Electrophysiological Properties of Isolated Photoreceptors from the Eye of *Lima scabra*

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ABSTRACT Photoreceptor cells were enzymatically dissociated from the eye of the file clam, *Lima scabra*. Micrographs of solitary cells reveal a villous rhabdomeric lobe, a smooth soma, and a heavily pigmented intermediate region. Membrane voltage recordings using patch electrodes show resting potentials around −60 mV. Input resistance ranges from 300 MΩ to >1 GΩ, while membrane capacitance is of the order of 50–70 pF. In darkness, quantum bumps occur spontaneously and their frequency can be increased by dim continuous illumination in a fashion graded with light intensity. Stimulation with flashes of light produces a depolarizing photoresponse which is usually followed by a transient hyperpolarization if the stimulus is sufficiently intense. Changing the membrane potential with current-clamp causes the early phase to invert around +10 mV, while the hyperpolarizing dip disappears around −80 mV. With bright light, the biphasic response is followed by an additional depolarizing wave, often accompanied by a burst of action potentials. Both Na and Ca ions are required in the extracellular solution for normal photoexcitation: the response to flashes of moderate intensity is greatly degraded either when Na is replaced with Tris, or when Ca is substituted with Mg. By contrast, quantum bumps elicited by dim, sustained light are not affected by Ca removal, but they are markedly suppressed in a reversible way in 0 Na sea water. It was concluded that the generation of the receptor potential is primarily dependent on Na ions, whereas Ca is probably involved in a voltage-dependent process that shapes the photoresponse. Light adaptation by repetitive flashes leads to a decrease of the depolarizing phase and a concomitant enhancement of the hyperpolarizing dip, eventually resulting in a purely hyperpolarizing photoresponse. Dark adaptation restores the original biphasic shape of the photoresponse.

INTRODUCTION

During the past few years the application of patch-clamp techniques to photoreceptor physiology has led to important advances in the study of cellular mechanisms of visual excitation. On-cell recording has allowed the demonstration of light-dependent, single-channel currents in both invertebrate (Bacigalupo and Lisman, 1983) and vertebrate cells (Matthews, 1987), while convincing evidence that cGMP is the

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final link in the transduction chain has been provided by measurements in excised membrane patches from rod outer segments (Fesenko et al., 1985). Traditional invertebrate preparations such as the *Limulus* ventral eye, which have provided a wealth of information on the visual process, including the first measurements of light-activated ionic channels, are not ideally suited for tight-seal recording because the presence of a surrounding layer of glial cells requires a laborious and delicate mechanical stripping procedure to expose the surface of the plasma membrane (Stern et al., 1982). An isolated invertebrate photoreceptor preparation would provide a convenient and useful model system to address many remaining questions in visual physiology.

In the course of a search for such a preparation, the mollusk *Lima scabra* appeared as a promising candidate because its eyes contain a large number of photoreceptors (in contrast, for example, with only five in each eye of *Hermissenda,* and three in *Balanus*). In addition, the cells appear to be relatively exposed, rather than imbedded in thick layers of connective tissue. The eyes of *Lima* (usually ~20–50) are located along the outer edge of the mantle, beneath a transparent layer of the mantle epithelium. Their basic morphology has been briefly described by Bell and Mpitsos (1968). According to these authors, the eye cup consists primarily of rhabdomeric photoreceptors surrounded by supporting cells containing screening pigment. A transparent structure, which had originally been classified as a lens, lies in front of the cup. Anatomical observations of sections through this structure revealed the presence of bundles of cilia and processes, presumably of neural origin. Mpitsos (1973) and McReynolds (1976) pointed out that this may actually be a second, distal retina, consisting of photoreceptors of the ciliary type. Axons from the photoreceptors in the two retinas give rise to separate branches of the afferent circumpallial nerve, which projects to the central ganglia of the animal.

