Limulus Ventral Eye

Physiological Properties of Photoreceptor Cells in an Organ Culture Medium

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ABSTRACT   Ventral photoreceptor cells bathed in an organ culture medium typically have resting potentials of −85 mV and membrane resistances of 35 MΩ and, when dark-adapted, exhibit large potential fluctuations (LPFs) of 60 mV and small potential fluctuations (SPFs) of <30 mV. LPFs appear to be regenerative events triggered by SPFs, the well-known quantum bumps. In the dark, SPFs and LPFs occur spontaneously. At intensities near threshold, the rate of occurrence is directly proportional to light intensity, indicating that SPFs and LPFs are elicited by single photon events. At higher intensities, SPFs and LPFs sum to produce a receptor potential that is graded over approximately a 9-log-unit range of light intensity. Amplitude histograms of the discrete potential waves are bimodal, reflecting the SPF and LPF populations. Histograms of current waves are unimodal. SPFs and LPFs are insensitive to 1 μM tetrodotoxin. *I-V* characteristics show initial inward currents of ~15 nA for voltage clamps to −40 mV and steady-state outward currents for all clamp potentials. Photoreceptor cells bathed in organ culture medium retain these properties for periods of at least 75 days.

INTRODUCTION

The ventral photoreceptor cells of *Limulus* are well suited for the study of basic phototransduction mechanisms (Millecchia et al., 1966). Located along the lateral olfactory nerve, these cells are often isolated from one another and are usually large enough to permit impalement with several microelectrodes. In addition, they may be illuminated directly without the interference of screening pigments or a lens structure. Partly because of these features, a great deal is now known about the physiology of the photoreceptors, including information on the ionic mechanisms contributing to the basic light response (Millecchia and Mauro, 1969 *a*, *b*), cellular processes underlying adaptation (Lisman and Brown, 1972, 1975; Brown and Blinks, 1974; Fein and DeVoe, 1973; Fein and Charlton, 1975; Fein and Lisman, 1975), and the sensitivity of the cells to chemical and pharmacological agents (Millecchia, 1969).

   In the present study we examine the properties of the ventral photoreceptor cells in an organ culture medium. The results are compared with what we and

others have found when recording from the cells in artificial seawater or *Limulus* Ringer's solutions. The impetus for our study came from experiments which showed that the physiological properties of the *Limulus* lateral eye *in situ* are significantly altered when the eye is excised from the animal (Barlow and Kaplan, 1971, 1977; Kaplan and Barlow, 1975). Later experiments by Kaplan et al. (1973) and Bayer (1975) demonstrated that the deleterious effects of excision can be averted by bathing the lateral eye in an organ culture medium.

We show that the same organ culture medium can maintain the physiological characteristics of ventral photoreceptor cells for periods of at least 75 days. Typical physiological characteristics in organ culture include resting potentials of −85 mV, cell membrane resistances of 35 MΩ, and large regenerative potentials at low levels of illumination. These properties are not typical of cells bathed in seawater.

METHODS

Biological Preparation

The lateral olfactory nerves, along which lie the ventral photoreceptors, were dissected from the animal and de-sheathed either in organ culture medium or in artificial seawater depending upon the experiment. The nerve preparation was then immersed in the appropriate bathing medium in a petri dish which was covered and stored in the dark at 2°C. On the experiment day, usually 1-7 days after the dissection, the nerve was removed from the petri dish and pinned with fine glass needles to the Sylgard base (Dow Corning Corp., Midland, Mich.) of a Plexiglas chamber. The connective tissue remaining on the cell bodies was softened by a 60-s treatment with 2% Pronase (Calbiochem, San Diego, Calif.) in buffered seawater (pH 7.3). The nerve was then placed in the recording chamber containing the desired bathing medium.

Composition of Bathing Media

Table I gives the composition of a 100-ml vol of the organ culture medium used in our experiments. This medium proved to be the most effective of the seven tested on the excised lateral eye of *Limulus* (Bayer, 1975). The effectiveness of a medium was assessed by the extent to which intracellular records from the lateral eye bathed in the medium resembled those from the lateral eye *in situ* (Kaplan et al., 1973; Barlow and Kaplan, 1977). We settled upon this indirect method for screening culture media primarily because ventral photoreceptor cells *in situ* are not accessible for intracellular recording. It appears reasonable to assume that the medium found most effective for lateral eye cells will also be effective for ventral photoreceptor cells.

The final salt concentrations in the culture medium in Table I are 410 mM NaCl, 9.0 mM KCl, 9.3 mM CaCl₂, 7.0 mM MgCl₂, and 23.8 mM MgSO₄. These values are based on the concentrations of major ions of *Limulus* serum (Parker and Cole, 1940). The medium had a pH of 7.3 and osmolality of 900 mosmol (Bayer, 1975). The concentrations of retinal and tocopherol are derived from the tissue culture studies of the ocular discs of *Drosophila* (Gottschewski, 1960). The glucose and penicillin-streptomycin concentrations are those used by Wolff (1963) for studying the structural integrity of explanted *Limulus* organs.

The final salt concentrations for the artificial seawater perfusate are 430 mM NaCl, 10 mM KCl, 10 mM CaCl₂, 20 mM MgCl₂, and 27 mM MgSO₄. The perfusate was buffered to pH 7.3 with 36 mM Tris-OH and HCl. The recording chamber for all experiments had a volume of ∼1 ml and was perfused with a gravity feed system kept at 20°C.

Electrical Recording and Voltage Clamp

Glass micropipettes were made from partition Theta tubing (William Dehn, Spencerville, Md.) and filled with 3 M KCl. Electrodes for recording the cell potential had resistances of 20-30 MΩ, measured in seawater. Electrodes for passing current under voltage-clamp conditions had a resistance of ~10 MΩ. The bath was connected to ground through a 3 M KCl-agar salt bridge, Ag-AgCl wire electrode combination. For experiments not involving voltage clamp, the recording electrode was connected to a high impedance DC bridge amplifier with capacitance compensation and 10-fold gain (Electronics Laboratories, The Rockefeller University). The amplifier contained a constant current source for passing currents into the cell through the recording electrode. The output of the

TABLE I

ORGAN CULTURE MEDIUM

Component	Volume
	ml
NaCl (2,750 mM)	10
KCl (37 mM)	10
CaCl$_2 \cdot$2 H$_2$O (80.5 mM)	10
MgCl$_2 \cdot$6 H$_2$O (70 mM)	10
MgSO$_4 \cdot$7 H$_2$O (230 mM)	10
Medium 199 w Hanks salts (10x)*	10
Horse serum*	10
Glucose (300 mM)	5
L-Glutamine (200 mM)‡	1
Retinol (*trans*)§ (2 g/liter)‖	1
Tocopherol§ (200 mg/liter)‖	1
Penicillin-streptomycin (10,000 Units/cm^3)	2
HEPES§ (50 mM)	1
TES§ (50 mM)	1
H$_2$O	18
Total volume	100

* Grand Island Biological Co., Grand Island, N.Y.

‡ Glutamine is unstable and readily divides into glutamic acid and ammonia. Therefore, glutamine was excluded from stock solutions and added directly to the culture at the time of an experiment.

§ Sigma Chemical Co., St. Louis, Mo.

‖ Because neither retinol nor tocopheral are directly water soluble, the following procedures were used to make up the stock solutions. 100 mg retinol was added to 1 ml 100% ethyl alcohol, and the solution was diluted 50 times with H$_2$O. 100 mg tocopherol (vitamin E) was added to 10 ml 100% ethyl alcohol. This solution was then diluted 500 times with H$_2$O.

amplifier could be displayed by a storage oscilloscope (D13, Tektronix, Inc., Beaverton, Ore.) and (or) by a pen recorder with a bandwidth of ~100 Hz (Brush model 220, Gould, Inc., Measurement Systems Div., Oxnard, Calif.).

