The Ventral Photoreceptor Cells of *Limulus*

II. *The basic photoresponse*

RONALD MILLECCHIA and ALEXANDER MAURO

From The Rockefeller University, New York 10021. Dr. Millecchia's present address is Biology Department, Reed College, Portland, Oregon 97202

ABSTRACT The ventral photoreceptors of *Limulus polyphemus* are unipolar cells with large, ellipsoidal somas located long both "lateral olfactory nerves." As a consequence of their size and location, the cells are easily impaled with microelectrodes. The cells have an average resting potential of −48 mv. The resting potential is a function of the external concentration of K. When the cell is illuminated, it gives rise to the typical "receptor potential" seen in most invertebrate photoreceptors which consists of a transient phase followed by a maintained phase of depolarization. The amplitude of the transient phase depends on both the state of adaptation of the cell and the intensity of the illumination, while the amplitude of the maintained phase depends only on the intensity of the illumination. The over-all size of the receptor potential depends on the external concentration of Na, e.g. in sodium-free seawater the receptor potential is markedly reduced, but not abolished. On the other hand lowering the Ca concentration produces a marked enhancement of both components of the response, but predominantly of the steady-state component. Slow potential fluctuations are seen in the dark-adapted cell when it is illuminated with a low intensity light. A spike-like regenerative process can be evoked by either the receptor potential or a current applied via a microelectrode. No evidence of impulse activity has been found in the axons of these cells. The ventral photoreceptor cell has many properties in common with a variety of retinular cells and therefore should serve as a convenient model of the primary receptor cell in many invertebrate eyes.

INTRODUCTION

The ionic basis for receptor potentials in photoreceptors has been investigated in only a few preparations, namely, the cells in the ommatidia of the horseshoe crab (Kikuchi, Naito, and Tanaka, 1962), the retinular cells of the crayfish (Eguchi, 1965), and the retinular cells of the honeybee drone (Fulpius and Baumann, 1969). These preparations all have the disadvantages of being quite small and embedded in a meshwork of pigment-containing cells and glial cells. In the studies cited above it was shown that sodium is im-

portant for the maintainance of the normal receptor potential. The receptor potentials were not completely abolished in the sodium-free solution, but were drastically reduced. It has been argued that the persistence of an attenuated response in sodium-free solutions might be due to the presence of sodium trapped in extracellular spaces not readily accessible to the bulk external solution (Kikuchi et al., 1962). On the other hand, it has also been suggested that other ions, in addition to sodium, may be involved in the production of the receptor potential, and it is these ions which give rise to the attenuated response in the sodium-free solution (Fulpius and Baumann, 1969).

In the present study the basic properties of the ventral photoreceptor cells of *Limulus* have been investigated. The large size of the cell soma and the apparent accessibility of the cell surface to the external environment make this photoreceptor an ideal preparation for the study of the ionic basis of photoreception. A preliminary report on some of this work has been given (Millecchia, Bradbury, and Mauro, 1966).

MATERIALS AND METHODS

The Biological Preparation

The ventral photoreceptors of *Limulus* (Millecchia et al., 1966; Clark, Millecchia, and Mauro, 1969) are unipolar neurons which are grouped into two lateral nerves—termed by Patten and Redenbaugh (1899) the "lateral olfactory nerves"—each of which is ensheathed within a blood vessel. The lateral olfactory nerves run anteriorly from the protocerebrum or forebrain to a "wart-like" structure on the soft exoskeleton (pleura) a few centimeters anterior to the paired chelicera. The neurons have large ellipsoidal somas which are 75–100 μ in diameter and 100–150 μ long. Some somas are scattered along the length of the nerve while others are clustered at the anterior end. In some instances, cell somas have been seen within the forebrain (Patten, 1912; Clark et al., 1969).

The desheathed nerves were placed in a small Plexiglas chamber filled with artificial seawater. The connective tissue remaining on the cell soma was softened with 0.5% Pronase (Calbiochem, Los Angeles, Calif.) in buffered seawater (pH 7.4) for 5 min.

The Electrical Recording Arrangement

The large cell bodies can be penetrated easily with conventional glass capillary micropipettes. The micropipettes were filled with 3 M KCl (DC resistance 15–20 MΩ) and connected to a calomel electrode via a 3 M KCl-agar salt bridge. The bath was connected to a second calomel electrode with a salt bridge. The outputs of the two calomel electrodes were fed into a unity gain, high input-impedance amplifier. The output of the amplifier was displayed on an oscilloscope which could be photographed for a permanent record. Currents could be passed into the cell either through a second micropipette inserted into the cell, or through the recording electrode using a bridge technique.

The Light Stimulus

The source of light was a 6 v, 15 w microscope illuminator lamp run on a voltage-regulated DC power supply (stable to within 0.5%). The light was focused onto a vane of an electromagnetic shutter which could be remotely driven to deliver pulses as short as 5 msec in duration. The beam was then collimated and projected onto a lens near the recording chamber which focused the beam onto the preparation through a polished wall of the Plexiglas chamber. The beam could be attenuated by interposing calibrated neutral filters between the source and the shutter. A beam splitter was placed in the collimated portion of the beam and deflected 8% of the beam onto a photovoltaic cell which monitored the relative intensity of the stimulus.

The absolute intensity of the system was measured with an ISCO Spectroradiometer which was calibrated against the ISCO Spectroradiometer Calibrator using a standard lamp with a color temperature of 2800°K. The unattenuated beam at the level of the preparation had an average intensity of 3.2 $\mu w/mm^2$ over the visible spectral range (400–700 nm). In terms of number of quanta per second at the surface of a soma (10^{-2} mm^2) this is approximately 10^{11} quanta/sec.

Artificial Seawater and Perfusion System

The recording chamber had a volume of about 2 ml. It could be continuously perfused with any solution while the level of the bath was maintained by drawing off the excess solution with an aspirator. The fastest and most complete exchange of solution was found to occur when small volume, multiple flushes were used (three 15 ml flushes). The artificial seawater was made by diluting 1 N stock solutions of the various salts. The final concentrations (mM) of the salts were: NaCl 435, KCl 10, $CaCl_2$ 10, $MgCl_2$ 20, and $MgSO_4$ 25. The isosmotic KCl seawater was made by replacing the NaCl with an equimolar concentration of KCl and the sodium-free seawater by replacing the NaCl with equimolar tris (hydroxymethyl) aminomethane chloride (pH 7.0), choline chloride, or LiCl. The calcium-free seawater was made by replacing the $CaCl_2$ with an equivalent amount of NaCl. The osmolarity of all the test solutions was checked with a freezing point depression osmometer and found to be within 2.5% of one another (1000 milliosmols).