Only a few electrophysiological investigations on the visual excitation process of this organism have appeared (Mpitsos, 1973; McReynolds, 1976; Cornwall and Gorman, 1983). Gross extracellular recordings made from bundles of fibers of the pallial nerve display both transient excitatory responses as well as "off" responses, i.e., inhibition of basal neural activity during light stimulation, followed by a burst of action potentials at the termination of the stimulus (Mpitsos, 1973). On the other hand, nerve fibers from the isolated distal retina seemingly show only the "off" response. Similar "off" discharges were also seen upon stimulation of extraocular portions of the mantle, suggesting the presence of dermal photoreceptors; however, they could never be identified morphologically. Intracellular measurements in the intact eye revealed both depolarizing and hyperpolarizing responses to light stimulation (Mpitsos, 1973), presumably arising from cells in the two retinas. Cornwall and Gorman (1983) used intracellular microelectrodes to record from the distal portion of the intact eye of *Lima,* and observed resting potentials around −45 mV and hyperpolarizing photoresponses with a saturating peak reaching about −70 mV. Experiments with current clamp and ionic substitutions revealed that the light response was accompanied by an increase of membrane conductance involving primarily K ions.

Depolarizing and hyperpolarizing photoresponses have also been documented in the eye of the scallop *Pecten irradians* (Gorman and McReynolds, 1969; McReynolds

and Gorman, 1970), making these organisms of considerable interest for physiological studies of visual transduction. However, there are inherent limitations in whole-eye preparations; these include the difficulty of positively identifying the cell type from which recordings are obtained (especially if no intracellular injection of marking dyes and subsequent histological examinations are performed), uncertainty about the extent of control of the extracellular environment, and the possible confounding that may result from synaptic interactions and/or electrotonic coupling between neighboring cells.

This report demonstrates that physiologically viable solitary photoreceptors can be obtained from *Lima* eyes by enzymatic dissociation, and shows a basic characterization of their photoresponse. A detailed study of voltage-dependent conductances is presented in the following paper, while the third article of this series will examine the photocurrent under voltage clamp.

METHODS

Subjects

Specimens of *Lima scabra* were obtained through Carolina Biological Supply Co. (Burlington, NC). Animals can be maintained for several weeks in an artificial sea water (ASW) aquarium (Instant Ocean; Aquarium Systems, Mentor, OH) at a temperature of 24°C.

Dissociation Procedure

The following protocol has been successfully used to obtain viable isolated photoreceptors: Eyes are dissected from the animal under dim red light illumination and enzymatically treated with 0.5% collagenase (type 1A; Sigma Chemical Co., St. Louis, MO) for 30–45 min and then with 0.2% trypsin (type III; Sigma Chemical Co.) for 20–30 min at 26°C. Subsequently, they are rinsed in cold ASW for 5–10 min. Dissociation is accomplished by gentle, repetitive suction and expulsion using a fire-polished Pasteur pipette. Sometimes trituration is performed in nominally 0 Ca ASW, which seems to facilitate cell dispersion. In such cases, exposure to low extracellular Ca is limited to no more than 1–2 min; otherwise, harmful effects can result (see below).

An aliquot of the cell suspension is transferred to the recording chamber, which is mounted onto the stage of an inverted microscope (Nikon Diaphot). To increase cell adhesion, the coverslip bottom of the chamber is treated overnight with a 0.1% solution of collagen in distilled water, then with a 0.5% solution of concanavalin A (Sigma Chemical Co.) in 1 M NaCl for 2 h (Bader et al., 1979), and rinsed with distilled water. Within 10–15 min after being transferred to the chamber most of the cells in the suspension are plated to the bottom, and the flow from a perfusion system that allows rapid solution changes is turned on at a rate of ~0.5–1.0 ml/min.

Scanning Electron Microscopy

Dissociated cells plated onto coverslips were fixed for 2 h in sea water containing 1% glutaraldehyde, diluted with distilled water to compensate for changes in osmolarity. After fixation the cells were dehydrated by immersion in ethyl alcohol solutions of increasing concentration (10, 25, 50, 75, 90, 95, and 100%, 5 min in each). Subsequently, they were critical-point dried, sputtered with gold palladium, and viewed with a JEOL-JSM 840 scanning electron microscope (SEM) at 15,000 V.