The circuit used for voltage-clamping single photoreceptor cells is given in Fig. 1. Two high impedance, capacity-compensated preamplifiers, P$_1$ (10-fold gain) and P$_2$ (unity gain), monitored the membrane potentials recorded from the voltage and current electrodes, respectively. Voltage-clamp records were taken only from cells for which the amplitude and time-course of recorded membrane events were the same for both electrodes. Following P$_1$ is a compensation phase-lead network which served to stabilize the closed-loop system and also to produce a critically damped cell response to

rectangular command inputs. Amplifier A_3 was included for isolation. The negative feedback amplifier combination of A_4 (Model 40j, Analog Devices, Inc., Norwood, Mass.) and A_5 (Model 1022, Teledyne Philbrick, Dedham, Mass.) provided an overall gain of up to 10,000. The time required to clamp the membrane potential to a final value was ~1 ms. A 3 M KCl-agar salt bridge connected the bath surrounding the nerve preparation to amplifier A_6 (40j, Analog Devices), the output of which was equal to the negative of the membrane current $(-I_M)$ times the feedback resistance. A_7 (40j, Analog Devices) amplified (10-fold) and inverted the output of A_6 and was followed by a low pass filter with a time constant of 0.1-10 ms. Command potentials were rectangular voltages delivered from a Tektronix 162 waveform generator and filtered with a time constant of
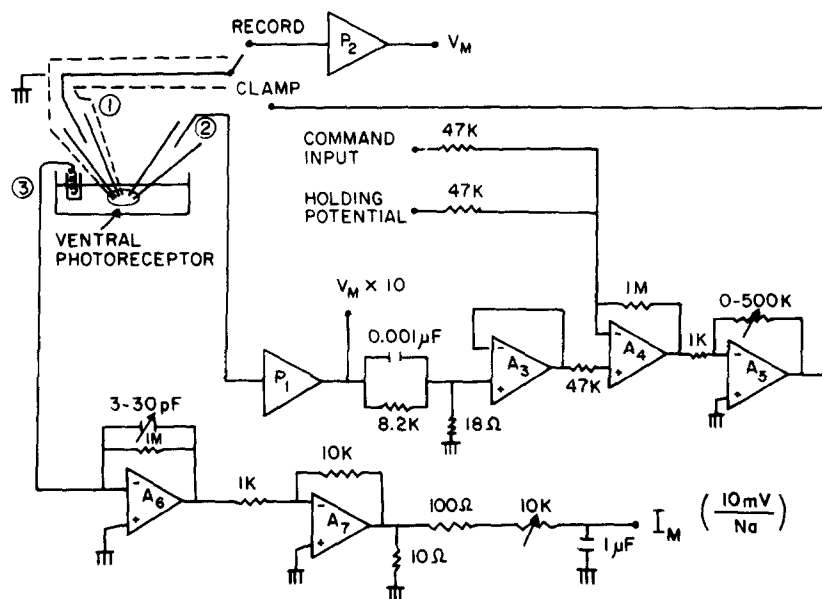

FIGURE 1. Schematic diagram of voltage-clamp circuitry. The cell voltage (V_M) was monitored by amplifiers P_1 (10-fold gain) and P_2 (unity gain). Under voltage clamp, voltages proportional to the cell potential and command inputs were compared by differential amplifier A_4. The output of A_4 was amplified and inverted by feedback amplifier A_5 which in turn passed current through the cell in a direction tending to minimize the voltage difference at the input of A_4. A salt bridge connected the bath of the recording chamber to the virtual ground input of A_6 which in combination with A_7 monitored the cell membrane current (I_M).

0.5 ms. To reduce capacitive coupling, an aluminum foil shield connected to ground surrounded the current passing electrode and lead.

Optical Stimulation and Calibration

The light source was a 45 W Quartzline lamp (type Q 45 T 2½ CL, General Electric Co., Schenectady, N.Y.) regulated at 6 A by a DC power supply. Condenser lenses focused an image of the filament in the aperture of a Uniblitz shutter (Vincent Associates, Rochester, N.Y.), which delivered flashes with rise times of 1 ms and durations as short as 5 ms. An aperture located at the condenser lens was imaged onto the stage of a compound

microscope, providing a uniform field of illumination at the level of the preparation. The unattenuated beam provided 70 μW/mm^2 between 400 and 700 nm which corresponds to about 10^{12} photons/s per cell (assuming a somal area of 10^{-2} mm^2). The optical system was calibrated with a silicon photodiode (PIN10DF, United Detector Technology, Inc., Santa Monica, Calif.). For threshold measurements the wavelength composition of the light was restricted to the region of peak spectral sensitivity for the ventral eye (Millecchia, 1969) by an interference filter (540±15 nm, Bausch & Lomb, Scientific Optical Products Div., Rochester, N.Y.). The transmission characteristic of the interference filter was determined with a spectrophotometer (model 14, Cary Instruments, Varian Associates, Palo Alto, Calif.). With the filter in place, the output of the unattenuated beam was 1.8 μW/mm^2 at the level of the preparation. This corresponds to a flux of 4.8 × 10^{10} photons/s per cell. The beam was attentuated with neutral density filters (Oriel Corp of America, Stamford, Conn.) which were also calibrated with the Cary spectrophotometer.

RESULTS

Organ Culture Medium vs. Seawater

RESTING POTENTIAL AND MEMBRANE RESISTANCE   When a single micropipette penetrated a dark-adapted ventral photoreceptor cell in the organ culture medium, a resting potential of between −65 and −120 mV was recorded. The average resting potential from a random sample of 20 cells was −85.3 ± 10.2 mV. The membrane resistance of these cells, measured at resting potential with small current pulses from a bridge amplifier, ranged from 20 to 50 MΩ with an average value of 35 ± 7.3 MΩ. The time constant of the cells, determined with 0.2-nA depolarizing current steps, was ~350 ms. The cell capacitance, calculated from these values, was 0.01 μF.

In seawater, cells typically had a resting potential of ~ −55 mV, a membrane resistance at resting potential of 8 MΩ, and a time constant of 75 ms. These values are in general agreement with those of other studies using a bathing medium of artificial seawater (e.g., Millecchia and Mauro, 1969 *a*). If current was passed to hyperpolarize a cell 10-15 mV below resting potential, the resistance often increased to 20-30 MΩ. When this did not occur, the cell was often relatively insensitive to light. Similar effects have been reported by Lisman and Brown (1971) who found that *I-V* characteristics for the photoreceptor cells generally contained a slope resistance of 8-30 MΩ for 10 mV hyperpolarization from resting potential. They suggested that cells were damaged when their slope resistance was significantly smaller than this value.

DISCRETE WAVES   Fig. 2 shows a typical intracellular recording from a dark-adapted ventral photoreceptor cell in the organ culture medium. Dim illumination elicited two populations of discrete waves. These waves share many characteristics with those recorded from retinular cells *in situ* by Barlow and Kaplan (1977), and therefore we shall follow their terminology and refer to the two populations as large potential fluctuations (LPFs) and small potential fluctuations (SPFs). For an individual cell the LPFs were of constant amplitude, rising from the cell resting potential to ~ −25 mV transmembrane potential. The average LPF amplitude for a sample of 30 cells was 60 ± 12.8 mV. For all cells the distribution of sizes of SPFs was continuous. The range depends upon

the resting potential of the cell and extends roughly from 0 to {|Resting Potential (mV)| −60}. SPFs decayed exponentially with a time constant of 200–500 ms which appeared to be related to the membrane resistance of the cell. Both SPFs and LPFs occurred spontaneously in the dark and could be elicited with dim illumination.

In artificial seawater the photoreceptor cells typically exhibited a single population of discrete waves with amplitudes < ~15 mV. However, cells having a membrane resistance that was strongly dependent on membrane potential (see Lisman and Brown, 1971) could be hyperpolarized to produce both SPFs and LPFs. Hyperpolarization produced no change in the amplitudes of discrete waves for cells with membrane resistances which were relatively independent of membrane potential.

VIABILITY MEASUREMENTS   Fig. 3 shows the response of a ventral photoreceptor cell that was stored for 75 days in organ culture medium in darkness at
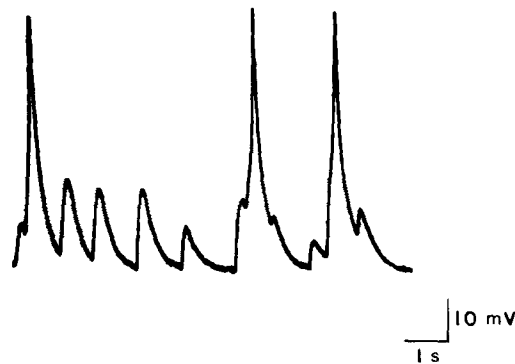

FIGURE 2.   LPFs and SPFs of a ventral photoreceptor cell bathed in the organ culture medium. Record taken after 30 min of dark adaptation. Cell was illuminated with white light at log I = −8.5. Shown are three LPFs of ~60 mV and a population of SPFs—the well-known quantum bumps. The cell resting potential was −85 mV.

2°C. The only apparent physiological change in this and in other preparations stored for more than 30 days was a slight decrease in the response to a fixed intensity flash. The decline in sensitivity for such preparations was usually <1 log unit. No changes in sensitivity were detected for cells stored <30 days in culture medium.

Photoreceptor cells stored in artificial seawater at 2°C in the dark remained viable for up to 4 days. Longer storage periods generally yielded cells with reduced resting potentials and lower responses to fixed test flashes. After ~6 days in seawater at 2°C in the dark, cells had no detectable resting potentials or light responses. These viability results are based on preparations stored in culture medium and in artificial seawater for various periods in the dark.