RESULTS

The Basic Properties of the Ventral Photoreceptors

The ventral photoreceptors in *Limulus* are large unipolar neurons which are extremely sensitive to light. When a microelectrode enters the cell, a resting potential of between −40 and −50 mv is recorded. The average resting potential from 173 cells was -48.2 ± 1.0 mv (mean ± SE). The effective resistance of these cells, measured with current pulses of *small* amplitude (<5 na) passed through a second electrode in the same cell, ranges from 5 to 10 MΩ. The potentials produced by these small current steps rise with a simple exponential time course (within experimental error) to the steady-

state value and these exponentials have time constants that range from 20 to 100 msec. The calculated capacitance of the cell is between 0.004 and 0.010 μF.

The surface area of the cell is about 5×10^{-4}cm^2, if we assume that the cell is a sphere with a 120 μ diameter. This implies that the specific capacitance of the cell is 8–20 μF/cm^2. However, the cell surface is not smooth but highly invaginated and has many microvillous projections (Clark et al., 1969); thus its area could easily be 20 times the spherical estimate, giving a specific capacitance of 0.4–1.0 μF/cm^2. This is a very rough estimate of the increase in the surface area; a more detailed estimate would have to take into account the tight junctions between microvilli and also between microvilli

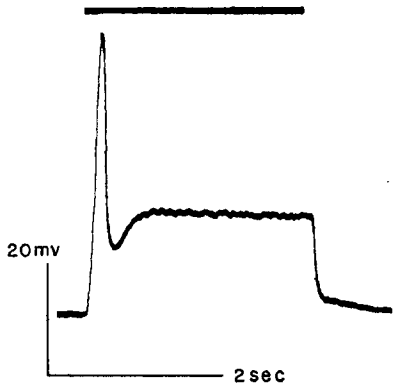

FIGURE 1. Typical receptor potential. The cell was stimulated with a 1.5 sec, moderately intense light flash. The upper trace is a photocell monitor of the stimulus; it also indicates the zero level for the potential.

and glial cells which would reduce the effective surface area; any spread of current down the axon must also be taken into account for this would increase the surface area. When these reservations are noted, the specific capacitance thus calculated is a rather reasonable value when compared with that of most biological membranes. The specific resistance of the cell, using the corrected value for the surface area, is 50,000–100,000 Ω cm^2, which is one to two orders of magnitude higher than that of most nerve or muscle cells.

The Photoresponse

The response of the cells to light (Fig. 1) consists of a slow depolarization (receptor potential)[1] with two phases, a transient phase (dynamic phase) and a steady-state phase (static phase or plateau). The transient phase on the upstroke usually evokes a single spike-like regenerative response similar to the regenerative response of the lateral eye in *Limulus* (Benolken, 1959, 1965; Fuortes and Poggio, 1963). The size and shape of the over-all response depend

[1] In the lateral eye of *Limulus* the light response has been called the ommatidial action potential (Hartline, Wagner, and MacNichol, 1952).

on the intensity and duration of the light and on the state of dark adaptation; namely, the length of time the cell has been in the dark prior to illumination. The different components of the response can be affected separately by changing these stimulus parameters.

DARK ADAPTATION Fig. 2 demonstrates the effects of dark adaptation on the response. The cell was maintained in the dark and then stimulated with a maximum intensity, 10 sec light flash; i.e. a conditioning flash. After a prescribed time interval a test flash of the same intensity and duration was

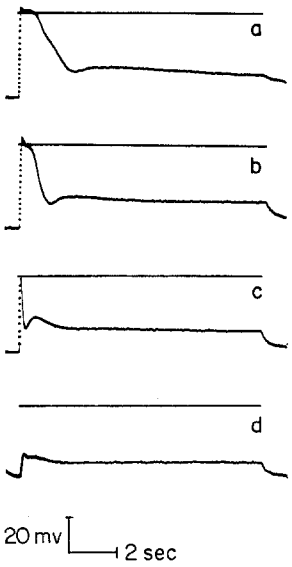

FIGURE 2. Effects of dark adaptation. The receptor potentials are from a cell which was first stimulated with a 10 sec, maximum intensity adapting light, then kept in the dark for (a) 1 hr, (b) 60 sec, (c) 5 sec, and (d) 0.8 sec. The light stimulus was the same for all the records and its duration is indicated by the length of the solid bar above each record; this bar also indicates the level of the zero potential.

presented and the response was observed. The response for a time interval of 1 hr in the dark is shown in Fig. 2 a. The different components of the response can be distinguished easily. The spike-like regenerative process is seen as a notch on the leading edge of the response. This is followed by a more prolonged but still transient depolarization, which then declines to a smaller, maintained steady phase of depolarization. In Fig. 2 b, c, and d, the responses are shown for decreasing intervals of time, namely, 60, 5, and 0.8 sec, respectively. In Fig. 2 b, the transient is not only smaller in amplitude, but also shorter in duration than in Fig. 2 a with the spike-like response remaining on the rising phase of the transient component. In Fig. 2 c, the transient is reduced to such an extent that the spike-like response is clearly seen as a separate cemponent. Finally, in Fig. 2 d, the transient is reduced so markedly that the spike-like response is no longer evoked, and all that remains is the steady phase of depolarization.

INTENSITY OF ILLUMINATION Fig. 3 shows the response of a cell to light flashes of different intensities, starting with the maximum available intensity and successively reducing the intensity by a factor of 10. In an effort to maintain a constant state of adaptation of the receptor between successive test flashes, the cell was exposed to a maximum intensity light flash of 10 sec

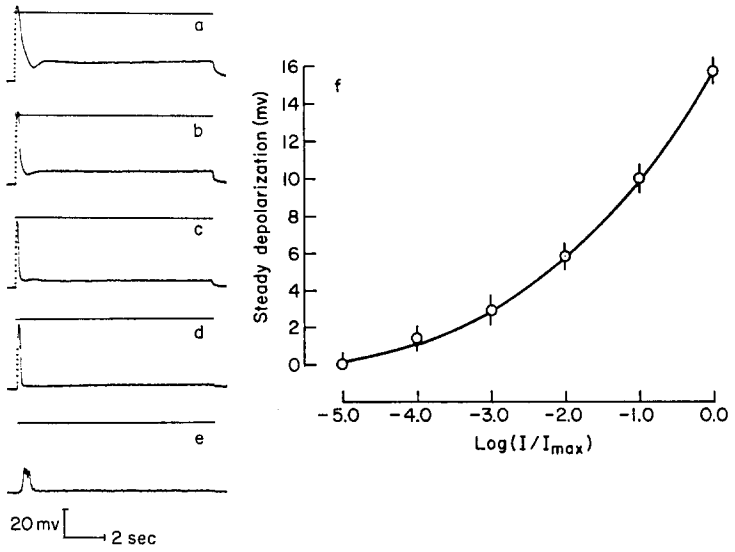

FIGURE 3. Effects of intensity of illumination. The receptor potential in (a) is in response to the maximum available intensity. The receptor potentials in (b) through (e) are evoked by light stimuli (b) 0.1, (c) 0.01, (d) 0.001, and (e) 0.0001 as intense as that used in (a). The bar above each record indicates the duration of the stimulus and the zero potential level. The state of adaptation of the cell was maintained constant between successive test flashes by exposing the cell to a maximum intensity light for 20 sec followed by 1 min of darkness. In (f) the amplitude of the steady phase of depolarization is plotted as a function of the $\log_{10}$ of the relative stimulus intensity (unattenuated intensity given the value of 0.0). The vertical bar on each datum point represents the measuring error, ± 0.5 mv.

duration followed by 1 min of darkness. The amplitude of the transient phase increases with the intensity of the light, but the exact nature of the relation is obscured by the presence of the spike-like response. The amplitude of the steady phase of depolarization also increases with the stimulus intensity; however, for this phase of the response the exact nature of the relation is easily obtained. A plot of the amplitude of the steady phase vs. the logarithm of the relative stimulus intensity is shown in Fig. 3 f. This relation is quite typical of photoreceptors (Rushton, 1961, 1965).

