situ:* (*a*) fire spontaneously in the dark; (*b*) fire irregularly in dim light; (*c*) respond to just a few absorbed photons; (*d*) exhibit wide-range intensity coding; and (*e*) produce an intensity function with a plateau region (Barlow and Kaplan, 1971; Kaplan and Barlow, 1975, 1976). These characteristics are not found in the responses of optic nerve fibers in the excised eye. Our working hypothesis is that two receptor mechanisms underlie the optic nerve responses *in situ*; excising the eye abolishes one of them.

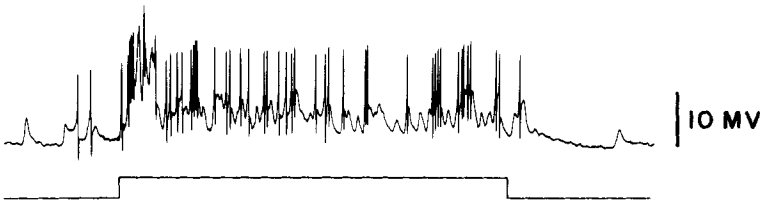

FIGURE 10. Intracellular response from a dark-adapted eccentric cell to a 4.5-s flash of log I = −6.5. The generator potential contains a number of large fluctuations which appear to produce bursts of nerve impulses. Amplitude of impulses before and after the flash was attenuated from 58 to ~16 mV by the filtering characteristics of the chart recorder.

Intracellular recordings from retinular cells *in situ* support the two-mechanism hypothesis. Such recordings exhibit: (*a*) large potential fluctuations in the dark; (*b*) irregular potential fluctuations in dim light; (*c*) responses to single photon absorptions; (*d*) wide-range intensity coding; and (*e*) a plateau in the intensity function. These properties are analogous to those listed above for optic nerve responses, and they appear to reflect the activity of LPFs and SPFs, the two components of the receptor potential. LPFs occur in the dark and produce irregular potential fluctuations in dim light by amplifying the effects of single-photon events. We interpret the plateau in the intensity function as the transition from LPF dominance of the receptor potential to SPF dominance (see below). Since excising the eye usually abolishes LPFs, it seems reasonable to conclude that the LPF and SPF underlie the two "receptor mechanisms" we hypothesized from optic nerve recordings *in situ*.

Nature of the Large Potential Fluctuations (LPFs)

LPFs appear to be regenerative events. Properties which point to a regenerative process are the waveform of the LPF (Fig. 3), similar peak amplitudes of

depolarization in a given cell (Figs. 2–4), and the fact that LPFs can be generated as all-or-none potentials by small depolarizing current pulses. Unfortunately, no direct evidence for the regenerative nature of the LPF has yet been obtained from voltage-clamp experiments on retinular cells *in situ*. However, Bayer (1975) recently investigated the properties of *Limulus* ventral eye cells in organ culture media. Using standard microelectrode techniques, he recorded LPFs and SPFs of the type reported here for retinular cells. With depolarizing voltage clamps, he measured substantial inward currents indicative of an electrically excitable membrane capable of producing regenerative potentials. If the photoreceptor cell of the ventral eye is similar to the retinular cell of the lateral eye, then we would conclude that the retinular cell is capable of producing regenerative potentials, namely LPFs.

LPFs appear to be triggered by SPFs. This conclusion is based on the finding that LPFs are often preceded by slow depolarizing potentials (Figs. 1 and 3) and on the observation that the amplitude of the prepotentials corresponds to the peak of the SPF amplitude (Fig. 2). The fact that not all LPFs have prepotentials (Figs. 1 and 3) may result from the nature of electrical coupling between cells within the ommatidium.

Coupling between Ommatidial Cells

Tight packing between retinular and eccentric cells may lead to electrical coupling as first suggested by Tomita (1956) and later by Hartline (see Fuortes and Poggio, 1963, p. 449). Indeed, Smith et al. (1965) and Borsellino et al. (1965) found electrical coupling between all ommatidial cells in excised eyes, and Miller (1957), Lasansky (1967), and Fahrenbach (1969) found tight junctions between the microvilli of the cells. What effect, if any, does electrical coupling have on the responses recorded from cells *in situ*?