To determine the possible effects of light exposure on photoreceptor viability, preparations were stored at 20°C in culture medium and in artificial seawater for 24 h under relatively intense illumination (~10^{10} photons/s at the surface of

the photoreceptor). Fig. 4 shows that after 30 min of dark adaptation a cell from a preparation bathed in seawater exhibited a significantly lower response than a cell bathed in culture medium. These are typical results for preparations exposed to intense illumination for a 24-h period. Shorter exposure periods
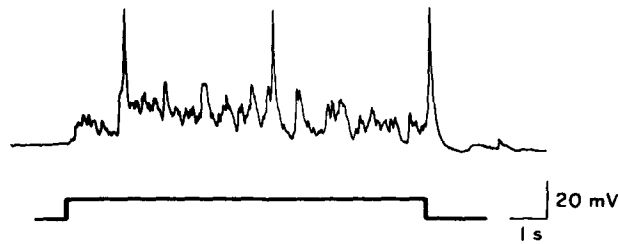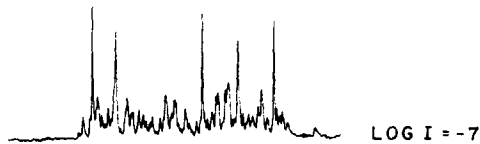
AFTER 75 DAYS IN ORGAN CULTURE

FIGURE 3.   Light response of a ventral photoreceptor after 75 days of storage in the organ culture medium. Cell was dark adapted for ~30 min before being illuminated with white light at log I = −7. Resting potential of the cell was −115 mV. The LPFs had an amplitude of ~90 mV.

ORGAN CULTURE

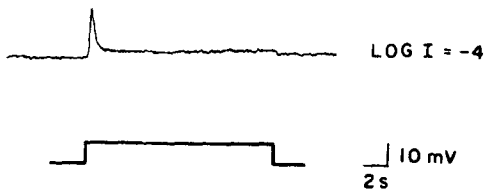LOG I = −7

ARTIFICIAL SEAWATER

LOG I = −4

FIGURE 4.   Effect of 24 h of light exposure on the responses of photoreceptor cells stored in culture medium and in artificial seawater. Responses are shown for one preparation stored in the culture medium and another stored in artificial seawater, both dark adapted for 30 min before the test flashes. The cell bathed in culture medium had a resting potential of −70 mV, generated LPFs, and exhibited a normal response to dim illumination. The cell in seawater had a resting potential of −30 mV, did not generate LPFs, and was relatively insensitive to light stimuli.

generally yielded smaller reductions in the responses from cells in seawater and near normal responses from cells in culture medium. Longer exposure periods were not tested.

INTENSITY-RESPONSE CHARACTERISTICS   Fig. 5 gives the responses of a typical cell in the culture medium to 10-s flashes covering a large range of intensities. The cell was dark adapted for 30 min at the beginning of the series and for 3 min between each test flash. The lowest test intensities elicited individual LPFs and SPFs. At moderate-to-bright intensities the receptor potential contained both transient and steady-state components.
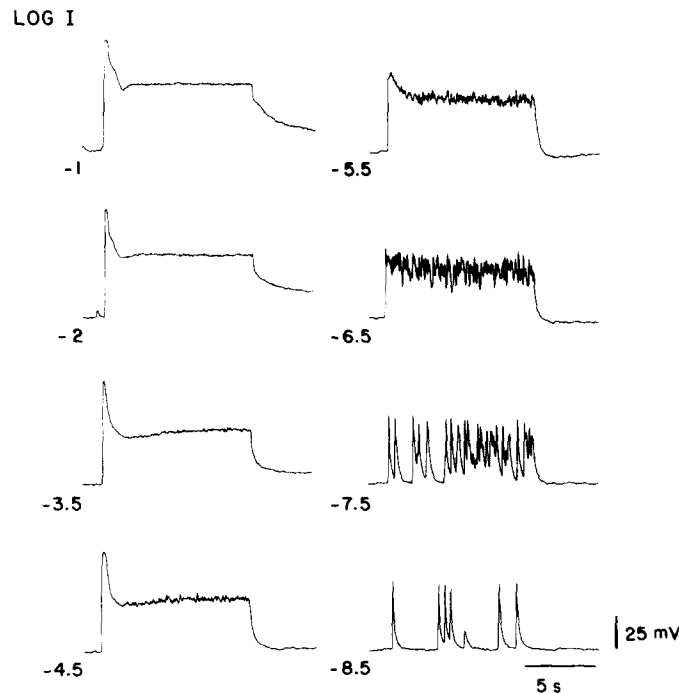
LOG I

FIGURE 5.   Responses of a ventral photoreceptor cell in culture medium over a large range of light intensities. Responses are shown to 10-s flashes of white light. At log I = −8.5 the response consists of LPFs and SPFs. At log I = −6.5 LPFs are not observed and a noisy receptor potential has formed which does not show a transient phase. A distinct transient is first observed at log I = −5.5. Cell resting potential was −85 mV.

Fig. 6 plots the time-averaged amplitude of the steady-state component of the receptor potential as a function of light intensity. The filled circles were measured from the records in Fig. 5 and the unfilled circles were taken from records of a representative cell in seawater. We note that integrating the receptor potential to produce the intensity function for steady-state response does not indicate the potential fluctuations that occur in the responses to dim light intensities (see Barlow and Kaplan, 1977). The intensity function for the cell in organ culture in Fig. 6 extends over at least a 9-log-unit range of light

intensity. At both high and low light intensities the cell's response grew roughly in proportion to log I. At intermediate intensities the time-averaged responses changed only slightly with light intensity. In contrast, the response of the cell in seawater was graded over about an 8-log-unit range, increasing in proportion to log I at intermediate and high intensities. The time-averaged potential was <1 mV for intensities dimmer than log I = −6. No plateau in the intensity-response function was apparent at intermediate intensities.
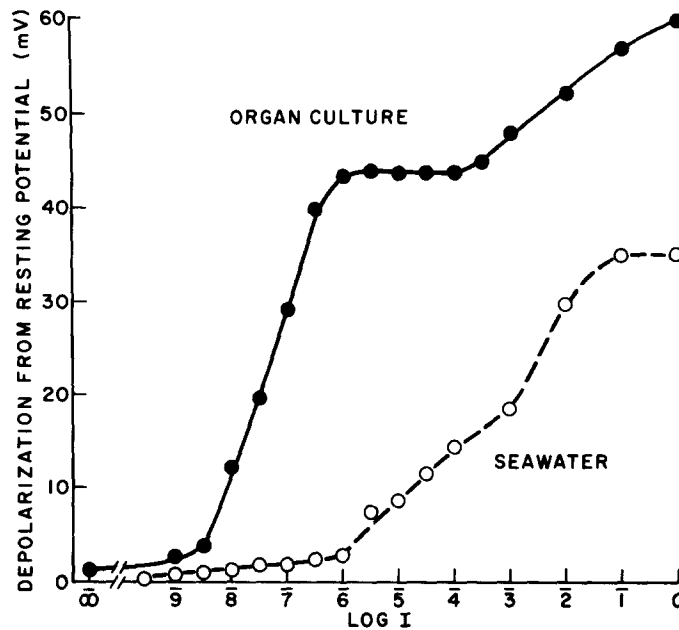

FIGURE 6.   Intensity function for steady-state response of dark-adapted cells in seawater and in culture medium. Steady-state response was defined as the time-averaged depolarization from resting potential over the last 5 s of a 10-s flash. Resting potential of the cell in culture medium was −80 mV and amplitude of its maximal steady-state response was 60 mV at log I = 0. Mean receptor potential in the plateau region was 45 mV above resting potential. Cell in seawater had a resting potential of ~ −55 mV and gave a maximal steady-state response of 35 mV at log I = −1 and 0. Data points at log I = −8.5, −9.0, and −∞ may be somewhat fortuitous because the responses at these intensities were variable. Time-averaged depolarization of the cell in seawater in the dark (log I = −∞) was only a fraction of a millivolt and is not plotted.

Table II summarizes the characteristic properties of cells in the culture medium and in artificial seawater. For the most part, the physiological results recorded from cells in artificial seawater are in agreement with those reported by other investigators (see, for example, Millecchia and Mauro, 1969 a; Lisman and Brown, 1971; Fein and DeVoe, 1973). We wish to emphasize that Table II gives typical properties for the two experimental conditions and that, with the exception of enhanced viability, all of the physiological properties which

characterize a cell in the culture medium are occasionally recorded from cells in seawater.

The physiological differences between ventral photoreceptor cells in seawater and those in culture medium (Table II) parallel to some extent the differences between retinular cells in excised eyes and those in eyes *in situ* (Barlow and Kaplan, 1977). Excising the lateral eye generally abolishes the LPFs and decreases both the membrane resistance and membrane potential of the retinular cells. However, such changes can be averted by bathing the excised lateral eye in culture medium (Kaplan et al., 1973) as we have done for the ventral photoreceptors.