LATENCY The latency of the light response, measured from the onset of the light stimulus to the first detectable inflection of the response, is greatly

affected by the state of adaptation of the cell and by the intensity of the stimulating light. A dark-adapted cell gives a larger light response with a longer latency than a light-adapted cell. Fig. 4 a shows two responses of a cell that was stimulated with a 50 msec, moderately intense light flash. The

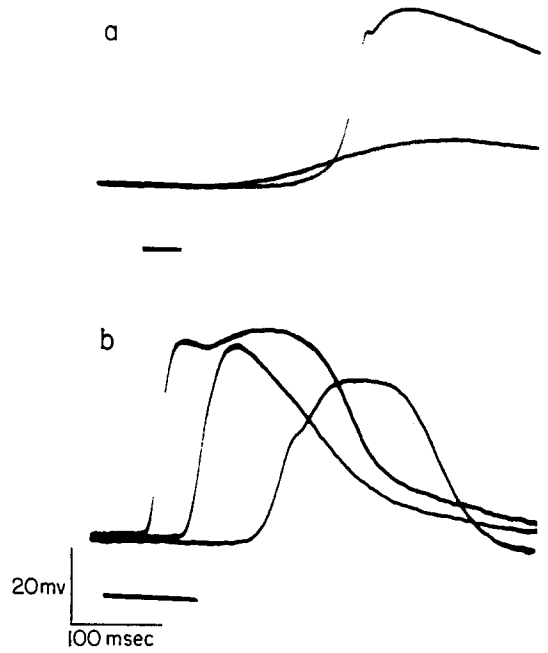

FIGURE 4. The latency of the receptor potential. In (a) two receptor potentials are superimposed; the larger response is from a cell after it had been in the dark for 60 sec and the smaller response is after only 20 sec in the dark. The stimulus was a 50 msec moderately intense light flash. In (b) three receptor potentials are superimposed. In this case the cell was maintained in the same state of adaptation by stimulating it with a 10 sec, maximum intensity adapting light followed by 1 min of darkness before each test flash. The test flashes were 100 msec long and at three different intensities (0.1, 0.01, and 0.001; relative to the unattenuated intensity). The largest response was evoked by the brightest light flash and the smallest reponse by the weakest. The short bars beneath the responses indicate the duration of the stimuli.

larger amplitude response, which was from the cell after it had been kept in the dark for 1 min, has a latency of about 150 msec. The smaller amplitude response, from the cell after it had been in the dark only 20 sec, has a latency of 100 msec.

When the cell is maintained in a constant state of adaptation and the intensity of the stimulating light is increased, the latency of the response decreases. In Fig. 4 b the responses of a cell to three different light intensities are superimposed photographically. The largest response has a latency of 40 msec, the next largest a latency of 75 msec, and the smallest a latency of 150

msec. These sets of data show that the effects of intensity and adaptation cannot be equated. A cell which is in two different states of adaptation can be made to give responses with identical amplitudes by adjusting the intensities of the two stimuli, but the latencies of the two responses will, in general, be quite different. We have not attempted to describe the effects of intensity and adaptation by a mathematical model as has been done in the case of the lateral eye of *Limulus* by Fuortes and Hodgkin (1964).

Spectral Properties

As is typical of most photoreceptors, the ventral photoreceptor cells are not equally sensitive to all wavelengths of light. The radiant energy needed to give a constant amplitude response varies with the wavelength of the illumination, with a minimum value (maximum sensitivity of the cell) at about 540 nm (Millecchia et al., 1966). This is similar to the spectral sensitivity of the lateral eye (Graham and Hartline, 1935) and medial eye (Wald and Krainin, 1963; Chapman and Lall, 1967) of *Limulus*, which contain a rhodopsin type of pigment. This pigment has been extracted from the lateral eye and has a maximum absorption at 520 nm (Hubbard and Wald, 1960). The absorption spectrum of the ventral photoreceptor cell was measured in vivo with a microspectrophotometer. These cells contain a photolabile pigment with an absorption maximum at 530 nm (Murray, 1966).

SLOW POTENTIAL FLUCTUATIONS An interesting feature of the response arises when the cell is stimulated with a very low intensity light (Fig. 3 e). The initial phase of the response is composed of many discrete depolarizations which fuse together. These discrete potential changes, or slow potential fluctuations, are similar to those found in the lateral eye of *Limulus* (Yeandle, 1957). The amplitude of these fluctuations increases as the cell becomes dark-adapted. Fig. 5 shows the response of a cell which was kept in the dark for about 15 min, then exposed to a very dim light (eight orders of magnitude weaker than the maximum intensity). In the dark the membrane potential is quite smooth, but as soon as the light is turned on (light indicated by the solid line) the fluctuations begin to appear. They occur at random intervals, and their amplitudes vary. Fuortes and Yeandle (1964) and Adolph (1964) have studied the time variations and amplitude distributions of the slow potential fluctuations in the lateral eye of *Limulus*. They analyzed the statistics of these events and concluded that the fluctuations represent the response of the cell to several quanta of light while the random occurrence of the fluctuations at low light intensities reflects the random arrival of the photons. This explanation is also applicable to the response of the ventral photoreceptor in *Limulus*. In Fig. 5 the average frequency of the potential fluctuations is slightly less than one fluctuation per second. The intensity of the illumination at the surface of the photoreceptor was about 10^3 quanta/sec.

If we assume that the cell absorbs about 0.5% of the incident light[2] then each potential fluctuation is the result of the absorption of approximately 5 photons.