Electrophysiological Recording

Initial intracellular measurements were performed with conventional fine-tipped microelectrodes pulled from omega dot capillary glass (type 27-32-1; Frederick Haer & Co., Brunswick, ME) on a Brown-Flaming horizontal puller (Sutter Instrument Company, San Francisco, CA) to a tip resistance of 100–150 MΩ when filled with 4 M KAc. In all subsequent recordings patch electrodes made from fiberless borosilicate glass were used instead. These were pulled in two stages on a vertical puller (model 700; David Kopf Instruments, Tujunga, CA) to a tip o.d. of 1–1.5 μm, and fire polished using the method described by Hamill et al. (1981). Electrodes were filled with a solution compatible with the cytosol, since exchange with intracellular constituents can be expected given the size of the tip orifice (Fenwick et al., 1982). The intracellular solution contained 300 mM KCl, 12 mM NaCl, 10 mM $MgCl_2$, 1 mM EGTA, 300 mM sucrose, and 10 mM HEPES buffered to pH 7.3. The electrode resistance measured in ASW ranged between 8 and 15 MΩ.

The composition of ASW was 480 mM NaCl, 10 mM KCl, 49 mM $MgCl_2$, 10 mM $CaCl_2$, and 10 mM HEPES (pH 7.8). In 0 Na, ASW Na was replaced with Tris, whereas in nominally 0 Ca ASW Mg was substituted for Ca. All experiments were conducted at room temperature (22–24°C).

The recording electrode was connected to a capacity-compensated, high-impedance differential amplifier (Thomas, 1977) equipped with a bridge circuit for injection of constant current. The reference electrode was an agar bridge (1% agar in 3 M KCl). A Huxley-type micromanipulator (Custom Medical Research Equipment, Glendora, NJ) was used to position the microelectrode while the cells were visualized with a TV camera (model 1350A; Panasonic) through the side port of the inverted microscope. A long-pass filter (50% transmission cut-off at 650 nm; Ditric Optics, Hudson, MA) was used to provide deep red, dim illumination. Small constant-current pulses (20–100 pA) were repetitively administered to monitor the resistance in series with the electrode. Upon making contact with the cell surface, gentle suction was applied through the side-port of the electrode holder (World Precision Instruments, New Haven, CT). When a high resistance seal was obtained (a criterion of 10 GΩ or more was usually adopted), the patch was broken by a brief, intense pulse of suction. Access to the interior of the cell was indicated by (*a*) a reduction of the measured resistance from a value >10 GΩ to several hundred megaohms (mainly the series combination of access resistance and input resistance of the cell); (*b*) a much slower rise time of the voltage signal induced by the current pulses, due to the charging of the cell capacitance; and (*c*) a dc shift in voltage (the cell's membrane potential). Photoreceptors were allowed to dark adapt for ~10 min before starting an experiment. Data were either directly recorded with a two-channel strip-chart recorder (model 2400; Gould Inc., Cleveland OH), or fed to a tape recorder (Racal Recorders, Southampton, UK) for subsequent play-back and analysis.

Optical Stimulation

Light stimulation was delivered through an optical bench consisting of a 100-W tungsten-halogen lamp (GTE Sylvania, Winchester, KY), a condenser lens, a heat-absorbing filter, an electromechanic shutter (Uniblitz; Vincent Associates, Rochester, NY), a set of calibrated neutral density filters, an adjustable pin-hole, and a field lens. The stimulating beam was brought into the light path of the microscope illuminator with a cube beam-splitter placed above the microscope condenser; this focused an image of the pinhole onto the preparation. The "full field" illumination therefore consisted of a circular region ~100–150 μm in diameter, such that only the target cell was illuminated. This precaution avoided exposing all the photoreceptors in the chamber to repetitive stimulation during the course of an experiment. The intensity of the unattenuated beam of light, measured with a calibrated radiometer

(United Detector Technology, Hawthorne, CA), was 240 $\mu W/cm^2$. White light was used for photostimulation. The shutter was operated through a driver circuit described previously (Cornwall and Thomas, 1979).

RESULTS

Morphology of Dissociated Cells

Fig. 1 *A* shows a typical photoreceptor, as viewed under Nomarski optics. Cells of this type are usually rather numerous and exhibit a smooth soma, an intermediate, heavily pigmented region, and a rhabdomeric lobe covered with microvilli. Typically the total length is ~25 μm and the width is ~10 μm. In addition to the rhabdomeric photoreceptors, clumps of small spherical cells are also frequently seen. These are lightly pigmented and are likely to be glia.

Scanning electron micrographs reveal finer morphological details of the photoreceptors (Fig. 1 *B*). In particular, the microvilli in the distal lobe of the cell can be appreciated. It is likely that their natural length is greater, and that during processing they are broken off. The stump of the axon, which in the intact animal projects along the circumpallial nerve, is clearly visible. Axons are almost invariably severed at the hillock during the dissociation process, and in over 20 cells examined in the SEM only one axon stump longer than 5 μm was seen. It is thus likely that all aspects of the responses recorded from the isolated cells originate in the soma or the rhabdomere.