LINEARITY OF RESPONSE RATE NEAR THRESHOLD Fig. 7 shows that near threshold the frequency of occurrence of discrete waves, LPFs and SPFs, is directly proportional to the light intensity incident on a single ventral photoreceptor in culture medium. Direct proportionality is indicated on the log-log coordinates by the straight line with a slope of 1.0. The detectable range of proportionality is 1.0–1.5 log units of light intensity. Above this range individual

TABLE II

PHYSIOLOGICAL PROPERTIES OF VENTRAL PHOTORECEPTOR CELLS IN ORGAN CULTURE MEDIUM AND SEAWATER

Physiological property	Artificial seawater	Organ culture	
Resting potential	−55	−85	
Resting membrane resistance, $M\Omega$	8	35	
Discrete waves, mV	<15	LPFs	SPFs
		60	<30
Shape of intensity function for steady-state response	No plateau	Plateau at intermediate light intensities	
Viability of photoreceptor			
Dark, *days*	<7	>75	
Light, *h*	<24	>24	

discrete waves were difficult to resolve and reliable measurements could not be made. Plotting the frequency of occurrence of LPFs as a function of light intensity yields a range of proportionality of about 0.75 log units. Direct proportionality between LPF rate and light intensity is strong evidence that a single photon absorption can elicit an LPF.

FREQUENCY-OF-RESPONSE CURVES Further information on the energy requirements at threshold can often be derived from frequency-of-response measurements of the type shown in Fig. 8. The filled circles give for each test intensity the proportion of flashes that elicited one or more discrete waves, LPFs or SPFs. The abscissa gives an estimate of the number of photons absorbed per flash by the visual pigment of the photoreceptor cell. The estimates are based on a somal cross-sectional area of 10^{-2} mm^2 and a visual pigment absorption of 0.5% (Murray, 1966). The curves are the Poisson sums, $P_{(n,a)}$ for $n = 1$ and $n = 2$ computed from the equation:

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

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. The shape of the curve is characteristic of the threshold n when the number of photons involved is small. This analysis provides a lower limit to the estimation of threshold (Pirenne, 1967). The good fit in Fig. 8 between theory and experiment suggests that one absorbed photon elicited a discrete wave. The abscissa values indicate, however, that the threshold for eliciting a discrete wave was 12 absorbed photons. This discrepancy, which is similar to that for other cells, may be a measure of the quantum efficiency of
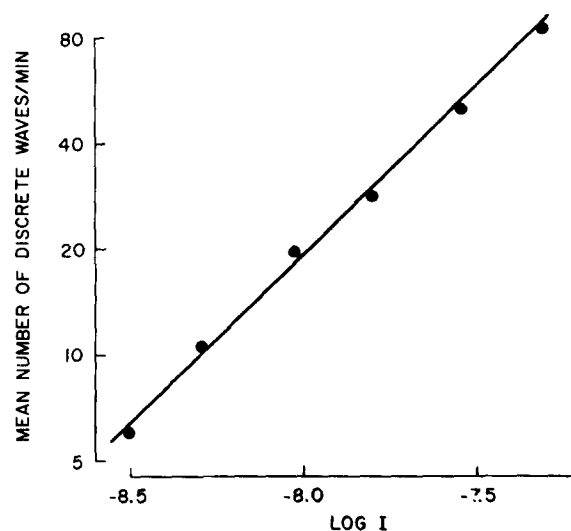

FIGURE 7.   Linearity in the rate of discrete waves of low light levels. Each data point represents the average total rate of discrete waves (LPFs and SPFs). For three lowest intensities the data points are based on 10 flash presentations of about 1 min each at 540 nm. For the three highest intensities, the data points represent averages over five flash presentations of about 1 min each. The variability in rate was about four discrete waves/min for each of the data points. Log I = −8.5 corresponds to about 50 absorbed photons/min. The line has a slope of 1.0. When SPFs are deleted from the data and only the rate of LPFs is plotted (not shown), the points deviate from the line for intensities above log I = −7.8. The close fit of the data to the line is consistent with the result of Fig. 8, single photons elicit single discrete waves.

eliciting discrete waves or it may reflect an error in estimating the number of absorbed photons.

Histograms of Discrete Waves

Dim illumination elicits both LPFs and SPFs from dark-adapted cells in organ culture. Under voltage clamp the cells respond with discrete waves of current, typically 50–100 ms in duration and 5 nA in amplitude. The peak amplitude of current waves for different cells ranged from ~2.5 to 10 nA. The total number of current waves elicited during a series of 60-s clamp periods was equal to the total number of voltage waves (LPFs and SPFs) elicited over equal periods

without clamp. A change from the voltage-clamped to the unclamped condition therefore produced no change in the average rate of discrete waves. Fig. 9 shows amplitude histograms of discrete waves for the clamped and unclamped conditions. The bimodal distribution for the unclamped condition reflects the LPF and SPF populations. The distribution under clamp is unimodal.

Fig. 10 gives the amplitude histograms of current waves for a cell in darkness and at three levels of light intensity. The spontaneous rate in the dark for the cell was ~10 current waves/min, a higher rate than normally found. The amplitudes of the current waves range up to 9 nA. The shapes of the histograms
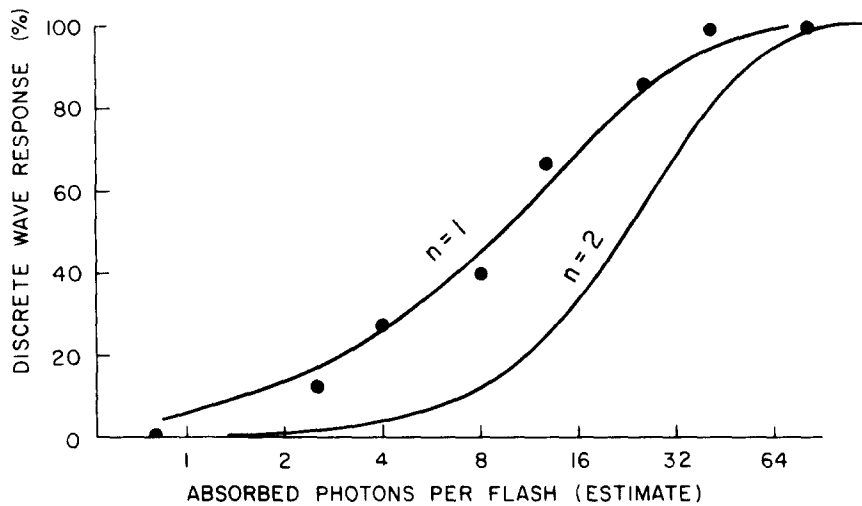

FIGURE 8. Frequency-of-response curve for a ventral photoreceptor cell in culture medium. The relative frequencies (●) of eliciting discrete wave responses, both SPFs and LPFs, at the flash intensities plotted on the abscissa. The two curves are the theoretical Poisson sums computed from Eq. 1 for $n = 1$ and 2. Each data point represents the percentage response to 25 flashes, wavelength 540 nm and duration 10 ms, minus the average rate of spontaneously occurring discrete waves. Responses were counted during the 1-s period after flash onset. Spontaneous events were noted during the 1-s period preceding the test flashes. Spontaneous events occurred in < 4% of the count periods. On the abscissa the estimated number of photons absorbed per flash is based on a 10^{-2} mm² cell soma and an absorption coefficient of 0.5%. One absorbed photon/flash corresponds to a 10-ms flash (540 nm) of log I = −6.4.

are approximately unimodal and are not strongly influenced by light intensity with the possible exception of current waves of <1.0 nA. Such waves appear relatively more numerous in darkness and at log I = −9 in Fig. 9; however, we do not know as yet if this population of waves is significantly distinct from that generated at higher intensities.

Large Potential Fluctuations (LPFs)

Fig. 11 shows an oscillograph record of six LPFs and one SPF elicited by brief flashes. The base line represents the resting potential of the impaled cell, −90

mV. The LPFs are often preceded by slow depolarizing potentials, presumably the SPFs. The slow prepotential is followed by a fast component which rises at 5 V/s and does not overshoot zero membrane potential. The repolarization phase has three components: the first decreases 8 mV at ~ −3 V/s; a notch precedes the second phase which decays at ~300 mV/s. The final phase (not shown here) decays exponentially with a time constant of 200–500 ms which appears to depend upon the membrane resistance of the cell. The LPF is often followed by an afterhyperpolarization of up to 5 mV.
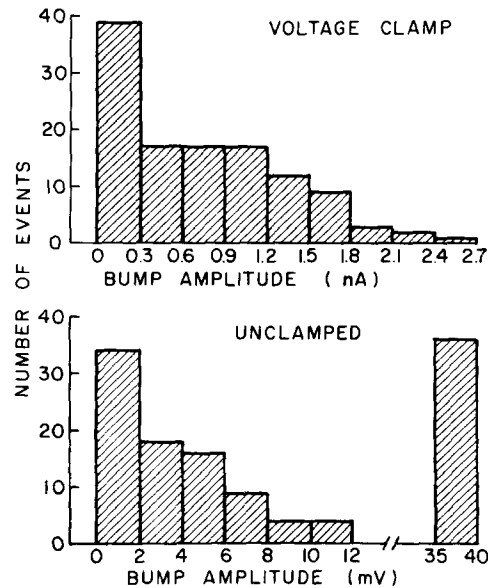

FIGURE 9. Amplitude histograms of discrete waves. Cell was bathed in the organ culture medium and illuminated with dim white light (log I = −8.5). At this intensity the total rate of discrete waves averaged ~0.5/s. Both LPFs and SPFs were elicited under the unclamped condition. There were no discrete waves (bumps) with amplitudes from 12 to 35 mV. When the same cell was voltage-clamped, a continuous distribution of current waves (bumps) between 0 and 2.7 nA was elicited. This range of current-wave sizes is smaller than normal. The data for each condition was collected over about a 5-min period. Over this period the total number of events (discrete waves) was 126 for the voltage clamp and 123 for the unclamped condition. LPFs and SPFs under the unclamped condition are reduced in size presumably as a result of trauma of the double impalement required for voltage clamp. Cell was clamped at the resting potential, −70 mV.