Our data do not provide a clear answer. The relatively tight cluster of LPF amplitudes in Fig. 2 suggests at least two possibilities. First, the LPFs generated in one retinular cell spread without attenuation to the other retinular cells within an ommatidium, i.e., all retinular cells are perfectly coupled. Second, each recorded LPF is generated by the impaled cell as would be the case if all retinular cells were electrically isolated from one another. If the latter were true, the depolarizing potentials recorded from an eccentric cell would outnumber by about 10:1 the LPFs recorded from single retinular cells (retinular cells outnumber eccentric cells by about 10:1 in an ommatidium). Our data show that the frequency of depolarizing potentials recorded from an eccentric cell is roughly the same as the frequency of LPFs recorded from a single retinular cell. This result suggests that a microelectrode in a single retinular cell records the LPFs generated by all retinular cells within an ommatidium. If this is the case, then the similarity of the peak amplitudes of the LPFs recorded from a single cell (Figs. 2–4) suggests that the electrical coupling between retinular cells is good. Smith and Baumann (1969) measured coupling ratios between 2:1 and 10:1 for cells in excised eyes; however better coupling may exist between cells in the eye *in situ*, particularly in the rhabdom region.

Both Dowling (1968) and we have noted that LPFs of maximum amplitude appear to be generated in a particular region of the cell. The region is character-

ized by high resistance. It may be located near the microvilli of the rhabdom. We have frequently observed that when LPF amplitudes are maximal, the intracellular record contains no sign of eccentric cell activity. For example, in Fig. 1 no eccentric cell spikes are observed in the retinular cell recording, in spite of the fact that depolarizing potentials from the retinular cell are evident in the eccentric cell record. The same is true for the recordings made by Dowling (1968). Apparently, depolarizing potentials propagate from retinular to eccentric cell but not in the reverse direction. This result is consistent with the observation by Smith and Baumann (1969) that the retinular-eccentric cell junction in the excised eye is singly rectifying.

Photon Sensitivity

A quantum bump (SPF) amplifies appreciably a single photon absorption in a retinular cell (Fuortes and Yeandle, 1964; Adolph, 1964); however, it alone cannot evoke an impulse in the eccentric cell. To do so, it must either summate with other SPFs or trigger off an LPF as suggested by Dowling (1968). In support of this idea are the data in Fig. 5 which show that the frequency of occurrence of LPFs is directly proportional to the incident light intensity. The results in Fig. 6 show that the thresholds for generating nerve impulses in an eccentric cell and for eliciting LPFs in a retinular cell are about the same and that the responses from both cells follow the Poisson sum for a single event. If our interpretation of the results is correct, then the following sequence of events may occur in a dark-adapted ommatidium in dim light: (*a*) a single-photon absorption in a retinular cell elicits an SPF; (*b*) the SPF triggers a regenerative potential, the LPF; (*c*) the LPF passes to the eccentric cell dendrite via a tight junction; (*d*) the LPF reaches the eccentric cell body as a small depolarizing potential; and (*e*) the depolarizing potential fires off one or more nerve impulses in the optic nerve. The LPF appears to be an important link in the chain of events initiated by a photon and ending with the discharge of impulses in an optic nerve fiber.

Intensity Function of the Receptor Potential

In a previous paper (Kaplan and Barlow, 1975) we presented a model for the shape of the intensity function of the generator potential of the eccentric cell. The model ascribed the shape of the function, especially the plateau, to a single receptor mechanism governed by the adapting-bump concept of Dodge et al. (1968). In brief, the model assumes that quantal bumps summate to produce the generator potential (Dodge et al., 1968; Borsellino and Fuortes, 1968), light adaptation reduces the size of the quantal bumps (Dodge et al., 1968), the effects of light adaptation are localized at low light intensity (Fein, 1973; Fein and Charlton, 1975), and bump adaptation is a dual function of light intensity. This last assumption was required to produce the plateau. The model is relatively simple in that it is based on a single type of receptor response, the quantal bump.