Resting Potentials and Passive Properties

Early attempts to record membrane voltage with fine microelectrodes were only marginally successful: impalements rarely lasted for more than a few minutes, and resting potentials were modest, seldom reaching −50 mV. The use of patch electrodes in the whole-cell configuration proved much more fruitful (see Methods). With this technique the average resting potential was −62 mV (SD = 12.8, $n = 27$), and stable recordings could be made for periods of up to 2 h. Evidence supporting the claim that such methods can indeed maintain the cell in better health, as compared with conventional microelectrode impalements, has been presented by Pelzer et al. (1984). Resting potential often declined somewhat (usually 5–10 mV) during the period of dark adaptation that preceded the beginning of the experiment, but then stabilized.

The input resistance of dissociated photoreceptors is in the range $3–10 \times 10^8\ \Omega$, as measured from the steady-state voltage change in response to injection of small pulses of constant current. The membrane capacitance was determined in a few cells from the time constant of the exponential relaxation after a current step, and is of the order of 50–70 pF. This value is approximately a factor of 6 higher than would be predicted from the surface area of a prolate spheroid of dimensions comparable to the cells, assuming a specific membrane capacitance of 1 $\mu F/cm^2$. Given the infoldings of the plasma membrane in the rhabdomeric region, such a discrepancy is readily accounted for. In the dark, injection of pulses of constant current depolarizing the cell membrane above −20 mV can trigger a regenerative response (not shown).