Fig. 12 shows responses to 10-ms flashes of different intensities. The variability and average latency of the responses are inversely related to intensity. For example, the dimmest flashes (log I = −5.5) elicited LPFs with latencies ranging from 120 to 220 ms, whereas the most intense flashes elicited responses with a nearly constant latency of ~90 ms. The sequence of recordings shows a transition from the unitary LPF response at low intensities to a more complex response at higher intensities. The complex responses appear to be composed of a single LPF followed by one or more SPFs which sum with the repolarizing
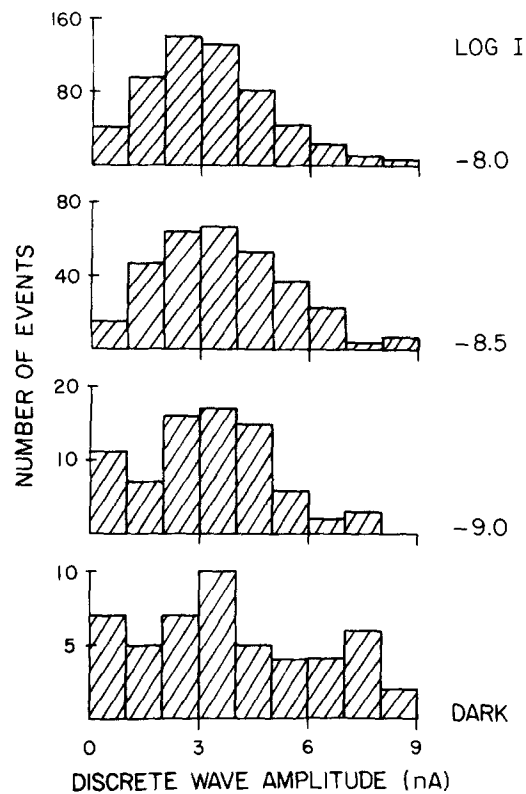
FIGURE 10. Histograms of current-wave amplitudes as a function of light intensity. Cell was bathed in organ culture medium and clamped at the resting potential, −75 mV. Current-wave responses were averaged over about a 3-min period for each of the four conditions. Individual waves were readily differentiated under each of the four experimental conditions. At the highest test intensity, log I = −3, current waves were elicited at an average rate of ~3.5 waves/s.
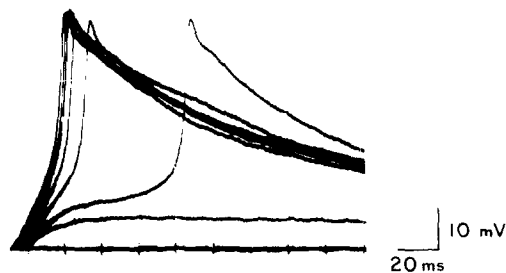

FIGURE 11. The shape of the LPF. Oscillograph recording showing six LPFs and one SPF from a single cell bathed in culture medium. The base line represents the resting potential of the cell, −90 mV. Responses were elicited by 10-ms flashes of monochromatic light (540 nm, log I = −5.5). Note that an LPF generally consists of a depolarizing prepotential, a rapid depolarization phase, and a repolarization which has an inflection (notch) at ~8 mV from the LPF peak. The final exponential decay of the LPF is not displayed. The prepotential is presumably an SPF.

phase of the LPF. The relatively smooth repolarization phase at log I = −3.5 may reflect the summation of a number of SPFs of reduced amplitude. Such a reduction in SPF amplitude could result in part from the decrease in gain (current/photon) that occurs after the onset of the light stimulus (Lisman and Brown, 1975).

The amplitude of the LPF can be reduced by a preceding LPF. Fig. 13 shows that the magnitude of the amplitude reduction is a function of the interval between two LPFs. Intervals between flashes near threshold were varied from 300 ms to 1.5 s. For the shortest interval of 300 ms, the second flash did not
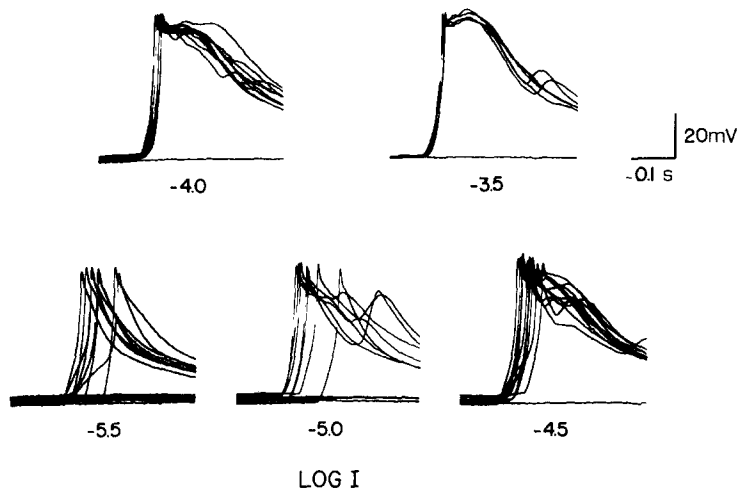

LOG I

FIGURE 12.   Responses of a ventral photoreceptor cell in culture medium to brief flashes. Records were taken from a storage oscilloscope which was triggered by the 10-ms flash of monochromatic light (540 nm). Flash onset occurred at the beginning of the traces. The base line in each record represents the cell resting potential (−90 mV). The cell responded to ∼50% of the flashes of log I = −5.5, which corresponds to an absorption of ∼10 photons/flash per cell. The cell responded to every flash of log I = −4.5 and greater. Note that as light intensity was increased, the response changed from unitary LPFs to a more complex response. At log I = −3.5 the transient component of the receptor potential has begun to form after a notch in the repolarization phase of the LPF. Also note the smaller variability and shorter latency of the responses to the higher flash intensities.

produce an LPF but instead elicited what appears to be an SPF on the falling phase of the first LPF. After a 500-ms interval, the second flash produced an LPF with an amplitude reduced by 5 mV. With progressively longer intervals the amplitude of the second LPF gradually increased and after ∼1.5 s no changes in amplitude were evident. Similar results have been obtained when the first LPF was produced by anodal break excitation with hyperpolarizing currents of ∼0.2 nA.

Tetrodotoxin at concentrations of up to 1 μM did not affect either the size or shape of the LPF. A similar result was observed by Dowling (1968) for eccentric cells of the lateral eye, and also Chappell and Dowling (1972) reported a

tetrodotoxin-resistant component in the spike-like response recorded from the dragonfly.

In summary, the data in Figs. 11, 12, and 13 are consistent with the idea that LPFs are regenerative potentials triggered by SPFs. This point is taken up in the Discussion.

Membrane Current Measurements

LIGHT-INDUCED CURRENTS   Fig. 14 gives the intensity functions for the transient and steady-state responses of a cell bathed in the culture medium. The unclamped receptor potentials (●) and clamped membrane currents (○) are plotted as functions of light intensity. We note that integrating the receptor response to produce the steady-state functions does not show either the voltage or current fluctuations that are characteristic of the responses to low light intensities.
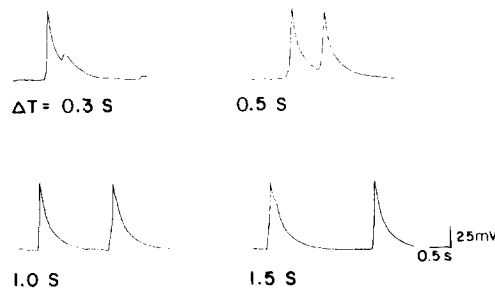

ΔT= 0.3 S   0.5 S

I.0 S   I.5 S   25mV
                              0.5s

FIGURE 13.   LPF adaptation. Shown are the responses of a cell in culture medium to pairs of 10-ms flashes of white light of log I = −7.5. The flash intensity was chosen to elicit an LPF response ~75% of the time. Results for four interflash intervals are shown. The response to the first flash of each series is an LPF of ~60 mV. The response to the second flash shows some evidence of adaptation for intervals <1 s. The second flash appears to have elicited an SPF at the shortest interval (0.3 s).