We now know from intracellular recordings *in situ* that there are two types of responses: the quantal bump (or SPF), and the LPF. This added information does not change the essential nature of our model, rather it allows us to be more specific with regard to the mechanisms of adaptation. Instead of assuming that bump adaptation is a dual function of light intensity, we suppose that light

adaptation differentially influences the two types of receptor responses. According to this view, both SPFs and LPFs contribute to the steady-state receptor potential; the individual contributions are determined by the incident light intensity. The SPF contribution is governed by the basic features of the adapting-bump model of Dodge et al. (1968), namely, the mean value of the SPF contribution of the receptor potential is the product of bump amplitude, rate, and duration. The LPF contribution incorporates several results of this paper: LPF amplitudes are nearly constant at low light intensities (Fig. 7), rate of occurrence of LPFs is directly proportional to light intensity at low intensities (Fig. 5), and LPFs are abolished at moderate intensities (Fig. 4). The disappearance of LPFs at moderate intensities may account for the plateau in the intensity function (Fig. 8). According to this view, LPFs are the principal component of the receptor potential at low intensities and SPFs predominate at high intensities.

Responses from Excised Eyes

In the dark or under dim illumination, two types of potential waves are normally recorded from the excised *Limulus* eye. Adolph (1964) called them "slow" and "fast" waves, and Borsellino and Fuortes (1968) labeled them "S" and "L". Fuortes and O'Bryan (1972) suggested that these two types of waves were produced by two distinct receptor processes within the retinular cell. The small, slow waves are generally less than 2 mV in amplitude and the large, fast waves are usually not more than 5 mV in amplitude. The two types of waves may be remnants of the SPFs and LPFs recorded from the eye *in situ*. On the other hand, both types of waves in the excised eye may be related to SPFs of the intact eye and no potential in the excised eye corresponds to the LPF. If this were the case, then the lower mode of the histogram in Fig. 2 would be comprised of two modes, which it is not. The relationship between potentials in excised and intact eyes remains an open issue.

In general, our experience is that excising the eye or cutting off its blood supply adversely affects the mechanisms that produce the receptor potential. However, the effects of excision are not instantaneous (Barlow and Kaplan, 1971). Under optimal conditions the characteristics of the eye *in situ* may survive for a short time in the excised eyes of adult animals. Bathing retinal slices in organ culture media (Kaplan et al., 1973) retards the deleterious effects of excision for at least 24 h. As mentioned above, Dowling's recordings from excised eyes of small *Limuli* are similar to those we obtained from adult eyes *in situ*. Perhaps eyes of small *Limuli* are more resistant to the effects of excision.

A nonpropagated "spike" can be seen on the rising phase of the transient responses in Fig. 7. A similar type of spike has been recorded from excised eyes (Benolken, 1965; Yeandle, 1967; Wulff and Mueller, 1973). Hyperpolarizing the membrane by extrinsic current accentuated the light-evoked spike in the excised eye (Fuortes and Poggio, 1963). A spike-like potential was generated by anodal break currents in ventral photoreceptors (Millecchia and Mauro, 1969). Yeandle, Wulff and Mueller, and Millecchia and Mauro concluded that the spike originated in a different region of the cell from the transient response. It is possible that the mechanism which generates the spike in the excised eye is a remnant of

the mechanism which triggers LPFs in the eye *in situ*. After excision, the initial transient component may be the only natural depolarizing potential of sufficient rise time and amplitude to fire off the spike.

A word about sensitivity: Fuortes and Yeandle (1964) and Adolph (1964) clearly demonstrated that single photons elicited detectable responses (quantum bumps) in the retinular cells of excised eyes. The 5-log unit difference in sensitivity between intact and excised eyes (Barlow and Kaplan, 1971) results from the fact that a single quantum bump in an excised eye is too small to trigger impulses in the optic nerve fiber, whereas in the intact eye a quantum bump (SPF) can fire off an LPF which is large enough to trigger nerve impulses. The difference in sensitivity between intact and excised eyes is therefore apparent in optic nerve responses.

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