The Spike[3]

The spike-like potential which is seen on the leading edge of the light response can also be evoked with current applied by means of a microelectrode. The spike is seen most often at the cessation of a hyperpolarizing current. This

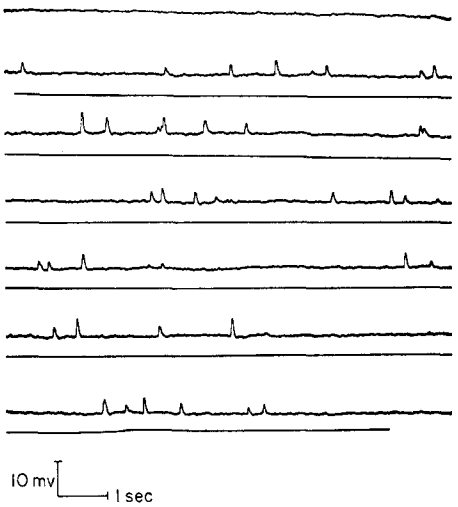

FIGURE 5. Slow potential fluctuations. The potential tracings, continuous from the end of one line to the beginning of the next, are from a cell kept in the dark for 15 min. Just after the start of the second trace the cell was exposed to a very weak light, eight orders of magnitude weaker than the unattenuated light (indicated by the bar beneath the potential tracings). The illumination was maintained until almost the end of the last potential tracing.

type of "anodal break" response is shown in Fig. 6 a; the cell was hyperpolarized with a 2 na current for 200 msec. The spike can also be evoked with a depolarizing current if the resting potential is greater than −50 mv of if the cell is polarized beyond this potential. The response shown in Fig. 6 b is from a cell which was polarized with a 2 na current and subsequently depolarized with a 10 msec, 3 na current. The amplitude of the spike is graded with the intensity of the depolarizing current, reaching a maximum value of 35 mv in some cells. The duration of the spike, at half-amplitude, varies inversely with the amplitude. The range we have observed is from 20 msec, for very large spikes, to over 100 msec for very small ones. This is in sharp contrast to the very brief 3–5 msec spikes evoked in the honeybee eye (Baumann, 1968).

We have adapted the working hypothesis that the spike occurs in a region

[2] The per cent absorption was calculated from the absorption difference spectrum of a fully dark-adapted ventral photoreceptor (Murray, 1966).

[3] This component of the response also has been referred to in the literature as the "regenerative response," the "sharp transient," and the "initial pulse."

of the cell distinct from that of the light response. This hypothesis is supported by the fact that in a few instances recordings have been made from cells in which there was no light response, but spike potentials could be evoked easily with current.

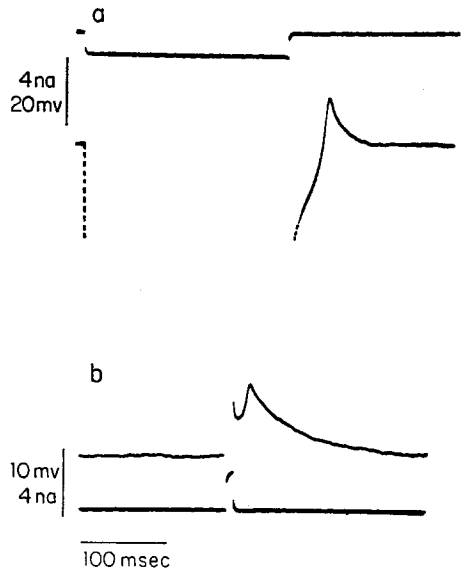

FIGURE 6. The spike-like potential. In (a) the cell was hyperpolarized with a 2 na current (upper trace) for 200 msec. The cell's potential (lower trace) in response to the hyperpolarizing current went off the screen of the oscilloscope but when the current was removed the potential recovered with an "overshooting anodal break" response. In (b) the cell was first hyperpolarized with a 2 na current, then a 10 msec, 3 na depolarizing current (lower trace) was passed into the cell. The spike potential in response to the depolarizing current pulse is shown in the upper trace.

The spikes are unaffected by tetrodotoxin up to a concentration of 10^{-5} g/ml [100 to 10,000 times the concentration needed to abolish the sodium conductance increase associated with action potentials in lobster giant axon (Narashi, Moore, and Scott, 1964), the squid giant axon (Nakamura, Nakajima, and Grundfest, 1965), and in the frog nerve (Hille, 1968)], and no evidence for propagated spike activity has been found in the axons of these cells.

Ionic Studies

EFFECTS OF POTASSIUM ON MEMBRANE POTENTIAL Potassium is the only ion that has an appreciable effect on the membrane potential; all the other ions normally found in seawater have little or no effect on the membrane potential. Decreasing the [K] has a variable effect on the membrane potential in that some cells respond with hyperpolarization while others respond

with depolarization of several millivolts. Increasing the external [K] depolarizes the cell very rapidly. The cell begins to depolarize as soon as the exchange of high K seawater for normal seawater begins, and is completely depolarized within 15–30 sec (the time needed to flow 15 ml of solution through the 2 ml recording chamber). Fig. 7 is a plot of the membrane potential vs. the logarithm of the external [K]. As is typical for most nerve and muscle cells, the membrane potential is quite insensitive to changes in the external [K] in the lower concentration range (10 mM and below), but

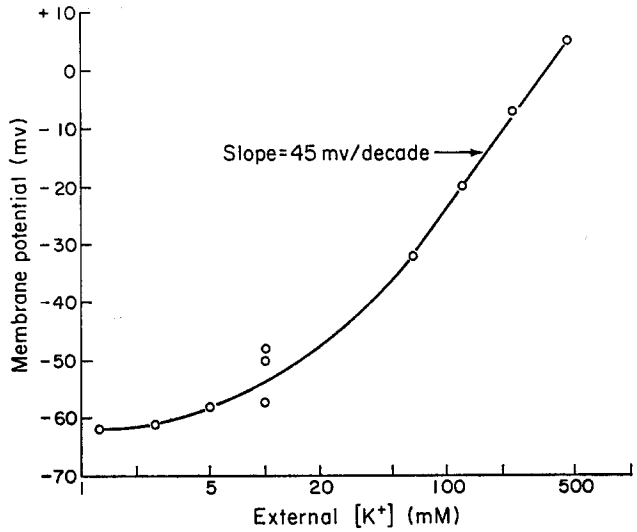

FIGURE 7. Semilogarithmic plot of the membrane potential vs. the external potassium concentration for one cell.

changes dramatically with [K] in the higher range (75 mM and above). The maximum slope of the curve for this particular cell is about 45 mv per 10-fold change in the external [K]. However, the average slope in this range for 10 cells was closer to 25 mv/decade, which is less than one-half the value expected for a membrane exclusively permeable to K (58 mv/decade).

EFFECT OF IONS ON THE LIGHT RESPONSE Sodium and calcium are the only ions in the external solution that have a direct effect on the light response. High potassium decreases the light response, but only indirectly, by depolarizing the cell. If the cell is repolarized in the presence of high potassium (and normal sodium and calcium), the cell will respond normally to light.