Both the steady-state response functions in Fig. 14 contain a plateau region at the midrange of light intensities. It is interesting to note that a plateau region is also characteristic of the intensity-response functions recorded from retinular cells of the lateral eye *in situ* (Barlow and Kaplan, 1977). Both of the transient curves are monotonic increasing functions in the midrange of intensities. At high intensities the amplitude of the initial transient potential saturates, whereas that of the current increases at a nearly constant rate. Transient responses were not detectable for intensities below the plateau region. Measurements of light-induced currents were not made from cells bathed in seawater.

VOLTAGE-INDUCED CURRENTS   Fig. 15 gives the current responses of a photoreceptor cell under voltage clamp to a series of rectangular command potentials. The dark-adapted cell was bathed in the organ culture medium throughout the experiment. The cell membrane potential was held at the resting potential, −80 mV. For hyperpolarizing (not shown here) and small (<20 mV) depolarizing command steps away from resting potential, the mem-

brane behaved as a passive resistance of ~30 MΩ. The membrane currents were <1.0 nA. For depolarizing command steps >20 mV, there were two components of the membrane current, an initial component, and a delayed outward component. The initial current for command potentials between −60 and −25 mV was inward. The initial current reversed at −25 mV and became outward
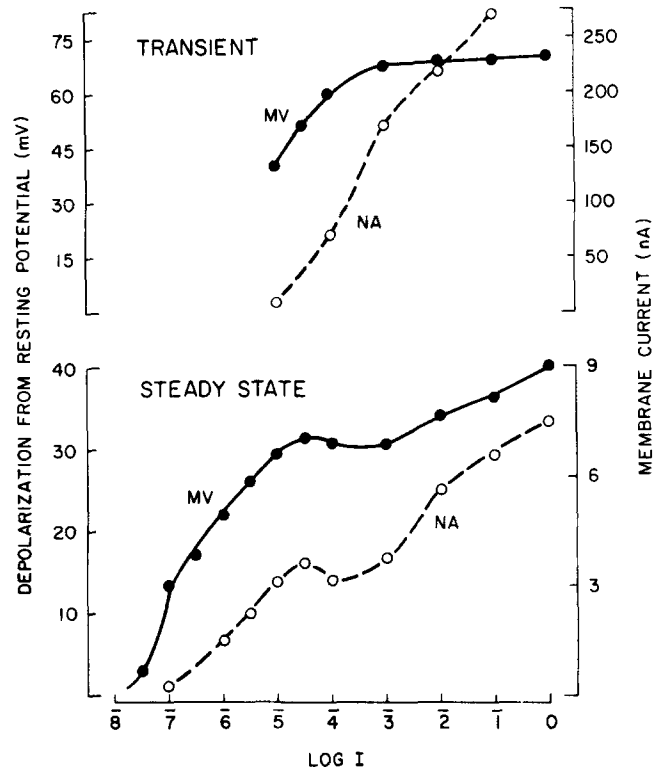

FIGURE 14. Intensity-response functions of a ventral photoreceptor cell for unclamped and voltage-clamp conditions. Cell was bathed in culture medium and exhibited LPFs. Under voltage clamp the holding potential was at −70 mV, the cell resting potential. Cell was dark-adapted for 15 min before beginning the series and for 2 min between successive trials. Data points were taken from the least intense (log I = −7.5) to the most intense (log I = 0) flashes, switching alternately from the unclamped to the voltage-clamp condition. Transient responses were not observed for intensities below log I = −5. Cell voltage could be clamped during the transient response for intensities up to and including log I = −3. For log I = −2 and −1 the data points underestimate the transient current inasmuch as the cell voltage could not be held to within better than 5 mV of the holding potential during the transient phase.

for more positive commands. These results are more clearly seen at the higher gain setting in the lower half of Fig. 15. The amplitude, time-to-peak, and total duration of the initial current depended upon the command potential. The time necessary to reach peak initial current decreased from 20 ms for a

command potential of −65 mV to ~5 ms for command potentials more positive than 0 mV. The maximum amplitude of the peak inward current was approximately 15 nA for command voltages of −40 mV.

Fig. 16 shows typical current-voltage (*I-V*) relations for the ventral photoreceptor cell measured at two times after the onset of voltage commands: 5-20 ms for the peak initial current and 50 ms for the delayed current. For clamp potentials between −100 and −70 mV, the membrane currents are time-independent. Between −70 and −25 mV, the *I-V* relation for the initial current
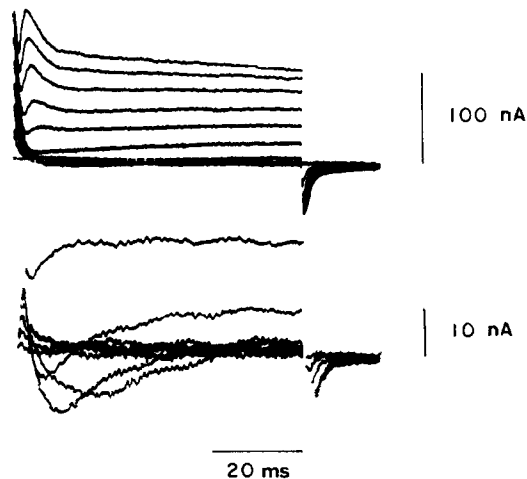

20 ms

FIGURE 15. Current responses of a ventral photoreceptor cell to depolarizing clamps. Direction of inward current is down. Holding potential of the cell was −80 mV. Lower record shows currents induced by command potentials of −80, −70, −60, −50, −40, −30, and −20 mV. Upper record show currents to commands from −80 to +30 mV in 10-mV steps. Beginning of each trace represents the onset of the command pulse. Earliest detectable current is the tail of the capacitive current surge. In the lower record the initial membrane current to commands of −80, −70, and −60 mV is passive. To commands of −50, −40, and −30 mV, the current is inward. The current reverses at ~ −25 mV. The latency to the peak initial current decreases as the command voltage is increased. In the upper record the initial inward currents cannot be resolved. The cell was bathed in culture medium and generated LPFs when not under voltage clamp.

is highly nonlinear. Over this region the clamp currents are inward, indicating an electrically active membrane process. For membrane potentials more positive than −25 mV, the *I-V* relation for the initial current is nearly linear with a slope resistance of ~0.25 MΩ. The *I-V* relation for the delayed outward current is approximately linear for membrane voltages more positive than ~ −40 mV, with a slope resistant of ~1 MΩ.

DISCUSSION

Taken as a whole, our results underscore an earlier finding of Barlow and Kaplan (1971); namely, the physiological properties of *Limulus* photoreceptors bathed in artificial seawater may not accurately reflect the properties of

receptors *in situ*. Our recordings from ventral photoreceptor cells in organ culture medium match closely those from retinular cells of the lateral eye *in situ* (Barlow and Kaplan, 1977), whereas recordings from ventral photoreceptors in seawater generally do not. These observations may have general application.

Our point is not that *Limulus* photoreceptors or any other physiological preparation must be maintained in a culture medium in order to yield meaningful results. Indeed, the data recorded from excised *Limulus* photore-
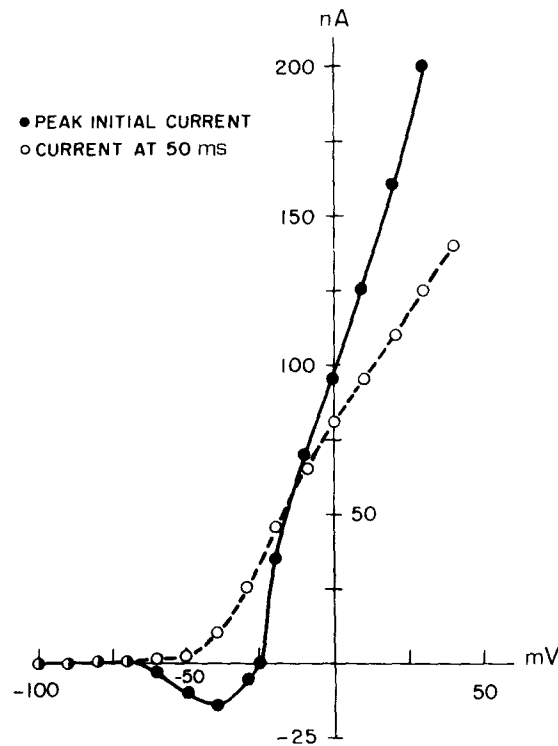

FIGURE 16. *I-V* characteristics for a ventral photoreceptor cell in the culture medium. (●) The initial membrane currents to rectangular command voltages. (○) The currents at 50 ms. Time from the onset of the clamp command to the peal initial current varied from 20 ms at −65 mV to ~5 ms for commands more positive than 0. Holding potential was at −80 mV, the cell resting potential. When not under voltage clamp the cell elicited LPFs. Cell was dark-adapted for ~30 min before the experiment.

ceptors in seawater have contributed significantly toward understanding basic mechanisms of visual excitation, visual adaptation, and neural integration. Rather we suggest that bathing a preparation in an organ culture medium may improve the quality of results and thus benefit several areas of research in visual physiology.