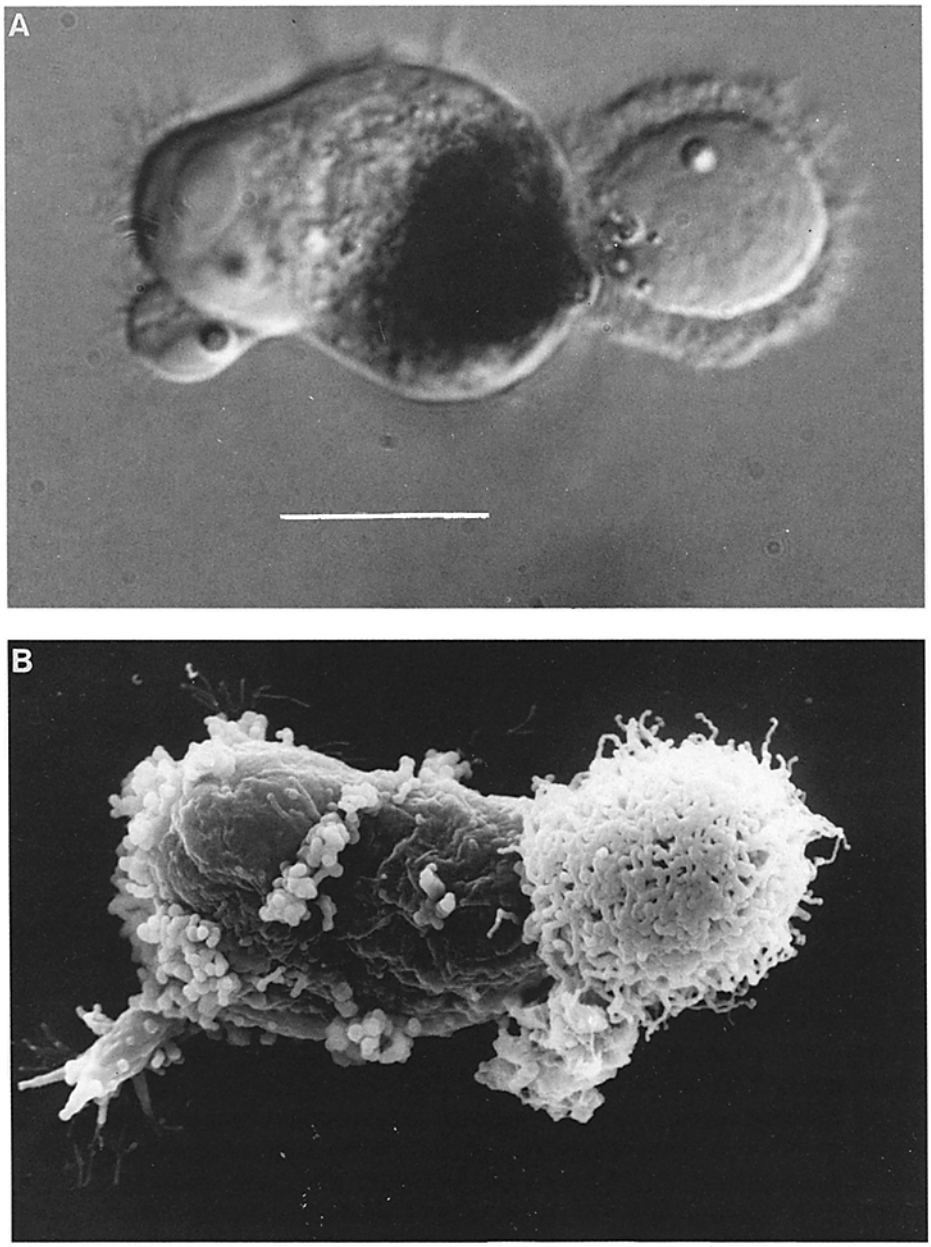

FIGURE 1. (*A*) Example of a solitary photoreceptor dissociated from the eye of *Lima*. The cell was viewed under Nomarski differential interference contrast optics, with a 100× oil-immersion objective. (*B*) Scanning electron micrograph of an isolated photoreceptor cell. The rhabdomeric lobe covered with microvilli is clearly visible, and the stump of the severed axon can also be discerned in the lower left part of the cell. Calibration bars = 10 μm.

A few attempts were made to record from clumps of the smaller, round cells. Resting potentials were found to be significantly more negative (around −80 mV), but no active responses could be elicited by depolarizing current injection or light stimulation. These cells were not studied further.

Light Response

After a few minutes of dark adaptation most cells begin to produce discrete waves ("quantum bumps"), usually between 2 and 25 mV in amplitude, with a rate that is typically ~ 1–2/s.

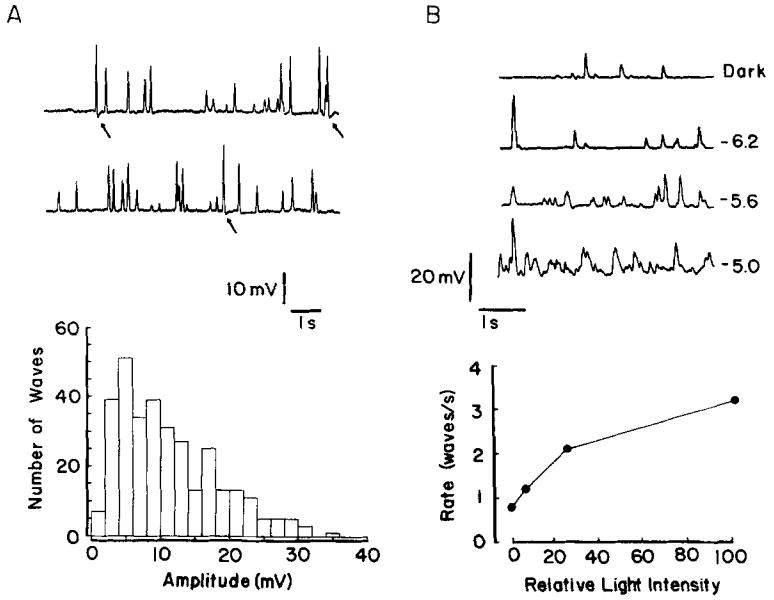

FIGURE 2. Discrete waves in *Lima* photoreceptors. The fluctuations displayed in *A* were recorded in the dark after several minutes of dark adaptation. The largest waves appear to trigger a regenerative response, usually followed by a brief hyperpolarizing overshoot (*arrows*). The amplitude distribution is shown in the histogram in the bottom left part of the figure, and was based on 322 recorded bumps. (*B*) Light-induced increase in the frequency of quantum bumps. The traces show spontaneous waves recorded in the dark, and the effect of dim, sustained steps of light (the relative light intensity in log units is indicated at the right of each record). The rate of production of quantum bumps as a function of the relative intensity of the stimulus is plotted at the bottom.