LOW SODIUM If the external sodium is removed and replaced by Tris, choline, or lithium, the light response is transiently abolished but partially recovers after a few minutes. Fig. 8 is a plot of the amplitudes of the initial

phase and the steady-state phase of the light response vs. the time the cell was in sodium-free Tris-seawater. The cell was tested every 10 sec with a 2.5 sec moderate intensity light flash. This procedure served two purposes:

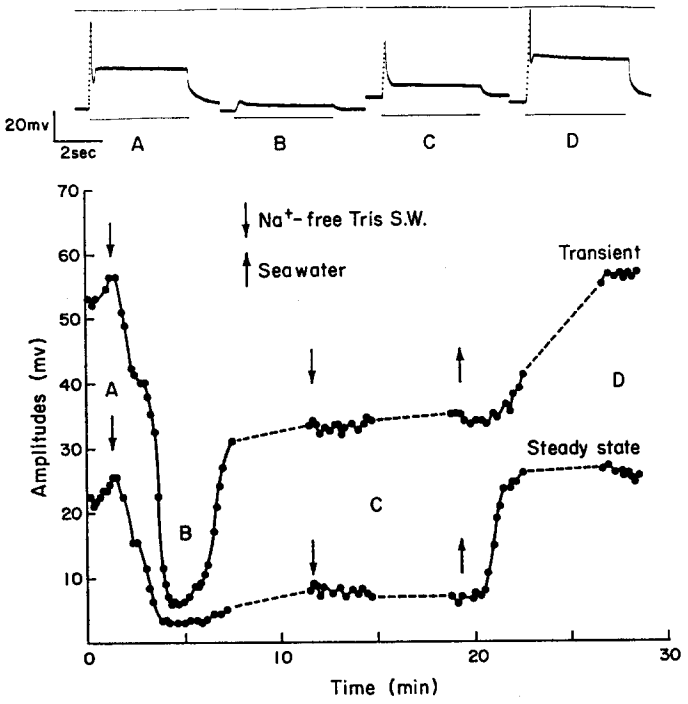

FIGURE 8. Effect of low external sodium concentration on the amplitude of the receptor potential. The upper records are sample receptor potentials at different times during the process of exchanging sodium-free seawater for normal seawater, (A) normal seawater, (B) initial blockage phase in sodium-free seawater, (C) later "recovery" phase in sodium-free seawater, and (D) return to normal seawater. The line above each record indicates the zero potential level, the line below each record indicates the duration of the light stimulus. The cell was stimulated every 10 sec with a constant intensity light, 2.5 sec in duration, throughout the experiment. The lower plot is the amplitudes (measured from the resting potential) of the transient phase of the receptor potential (upper data points) and of the steady phase (lower data points) as a function of time. The downward arrows indicate the start of the perfusion of the 2 ml recording chamber with 20 ml of sodium-free, Tris-Cl seawater. The upward arrows indicate the reintroduction of normal seawater into the chamber.

it kept the cell in a constant state of adaptation and also "continuously" monitored the effects of the sodium-free solution on the light response. Within 5 min after the start of the perfusion the entire response was reduced to 10% of its initial value. In some cells the response is completely abolished for several minutes. After about 10 min, the response had partially recovered, the amplitude of the transient phase was about 60% of the starting value, and

the amplitude of the steady phase was about 30% of its starting value. This response was unaffected either by repeated replacements of the bathing solution with fresh sodium-free solution or by continuous perfusion of the chamber with sodium-free solution.

The initial phase of the light response during the partial recovery stage is disproportionately larger than the steady phase. It is much shorter in duration than the normal response and seems to be predominantly due to the spike-like potential. In some cells the exposure to a sodium-free medium causes the spike and transient phase to be more clearly separated in time. This situation occurs not only during the partial recovery stage in the sodium-free solution, but also after the cell is returned to the normal sodium seawater. In most cells the amplitudes of all phases of the light responses recovered to better than 80% of their initial values when the cell was returned to seawater.

The slow potential fluctuations seen in the dark-adapted cell are also affected by lowering the external sodium concentration. The amplitudes of the slow potential fluctuations are reduced in the low sodium medium just as is the amplitude of the full size receptor potential. There is no sign of a partial recovery stage but this could be simply due to the small size of the fluctuations (10–20% partially recovered fluctuations would be below the noise level of the system).

CALCIUM The light response of the ventral photoreceptor is greatly affected by lowering the external calcium concentration. If the cell is in a solution which lacks calcium the amplitudes of various phases of the responses are enhanced. The initial transient phase is increased by only about 25%, but the amplitude of the steady phase is increased by as much as three times its normal value. The resulting response shows almost no difference between the initial and steady-state phases. If a cell is left in a calcium-free solution for less than 10 min these effects are completely reversible. If, however, the cell remains in the low calcium solution longer than 15 min the light response begins to diminish and becomes irreversibly abolished after 20–30 min.

Fig. 9 is a sequence of light responses from a cell which was exposed to a low calcium seawater. Fig. 9 a is the response of this cell in normal seawater. The bathing solution was then exchanged for an artificial seawater which lacked calcium. Fig. 9 b shows the response of the cell after 3 min in the low calcium solution (the same test stimulus as in Fig. 9 a). The light response of this cell did not return to its former size when it was again bathed in the normal seawater. The steady phase was reduced to about 65% of its initial value and the initial phase was reduced to about 80% of its starting value (Fig. 9 c). The recovery of the response depends critically on the length of time that the cell remains in the low calcium solution and also to what extent the external calcium concentration is lowered. If a chelating agent such as

EDTA is added to the low calcium seawater to reduce the available calcium further, the enhancement of the response occurs much more rapidly and the recovery in normal seawater is much poorer even after short exposures to the EDTA. It might be noted here, that although EDTA also reduced the effective magnesium concentration this would not affect the response since by direct measurement the light response is insensitive to large variations in the external magnesium concentration.

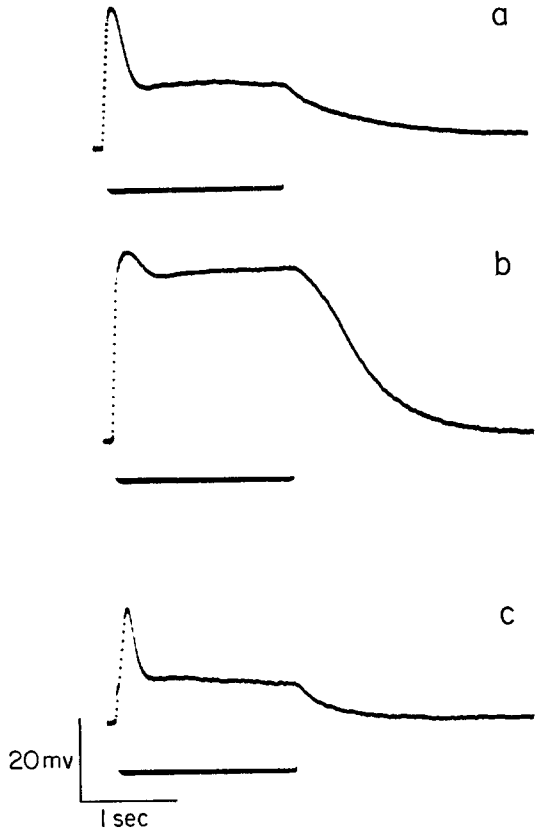

FIGURE 9. Effect of low external calcium on the receptor potential. The responses of a cell in (a) normal seawater, (b) calcium-free seawater, and (c) normal seawater again are shown. The intensity and the duration of the light stimulus (bar below each record) were the same throughout the experiment; 2 sec light flash delivered every 10 sec.