We discuss below the general physiological characteristics of ventral photoreceptors in culture medium and how these characteristics compare with those reported elsewhere for *Limulus* photoreceptors.

Discrete Waves

Fig. 2 shows that a ventral photoreceptor cell in culture medium exhibits two types of discrete waves, LPFs and SPFs. Kaplan et al. (1973) recorded LPFs and SPFs from retinular cells of preparations bathed in an organ culture medium, and Barlow and Kaplan (1977) recorded both types of potentials from the lateral eye *in situ*. Under optimal conditions, Dowling (1968) was able to record similar potentials from retinular cells of excised lateral eyes.

Most studies of discrete wave activity in *Limulus* photoreceptors, however, have not reported LPFs and SPFs (for a review, see Fuortes and O'Bryan, 1972). These studies, which were carried out on excised eyes in artificial seawater, frequently reported two types of discrete waves: either "fast" and "slow" waves (Adolph, 1964), or "S" and "L" waves (Borsellino and Fuortes, 1968; Srebro and Behbehani, 1971). The results are not in general agreement. Adolph (1964) proposed that the fast wave was a regenerative response based on the fact that it appeared to be preceded by a prepotential. Borsellino and Fuortes (1968) found that L waves predominated in some cells, S waves predominated in others, and frequently one wave type would be absent altogether. They found a greater dispersion of latencies for L waves than for S waves. To explain the properties of the generator potential, they proposed a model of discrete wave activity which basically was an extension of an earlier compartment model proposed by Fuortes and Hodgkin (1964).

In a similar study Srebro and Behbehani (1971) found the latency distributions for S and L waves to be the same. They indicated that the model proposed by Borsellino and Fuortes was insufficient to account for this result and proposed instead a scheme in which L waves represent propagated, regenerative responses, and S waves, nonpropagated responses. They subsequently rejected the idea that the L wave was a regenerative response (Behbehani and Srebro, 1974).

In each of the above studies the data were recorded from photoreceptors bathed in artificial seawater. The different results and interpretations may therefore reflect the physiological state of the individual preparations. Our results in Figs. 4 and 6 indicate that the characteristics of a ventral photoreceptor cell change after the cell is placed in artificial seawater. Both membrane potential and cell resistance are generally reduced in size (see Table II), and as a consequence a given current wave would produce a reduced voltage wave. The relatively small sizes of fast and slow waves reported in the above studies may therefore result from a shift in resting potential. For example, a shift in resting potential entirely into or through the negative resistance region of the *I-V* characteristic (Fig. 16) would reduce or perhaps eliminate the effectiveness of a current wave in eliciting a regenerative response. The deteriorated condition of some cells in seawater may be sufficient to eliminate the electrical excitability of the photoreceptor membrane.

Behbehani and Srebro (1974) reported bimodal distributions of discrete wave sizes for both voltage-clamp and unclamped recordings from ventral photoreceptors in seawater. They found that the percentage of small waves increased when a second electrode was inserted into a cell and suggested that the

population of smaller waves resulted from damaged patches of cell membrane. Our results in Fig. 9 show that ventral photoreceptors in an organ culture medium yield a bimodal distribution of discrete waves for the unclamped condition and a unimodal distribution under voltage clamp. However, we also found an increase in small waves (≤1 mV) upon penetration of a cell with a second electrode, but the increase was not significant relative to the frequency of SPFs and LPFs. One effect of bathing cells in an organ culture medium may be to minimize the relative number of damaged membrane patches.

Yeandle and Spiegler (1973) reported that a ventral photoreceptor cell generates two statistically independent classes of waves, large light-evoked waves and small spontaneous waves. They found that increases in light intensity increased the relative proportion of large waves. For singly impaled cells in organ culture, we found that both classes of discrete waves, LPFs and SPFs, occur spontaneously in the dark and may be elicited by light. The frequency of occurrence of LPFs relative to SPFs decreases with light intensity, an effect which appears to reflect the regenerative nature and refractory characteristic of the LPF (Fig. 13). For doubly impaled cells we observed a slight increase in the proportion of small waves, an effect which may result from membrane damage. The relative frequency of the smallest population of current waves (≤1 nA) decreases with increasing light intensity (Fig. 10), a result similar to that reported by Yeandle and Spiegler (1973).

Nature of the Large Potential Fluctuations (LPFs)

LPFs appear to be regenerative events produced by the electrical excitability of the photoreceptor membrane. Properties which point to a regenerative process are as follows. First, the peak of the depolarizing potential of the LPF is followed initially by a rapid phase of repolarization (8 mV) and then by a slow phase of repolarization (Fig. 11). Second, the peak amplitude of the LPF depolarization has a fixed value of $\sim$ −25 mV. Third, depolarizing voltage clamps reveal substantial inward currents indicative of an electrically excitable membrane (Fig. 16). And finally, small current pulses which depolarize the photoreceptor membrane potential into the "negative resistance" region of the I-V characteristic, between −60 and −40 mV, generate all-or-none LPFs. The membrane behaves passively for depolarizations from resting potential to < −60 mV, and LPFs are not elicited.

The peak amplitude of the LPF depolarization (−25 mV; Fig. 11) has about the same value as the reversal potential for the initial inward current observed under voltage clamp (Fig. 16). Lisman and Brown (1971) reported a similar value for the reversal potential of inward current. Such a reversal potential is not readily interpreted in terms of a single ion. The equilibrium potentials of sodium and calcium are too high and those of potassium and chloride appear too low. It is possible, however, that the observed reversal potential reflects the increase in conductance to two ions such as calcium and potassium.

LPFs appear to be triggered by SPFs. This result is suggested by the finding that LPFs are often preceded by slow depolarizing potentials (Figs. 2, 11, and 12). Several LPFs in Fig. 12 (−5.5 log I) appear to be triggered by depolarizing

prepotentials of ~20 mV which is approximately the peak amplitude of the SPFs recorded from this cell. Barlow and Kaplan (1977) also observed that SPFs appear to trigger LPFs in retinular cells of the *Limulus* lateral eye.

THE SPIKE-LIKE RESPONSE   Responses of ventral photoreceptors to increases in flash intensity change from a unitary LPF to a response with the fast rising phase of the LPF followed by a graded, depolarizing component (Fig. 12). At the highest test intensities the graded component overshadows the LPF. What remains is an inflection on the rising phase of the response. For flashes of greater duration, this combined response is the transient of the receptor potential.

Others have detected the initial fast rising phase of receptor potential and generally termed it the spike-like response (Benolken, 1959; Yeandle, 1967; Millecchia, 1969; Wulff and Mueller, 1973). A similar spike-like response has been reported for retinular cells of the honeybee drone eye (Bauman, 1968) and for cells of the dragonfly eye (Chappell and Dowling, 1972). In *Limulus* the differences between the spike-like responses and LPFs appear minor. Both potentials may result from the same regenerative membrane process. The fact that the spike-like response can be recorded from *Limulus* cells which do not exhibit LPFs may reflect changes in the membrane characteristics. Specifically, in healthy cells with large membrane resistance, small currents (5 nA) can shift the transmembrane potential rapidly through the negative resistance region of the I-V characteristics and thereby elicit a regenerative response. In partially deteriorated cells having lower membrane resistance, small currents are ineffective, but large currents can elicit regenerative responses for cell resting potential $>$~ -40 mV, the potential at which the slope of the initial current function changes sign.

Photon Sensitivity at Threshold

Yeandle (1958) first observed discrete waves in the lateral eye of *Limulus* and suggested that they resulted from the absorption of single photons. He based his conclusion on the random occurrence of discrete waves at low light levels and the fact that the probability of their occurrence as a function of flash intensity could be described approximately by a Poisson sum of $n = 1$. Supporting evidence has been reported by Adolph, 1964; Fuortes and Yeandle, 1964; Borsellino and Fuortes, 1968; and Barlow and Kaplan, 1977.

The generation of discrete waves by single photon events is also apparent in our records from ventral photoreceptor cells. Note that in Fig. 8 the Poisson curves indicate a threshold of one photon, but the abscissa gives a value of 12 absorbed photons. Such discrepancies were common and may indicate a low value for the quantum efficiency of eliciting discrete waves or an error in estimating the number of absorbed photons.