In Fig. 2 *A* a representative record is displayed. The amplitude distribution of the quantum bumps is skewed, as shown in the histogram in the bottom part of the figure, and for this cell the mean value is 11 mV. Small waves (< 20 mV) relax smoothly back to baseline, whereas larger ones usually have a spikelike appearance and are followed by a brief after-hyperpolarization, as though they triggered a regenerative response (*arrows*). The time intervals between waves follow an approximately exponential distribution (not shown). The rate of quantum bumps can be

increased by dim background light stimulation in a way that is graded with stimulus intensity (Fig. 2 *B, top*). At low intensities the frequency is an approximately linear function of the rate of incident photons, but with brighter lights it becomes markedly sublinear (Fig. 2 *B, bottom*). Similar results were obtained in two other cells.

Fig. 3 shows the effect of presenting steps of dim light of increasing intensity and illustrates the way light-evoked bursts of discrete waves gradually give rise to a phasic depolarizing receptor potential. At higher stimulus intensities not only does the receptor potential acquire a smoother time course, but a hyperpolarizing dip (clearly overshooting the resting potential) also becomes evident. The voltage traces in Fig. 4 *A*, recorded in a different cell, reveal additional features of the photoresponse elicited by brighter flashes of light. The amplitude of the early spikelike depolarization reaches saturation, and the hyperpolarizing dip is followed by a second, more sluggish depolarizing wave accompanied by a train of action potentials. Very bright

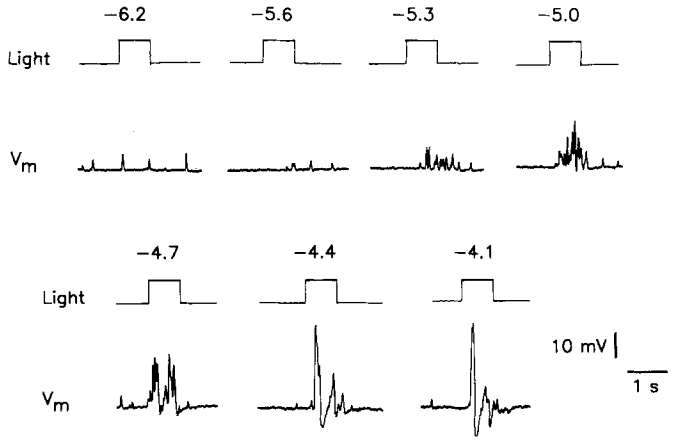

FIGURE 3. Transition from light-induced discrete waves to a macroscopic light response. Presentation of a dim light for 1 s increases the frequency of quantum bumps in a fashion graded with light intensity. Steps of brighter light result in a burst of overlapping waves, leading to the appearance of a transient multiphasic receptor potential.

flashes are always followed, in addition, by a third depolarization, which develops slowly (over the course of many seconds) and can last for more than 1 min. This slow component was not examined further in this study. All of the features of the complex light response evoked by a brief, intense stimulus can be appreciated in Fig. 4 *B*, including the initial phase of the prolonged after-depolarization. In most experiments reported below light intensity was adjusted to elicit only the early phases of the response, because after stimulation with brighter stimuli a long period of dark adaptation is required to recover sensitivity.

Ionic Basis of the Photoresponse

A number of ion substitution experiments were performed to elucidate the nature of the conductance changes underlying the light response. Replacement of extracellular Na with Tris on an equimolar basis nearly abolished the light response (first two records in Fig. 5), the effect being fully reversible in most cases (last record).