The slow potential fluctuations are also affected by reducing the external calcium concentration. The fluctuations can be four times as large as those in normal calcium (Fig. 10). The frequency of the fluctuations appears to be unaffected by lowering the external calcium concentration, but this has not been thoroughly investigated.

The amplitudes of the various phases of the light response are rather insensitive to increases in the external calcium concentration. The transient and steady phases are decreased by only 25% when the calcium concentration is increased by as much as fivefold (to 50 mM). The slow potential fluctuations are also quite insensitive to increases in the external calcium.

ANIONS Sulfate, acetate, isethionate, and propionate were used as the major anions in the artificial seawater to investigate the role chloride might play in the generation of the receptor potential. Of the four anions tested, only propionate had an effect on the resting potential and the receptor potential. The cell was rapidly depolarized 15–20 mv in the propionate and the receptor potential was concomitantly reduced. The recovery of the receptor potential was usually quite poor. The effects did not seem to be due to the removal of chloride, but rather to the presence of propionate.

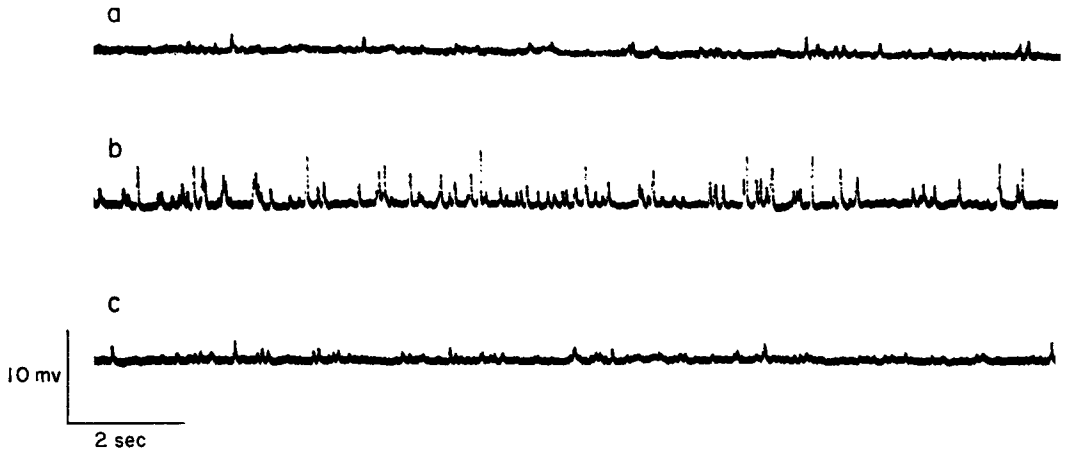

FIGURE 10. Effect of low external calcium on the slow potential fluctuations. Responses of the cell to a constant low level of illumination in (a) normal seawater, (b) calcium-free seawater, and (c) normal seawater again are shown.

DISCUSSION

The general properties of the ventral photoreceptor cells of *Limulus* are quite similar to those of most other invertebrate photoreceptors. Qualitatively, the over-all shape of the receptor potential resembles that of photoreceptor potentials seen in other arthropods, in annelids, and in some molluscs. The dependence of the response on the stimulus intensity and duration, or on the state of adaptation of the cell, is not unique, and the slow potential fluctuations and the single current-evoked spike-like process have also been seen in many of the other photoreceptors. But the ventral photoreceptors of *Limulus* are much larger and more accessible than any of the other photoreceptors. Thus we have a photoreceptor whose properties can be easily studied and which can serve as a model photoreceptor. There are also two other photoreceptor systems in *Limulus* which are composed of cells with large somas similar to the ventral photoreceptors, namely, the "endoparietal eyes" and "rudimentary lateral eyes" (Patten, 1912; DeMoll, 1914; Hanström, 1926). Although our studies on these cells have not been thorough, all the proper-

ties which we have observed are similar to those of the ventral photoreceptors.

The absence of propagated nerve impulses either with a light stimulus or an applied current appears to be a property common to many photoreceptor cells. Washizu (1964) in his study of the retinular cells in the eye of *Calliphora* failed to find any evidence of nerve impulses. Indeed, he cited this property as a distinct advantage since the primary electrical response could be studied without being hindered by impulse activity. In the ommatidium of the lateral eye of *Limulus* it is generally agreed that the retinular cells give rise to a generator potential with a single regenerative spike which fails to leave the cell. The impulses which are recorded from an ommatidium originate in the eccentric cell. This has been established by Behrens and Wulff (1965) in their studies of ommatidia which are found by chance to be free of eccentric cells as confirmed by intracellular staining and histological examinations. Very recently Baumann (1968) working with the eye of the honeybee drone reported only five instances of repetitive firing of nerve impulses out of 1000 cells; he ascribed the repetitive firing to damaged preparations. Finally Eichenbaum and Goldsmith (1968) in a tissue transplant study of embryonic cockroach eye tissue found no evidence of impulse activity from the earliest stages to maturation.

The absence of impulse activity in an axonal structure does not imply that the neuron as a whole fails to serve a sensory function. For example, Gwilliam (1963, 1965) has shown that the lateral ocelli of the barnacle, whose axons do not conduct impulses, activate the motor neurons to the adductor muscle which mediates the shadow reflex. Recently, Ripley, Bush, and Roberts (1968) and Bush and Roberts (1968) demonstrated the existence of a muscle receptor in the leg of the crab which responds to stretching without producing propagated impulses, yet causes propagated activity in the motor neurons in its reflex arc. Both these systems are able to stimulate their second-order neurons because their axons are relatively short; i.e., the passive electrotonic spread of the receptor potentials are not decremented appreciably.

The paradox still remains as to how the ventral photoreceptors of *Limulus* exert their effects on second-order neurons. The nearest proximal synapse is probably several centimeters from the cell bodies, making passive spread an unlikely means of activation.

Ionic Studies

POTASSIUM The membrane potential of the ventral photoreceptors is relatively insensitive to changes in the external [K] at the lower concentration range, in a way that is similar to many other cells, for example, the squid axon (Curtis and Cole, 1942; Hodgkin and Katz, 1949), the frog muscle fiber (Ling and Gerard, 1950), the myelinated nerve of the frog (Huxley and

Stämpfli, 1951), and the lobster axon (Julian, Moore, and Goldman, 1962). Several different mechanisms could explain this relative insensitivity. One possibility is that a nonspecific "shunt" occurs in the membrane, as a result of damage caused by the insertion of the microelectrode. But the fact that the effective resting resistance of the ventral photoreceptors is very large (10 MΩ for a 100 μ cell) tends to rule out this possibility.