Stronger evidence that a single photon event can elicit a discrete wave is the linearity of the response rate in Fig. 7. Rough estimates of the number of absorbed photons in Figs. 7 and 8 yield quantum efficiencies of ~0.1. Measurements from other ventral eye preparations yield values ranging from 0.03 to 0.13. Quantum efficiencies of 0.05–0.2 have been reported for cells of the lateral eye (Kaplan and Barlow, 1976).

Intensity-Response Function

The intensity function for the steady-state response of cells in the culture medium contains a prominent plateau (Fig. 6). This characteristic shape may have several contributing factors. First, the intensity function for membrane current contains a plateau over the same range of intensities (Fig. 14). Second, the steady-state I-V characteristic exhibits substantial rectification between -70 and -40 mV (Fig. 16). Over this voltage range the cell resistance decreases from ~ 30 MΩ to 1 MΩ and thereby reduces the effectiveness of light-induced current in producing membrane depolarization. Third, LPFs are the principal component of the receptor potential at low intensities and SPFs predominate at high intensities (Fig. 5). The shift from LPFs to SPFs at intermediate intensities may influence the shape of the intensity function (Barlow and Kaplan, 1977). The first of these possibilities may not be an important factor because cells have been recorded which exhibit a plateau in the intensity function for receptor current but do not show a plateau in the function for receptor potential (Lisman, 1971). Membrane rectification could be a contributing factor because cells which exhibit substantial rectification for hyperpolarizing currents produce distinct plateaus. The shift from LPFs to SPFs may contribute in conjunction with membrane rectification inasmuch as small depolarizing currents generate LPFs only in cells with high membrane resistance.

REFERENCES

ADOLPH, A. R. 1964. Spontaneous slow potential fluctuations in the *Limulus* photoreceptor. *J. Gen. Physiol.* **48:**297–322.

BARLOW, R. B., JR., and E. KAPLAN. 1971. *Limulus* lateral eye: properties of receptor units in the unexcised eye. *Science (Wash. D.C.).* **174:**1027–1029.

BARLOW, R. B., JR., and E. KAPLAN. 1977. Properties of visual cells in the lateral eye of *Limulus in situ:* intracellular recordings. *J. Gen. Physiol.* **69:**203–220.

BAUMAN, F. 1968. Slow and spike potentials recorded from retinular cells of the honeybee drone in response to light. *J. Gen. Physiol.* **52:**855–875.

BAYER, D. S. 1975. *Limulus* ventral eye: physiological properties of photoreceptor cells in organ culture. Doctoral Dissertation. Syracuse University, Syracuse, N.Y.

BEHBEHANI, M., and R. SREBRO. 1974. Discrete waves and phototransduction in voltage-clamped ventral photoreceptors. *J. Gen. Physiol.* **64:**186–200.

BENOLKEN, R. M. 1959. Light- and dark-adaptation studies on the graded receptor potential of the *Limulus* eye. Doctoral Dissertation. The Johns Hopkins University, Baltimore, Md.

BORSELLINO, A., and M. G. F. FUORTES. 1968. Responses to single photons in visual cells of *Limulus. J. Physiol. (Lond.).* **196:**507–539.

BROWN, J. E., and J. R. BLINKS. 1974. Changes in intracellular free calcium concentration

during illumination of invertebrate photoreceptors. Detection with aequorin. *J. Gen. Physiol.* **64**:643–665.

CHAPPELL, R. L., and J. E. DOWLING. 1972. Neural organization of the median ocellus of the dragonfly. *J. Gen. Physiol.* **60**:121–165.

DOWLING, J. E. 1968. Discrete potentials in the dark-adapted eye of *Limulus*. *nature (Lond.).* **217**:28–31.

FEIN, A., and J. S. CHARLTON. 1975. Local adaptation in the ventral photoreceptors of *Limulus*. *J. Gen. Physiol.* **66**:823–836.

FEIN, A., and R. DeVOE. 1973. Adaptation in the ventral eye of *Limulus* is functionally independent of the photochemical cycle, membrane potential, and membrane resistance. *J. Gen. Physiol.* **61**:273–289.

FEIN, A., and J. LISMAN. 1975. Localized desensitization of *Limulus* photoreceptors produced by light or intracellular calcium ion injection. *Science (Wash. D.C.).* **187**:1094–1096.

FUORTES, M. G. F., and A. L. HODGKIN. 1964. Changes in time scale and sensitivity in the ommatidia of *Limulus*. *J. Physiol. (Lond.).*, **172**:239–263.

FUORTES, M. G. F., and P. M. O'BRYAN. 1972. Responses to single photons. *In* Physiology of Photoreceptor Organs, Handbook of Sensory Physiology, Vol. VII/2. M. G. F. Fuortes, editor. Springer-Verlag, Berlin. 321–338.

FUORTES, M. G. F., and S. S. YEANDLE. 1964. Probability of occurrence of discrete potential waves in the eye of *Limulus*. *J. Gen. Physiol.* **47**:443–463.

GOTTSCHEWSKI, G. H. M. 1960. Morphogenetische Untersuchungen an *In Vitro* wachsenden Augenanlagen von *Drosophila melanogaster*. *Wilhelm Roux' Arch. Entwicklungsmech. Org.* **152**:204.

KAPLAN, E., and R. B. BARLOW, JR. 1975. Properties of visual cells in the lateral eye of *Limulus in situ*. Extracellular recordings. *J. Gen. Physiol.* **66**:303–326.

KAPLAN, E., and R. B. BARLOW, JR. 1976. Energy, quanta, and *Limulus* vision. *Vision Res.* **16**:745–751.

KAPLAN, E., D. S. BAYER, and R. B. BARLOW, JR. 1973. *Limulus* lateral eye: intracellular recordings *in situ* and in organ culture. *Biol. Bull. (Woods Hole).* **145**:442.

LISMAN, J. 1971. An electrophysiological investigation of the ventral eye of the horseshoe crab, *Limulus polyphemus*. Doctoral Dissertation. Massachusetts Institute of Technology, Cambridge, Mass.

LISMAN, J. E., and J. E. BROWN. 1971. Two light-induced processes in the photoreceptor cells of *Limulus* ventral eye. *J. Gen. Physiol.* **58**:544–561.

LISMAN, J. E., and J. E. BROWN. 1972. The effects of intracellular iontophoretic injection of calcium and sodium ions on the light response of *Limulus* ventral photoreceptors. *J. Gen. Physiol.* **59**:701–719.

LISMAN, J. E., and J. E. BROWN. 1975. Light-induced sensitivity changes in *Limulus* photoreceptor. *J. Gen. Physiol.* **66**:473–488.

MILLECCHIA, R. 1969. The ventral photoreceptor cells of *Limulus*: an electrophysiological study. Doctoral Dissertation. The Rockefeller University, New York.

MILLECCHIA, R., R. BRADBURY, and A. MAURO. 1966. Simple photoreceptors in *Limulus polyphemus*. *Science (Wash. D.C.).* **154**:1199–1201.

MILLECCHIA, R., and A. MAURO. 1969 *a*. The ventral photoreceptor cells of *Limulus*. II. The basic photoresponse. *J. Gen. Physiol.* **54**:310–330.

MILLECCHIA, R., and A. MAURO. 1969 *b*. The ventral photoreceptor cells of *Limulus*. III. A voltage-clamp study. *J. Gen. Physiol.* **44**:331–351.

MURRAY, G. 1966. Intracellular absorption difference spectrum of *Limulus* extra-ocular photolabile pigment. *Science (Wash. D.C.).* **154:**1182–1183.

PARKER, R. C., and W. H. COLE. 1940. Studies of the body fluids and sera of some marine invertebrates. *Bull. MT. Desert Isl. Biol. Lab.*

PIRENNE, M. H. 1967. Vision and the Eye. Chapman and Hall Ltd., London. 224 pp.

SREBRO, R., and M. BEHBEHANI. 1971. A stochastic model for discrete waves in the *Limulus* photoreceptor. *J. Gen. Physiol.* **58:**267–286.

WOLFF, E. M. 1963. Sur l'explanation *in vitro* de fragments d'organes de limules (Xiphosura polyphemus). *Ann. Epiphyt. (Paris).* **14:**113.

WULFF, V. J., and W. J. MUELLER. 1973. On the origin of the receptor potential in the lateral eye of *Limulus. Vision Res.* **13:**661–671.

YEANDLE, S. 1958. Evidence of quantized slow potentials in the eye of *Limulus. Am. J. Ophthalmol.* **46:**82–87.

YEANDLE, S. S. 1967. Some properties of the components of the *Limulus* ommatidial potential. *Kybernetik* **3:**250–254.

YEANDLE, S., and J. B. SPIEGLER. 1973. Light-evoked and spontaneous discrete waves in the ventral nerve photoreceptor of *Limulus. J. Gen. Physiol.* **61:**552–571.