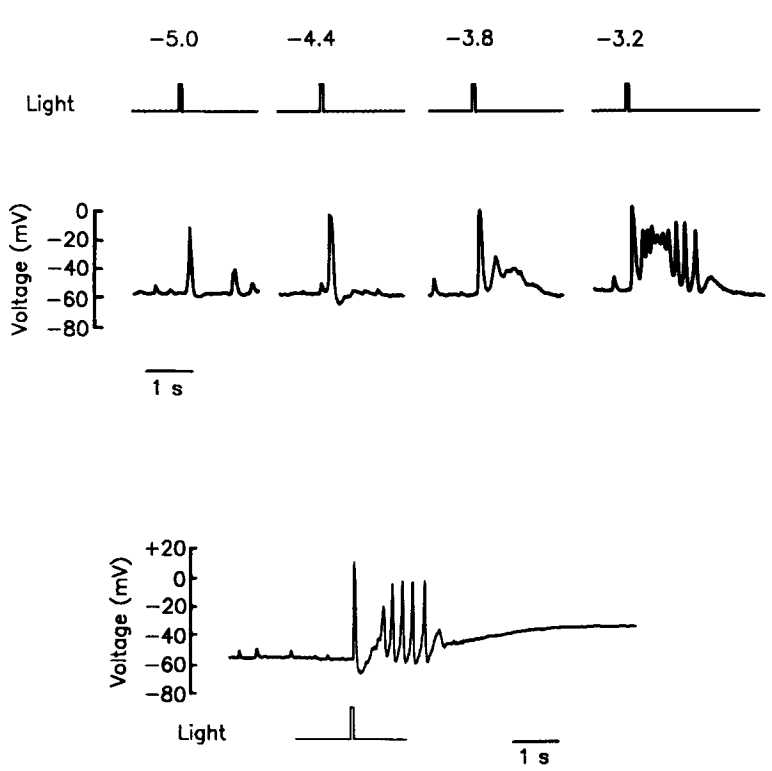

FIGURE 4. Time course of the photoresponse. (*A*) Brief flashes of increasing intensity induce a large depolarizing potential. A post-depolarization dip appears at moderate to bright intensities. A further increase in light intensity results in a second depolarizing wave accompanied by a burst of action potentials. (*B*) Example of response to a bright flash (−2.6 log) in a dark-adapted photoreceptor, showing all the characteristic features of the photoresponse of *Lima* cells, including the rising phase of the depolarizing afterpotential that slowly develops after the light response.

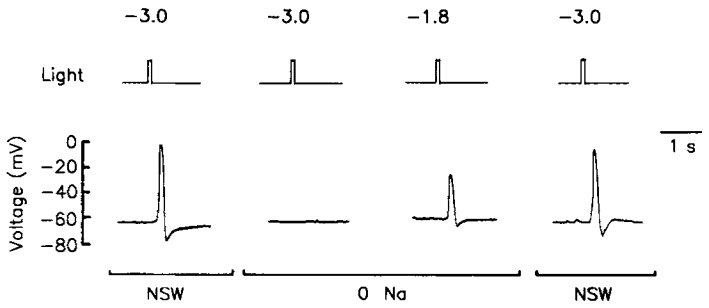

FIGURE 5. Effects of removal of extracellular Na on the photoresponse. A brief flash of light was administered as the cell was superfused with normal ASW. After Na was replaced with Tris, a test flash of the same intensity failed to evoke any response, although increasing the stimulus intensity still produced a substantial receptor potential. Upon returning to normal sea water, the cell recovered its initial responsiveness.

Increasing the test stimulus intensity while the cell was bathed in 0 Na ASW, however, still resulted in the production of a sizable photoresponse (Fig. 5, third trace). This inability to completely suppress the light response in 0 Na sea water was confirmed in four other cells.

A substantial reduction of the light response was also observed when photoreceptors were superfused with nominally 0 Ca ASW, replacing Ca^{2+} with Mg^{2+} (Fig. 6 *A*). Recovery of the photoresponse upon returning to normal ASW could be obtained, provided that the duration of exposure to 0 Ca ASW did not exceed ~5 min (n = 3).