Another mechanism is that other ions contribute to the resting potential; i.e., the membrane is not permselective to K ions. This has been a classical mechanism used in nerve and muscle physiology to "explain" the considerable deviation from the Nernst equation seen in the physiological range of K concentration (Adrian, 1956). A special case of this mechanism would be a "leakage" system of current which is in parallel with the potassium system, as is postulated for the giant axon of the squid and the node of Ranvier in the frog.

Still another hypothesis, worthy of consideration, is that not all of the cell's surface is accessible to the bulk external medium. If the cell membrane were exclusively permeable to potassium, but an appreciable portion of the cell surface were shielded from changes in the external solution, the potential would vary in the observed manner when the external [K] is changed. Indeed, ultrastructural studies have revealed a complex interrelationship between the photoreceptor cells and the closely apposed glial cells (Clark et al., 1969). The photocell surface is highly invaginated with microvillous projections arising from these invaginations. The glial cells send processes into these invaginations, forming intimate associations with the microvillous membranes. Tight junctions between the photocell membrane and the glial membrane have also been seen. This highly convoluted surface, closely coupled with the glial processes, could be the hypothetical barrier which limits the exchange of ions between the bulk external solution and extracellular spaces.

Two examples of cells whose membrane potentials are totally dependent on the external [K] are the glial cells in the central nervous system of the leech (Kuffler and Potter, 1964) and the glial cells in the optic nerve of the mud puppy, *Necturus* (Kuffler, Nicholls, and Orkand, 1966). In both instances, the resting potential of the glial cells is at the K equilibrium potential and changes as a function of the external potassium ion concentration as predicted by the Nernst equation for a membrane exclusively permeable to K, over the range of 1.5 to 75 mM (Kuffler and Nicholls, 1966).

SODIUM The light response of the ventral photoreceptors is affected by changes in the external Na concentration in a very complex way. Initially, the receptor potential seems to be totally dependent on Na, since it is completely abolished within a few minutes in a sodium-free solution. However, the receptor potential does not remain blocked but recovers partially while

the cell remains in the sodium-free medium. It will be noted in the following paper (Millecchia and Mauro, 1969) that while the receptor *potential* can recover 50–60% of its normal value in the sodium-free solution, the *currents* which underlie the receptor potential recover only 10% of their normal value. This fact should be kept in mind when evaluating similar phenomena; that is, potential changes are an adequate measure of the cell's membrane properties only when the conductances of the system are linear. Indeed if the conductances are voltage-dependent as in these cells the potential changes can be quite misleading.

One mechanism which would explain the partial recovery of the receptor potential is that the perfusion of the chamber with the sodium-free solution does not bring about a complete removal of the sodium ions whereupon "trapped" ions slowly diffuse to the cell surface. This mechanism does not explain the observation that once the receptor potential has partially recovered it cannot be reduced any further by continuously perfusing the cell with a sodium-free solution.

Another mechanism which could explain the partial recovery is that other ions contribute to the light response. The light response would initially be a pure sodium response, but when the external sodium is removed the cell becomes modified in such a way that it can now use other ions to produce the light response. This modification occurs slowly enough that initially a blockage phase occurs and then gradually the recovery phase develops.

A third hypothesis is that the glial cells which intimately surround the photocell "secrete" sodium into the extracellular space between the cells. Such a mechanism has been proposed by Treherne and Maddrell (1967) to explain the normal function of the nerve of the walking stick insect, despite the fact that its hemolymph contains high potassium and low sodium. If this mechanism is to explain the behavior of the ventral photoreceptors then the secretion process must be influenced by the external sodium concentration. To explain the time course of the cell's response to low sodium, the secretion would have to be essentially zero when the external sodium concentration is normal; then slowly increase when the external sodium concentration is reduced at such a rate as to give rise to the "blockage" phase and the partial recovery phase.

The ability of sensory receptors to continue to respond in sodium-free solutions has been seen in many systems, the Pacinian corpuscle (Diamond, Gray, and Inman, 1958), the crayfish stretch receptor (Edwards, Terzuolo, and Washizu, 1963; Obara and Grundfest, 1968; Obara, 1968), and the muscle spindle (Ottoson, 1964; Calma, 1965). The initial blockage phase has not been reported previously. The presence of this phase in the ventral photoreceptor cells may be due to the rapid exchange of the solution right at the cell's surface. If, in fact, the solution is exchanged slowly the initial

blockage phase is not observed and the light response is simply reduced to the level of the partial recovery phase.

Dr. H. K. Hartline was the first to observe a light-evoked electrical response in the "olfactory nerves" of *Limulus*. We would like to thank him for telling us about his observations, and for his encouragement and interest throughout the course of this investigation.

Received for publication 1 April 1969.

REFERENCES

ADOLPH, A. R. 1964. Spontaneous slow potential fluctuations in the *Limulus* photoreceptor. *J. Gen. Physiol.* **48**:297.

ADRIAN, R. H. 1956. The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol. (London).* **133**:631.

BAUMANN, F. 1968. Slow and spike potentials recorded from retinula cells of the honeybee drone in response to light. *J. Gen. Physiol.* **52**:855.

BEHRENS, M. E., and V. J. WULFF. 1965. Light-initiated responses of retinula and eccentric cells in the *Limulus* lateral eye. *J. Gen. Physiol.* **48**:1081.

BENOLKEN, R. M. 1959. Light- and dark-adaptation studies on the graded receptor potential of the *Limulus* eye. Ph.D. thesis. The Johns Hopkins University.

BENOLKEN, R. M. 1965. Regenerative transducing properties of a graded visual response. *Cold Spring Harbor Symp. Quant. Biol.* **30**:445.

BUSH, B. M. H., and A. ROBERTS. 1968. Resistance reflexes from a crab muscle receptor without impulses. *Nature (London).* **218**:1171.

CALMA, I. 1965. Ions and the receptor potential in the muscle spindle of the frog. *J. Physiol. (London).* **177**:31.

CHAPMAN, R. M., and A. B. LALL. 1967. Electroretinogram characteristics and the spectral mechanisms of the median ocellus and the lateral eye in *Limulus polyphemus*. *J. Gen. Physiol.* **50**:2267.

CLARK, A. W., R. MILLECCHIA, and A. MAURO. 1969. The ventral photoreceptor cells of *Limulus*. I. The microanatomy. *J. Gen. Physiol.* **54**:289.

CURTIS, H. J., and K. S. COLE. 1942. Membrane resting and action potentials from the squid giant axon. *J. Cell. Comp. Physiol.* **19**:135.

DEMOLL, R. 1914. Die Augen von *Limulus*. *Zool. Jahrb. Abt. Anat. Ontog. Tiere.* **38**:443.

DIAMOND, J., J. A. B. GRAY, and D. R. INMAN. 1958. The relation between receptor potentials and the concentration of sodium ions. *J. Physiol. (London).* **142**:382.

EDWARDS, C., C. A. TERZUOLO, and Y. WASHIZU. 1963. The effect of changes of the ionic environment upon an isolated crustacean sensory neuron. *J. Neurophysiol.* **26**:948.

EGUCHI, E. 1965. Rhabdom structure and receptor potentials in single crayfish retinular cells. *J. Cell. Comp. Physiol.* **66**:411.

EICHENBAUM, D. M., and T. H. GOLDSMITH. 1968. Properties of intact photoreceptor cells lacking synapses. *J. Exp. Zool.* **169**:15.

FULPIUS, B., and F. BAUMANN. 1969. Effects of sodium, potassium, and calcium ions on slow and spike potentials in single photoreceptor cells. *J. Gen. Physiol.* **53**:541.

FUORTES, M. G. F., and A. L. HODGKIN. 1964. Changes in time scale and sensitivity in the ommatidia of *Limulus*. *J. Physiol. (London).* **172**:239.

FUORTES, M. G. F., and G. F. POGGIO. 1963. Transient responses to sudden illumination in cells of the eye of *Limulus*. *J. Gen. Physiol.* **46**:435.

FUORTES, M. G. F., and S. YEANDLE. 1964. Probability of occurrence of discrete potential waves in the eye of *Limulus*. *J. Gen. Physiol.* **47**:443.

GRAHAM, C. H., and H. K. HARTLINE. 1935. The response of single visual sense cells to lights of different wave lengths. *J. Gen. Physiol.* **18**:917.

GWILLIAM, G. F. 1963. The mechanism of the shadow reflex in cirripedia. I. Electrical activity in the supraesophageal ganglion and ocellar nerve. *Biol. Bull.* **125**:470.

GWILLIAM, G. F. 1965. The mechanism of the shadow reflex in cirripedia. II. Photoreceptor cell response, second-order responses, and motor cell output. *Biol. Bull.* **129**:244.

HANSTRÖM, B. 1926. Das Nervensystem und die Sinnesorgane von *Limulus polyphemus*. Lunds Univ. Arsskr. Avd. 2. **22**(5):1.

HARTLINE, H. K., H. G. WAGNER, and E. F. MACNICHOL, JR. 1952. The peripheral origin of nervous activity in the visual system. *Cold Spring Harbor Symp. Quant. Biol.* **17**:125.

HILLE, B. 1968. Pharmacological modifications of the sodium channels of frog nerve. *J. Gen. Physiol.* **51**:199.

HODGKIN, A. L., and B. KATZ. 1949. The effects of sodium and potassium on the electrical activity of the giant axon of the squid. *J. Physiol. (London).* **108**:37.

HUBBARD, R., and G. WALD. 1960. Visual pigment of the horsehshoe crab, *Limulus polyphemus*. *Nature (London).* **186**:212.

HUXLEY, A. F., and R. STÄMPFLI. 1951. Effect of potassium and sodium on resting potential and action potential in single myelinated nerve fibers. *J. Physiol. (London).* **112**:496.

JULIAN, F. J., J. W. MOORE, and D. E. GOLDMAN. 1962. Membrane potentials of the lobster giant axon obtained by use of the sucrose-gap technique. *J. Gen. Physiol.* **45**:1195.

KIKUCHI, R., K. NAITO, and I. TANAKA. 1962. Effect of sodium and potassium ions on the electrical activity of single cells in the lateral eye of the horseshoe crab. *J. Physiol. (London).* **161**:319.

KUFFLER, S. W., and J. G. NICHOLLS. 1966. The physiology of neuroglial cells. *Ergeb. Physiol.* **57**:1.

KUFFLER, S. W., J. G. NICHOLLS, and R. ORKAND. 1966. Physiological properties of glial cells in the central nervous system of amphibia. *J. Neurophysiol.* **29**:768.

KUFFLER, S. W., and D. D. POTTER. 1964. Glia in the leech central nervous system. Physiological properties and neuron-glia relationship. *J. Neurophysiol.* **27**:290.

LING, G., and R. W. GERARD. 1950. External potassium and the membrane potential of single muscle fibers. *Nature (London).* **165**:113.

MILLECCHIA, R., J. BRADBURY, and A. MAURO. 1966. Simple photoreceptors in *Limulus polyphemus*. *Science.* **154**:1199.

MILLECCHIA, R., and A. MAURO. 1969. The ventral photoreceptor cells of *Limulus*. III. A voltage-clamp study. *J. Gen. Physiol.* **54**:331.

MURRAY, G. 1966. Intracellular absorption difference spectrum of *Limulus* extraocular photolabile pigment. *Science.* **154**:1182.

NAKAMURA, Y., S. NAKAJIMA, and H. GRUNDFEST. 1965. The action of tetrodotoxin on electrogenic components of squid giant axons. *J. Gen. Physiol.* **48**:985.

NARAHASHI, T., J. W. MOORE, and W. R. SCOTT. 1964. Tetrodotoxin blockage of sodium conductance increases in lobster giant axons. *J. Gen. Physiol.* **47**:965.

OBARA, S. 1968. Effects of some organic cations on generator potential of crayfish stretch receptor. *J. Gen. Physiol.* **52**:363.

OBARA, S., and H. GRUNDFEST. 1968. Effects of lithium on different membrane components of crayfish stretch receptor neurons. *J. Gen. Physiol.* **51**:635.

OTTOSON, D. 1964. The effects of sodium deficiency on the response of the isolated muscle spindle. *J. Physiol. (London).* **171**:109.

PATTEN, W. 1912. The Evolution of the Vertebrates and Their Kin. P. Blakiston's Son and Co., Philadelphia.

PATTEN, W., and W. A. REDENBAUGH. 1899. The nervous system of *Limulus polyphemus*. *J. Morphol.* **16**:91.

RIPLEY, S. H., B. M. H. BUSH, and A. ROBERTS. 1968. Crab muscle receptor which responds without impulses. *Nature (London).* **218**:1170.

RUSHTON, W. A. H. 1961. The intensity factor in vision. *In* Light and Life. W. D. McElroy and B. Glass, editors. The Johns Hopkins Press, Baltimore.

RUSHTON, W. A. H. 1965. The Ferrier Lecture: Visual adaptation. *Proc. Roy. Soc. Ser. B. Biol. Sc.* **162**:20.

TREHERNE, J. E., and S. H. P. MADDRELL. 1967. Axonal function and ionic regulation in the central nervous system of a phytophagus insect (*Carousius morosus*). *J. Exp. Biol.* **47**:235.

WALD, G., and J. M. KRAININ. 1963. The median eye of *Limulus*: An ultraviolet receptor. *Proc. Nat. Acad. Sci. U. S. A.* **50**:1011.

WASHIZU, Y. 1964. Electrical activity of single retinular cells in the compound eye of the blowfly *Calliphora erythrocephala* Meig. *Comp. Biochem. Physiol.* **12**:369.

YEANDLE, S. S. 1957. Studies on the slow potential and the effects of cations on the electrical responses of the *Limulus* ommatidium. Ph.D. thesis, The Johns Hopkins University.