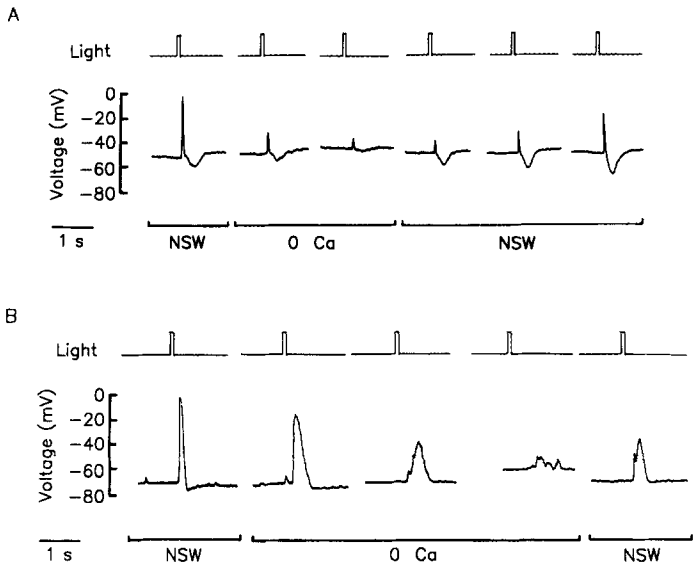

FIGURE 6. Effects of nominally 0 Ca sea water on photoresponse. (*A*) A 100-ms flash (−1.8 log) was delivered while the photoreceptor was bathed in ASW. The solution was then switched to one in which Ca was replaced with magnesium (no Ca buffers added). During exposure to 0 Ca (lasting ~4 min) the light response was greatly degraded, the effect being reversible. (*B*) Irreversible loss of light sensitivity after prolonged exposure to 0 Ca solution. A different photoreceptor was tested with a similar protocol, but exposure to the 0 Ca sea water was extended to ~10 min (notice the irregular time course of the photoresponse in 0 Ca sea water). Returning to the control solution resulted only in partial recovery. (Relative light intensity −3.9 log.)

Otherwise, an irreversible deterioration usually resulted (n = 5), as in the example shown in Fig. 6 *B*. 0 Ca ASW not only reduced the amplitude of the response, but also resulted in a characteristic "bumpy" appearance, reminiscent of the effect of 0 Ca ASW observed in *Limulus* ventral photoreceptors (Lisman, 1976).

The integrity of the light response in *Lima* photoreceptors appears to depend on the presence of both Na and Ca ions in the extracellular bathing medium. The contribution of these ions to the photoresponse, however, may concern fundamentally different processes: for example, light-activated vs. voltage-dependent conduc-

tance changes. Since these factors are confounded in the experiments described above, a paradigm was sought that would minimize the contamination of the photoresponse by voltage-dependent mechanisms. To this end, ion substitution experiments were performed using not only discrete flashes, but also prolonged, dim steps of light capable of inducing an increase in the frequency of quantum bumps without causing a net depolarizing shift in the cell membrane potential. In 0 Na ASW both the light-induced quantum bumps and the response to a brighter flash were markedly attenuated in a reversible way (Fig. 7, *A–C*). By contrast, discrete waves did not seem to be significantly altered by superfusion with 0 Ca ASW for a short time

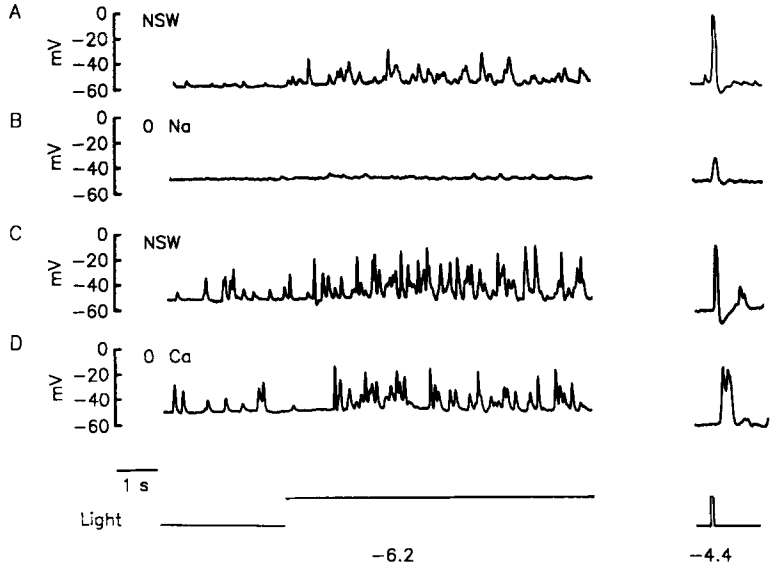

FIGURE 7. Production of discrete waves in response to dim, steady illumination, and responses evoked by moderately bright test flashes under different ionic conditions. Since the membrane potential changed somewhat during various solution changes, the brief test flashes were presented while constant current was injected in order to recover the initial level of membrane potential. (*A*) Cell bathed in normal sea water; (*B*) Na replaced by Tris; (*C*) return to normal sea water; (*D*) Ca replaced by magnesium. While removal of either Na^+ or Ca^{2+} has a deleterious effect on the photoresponse evoked by a brief flash, only elimination of Na^+ severely affects light-induced quantum bumps.

(Fig. 7 *D*), whereas the response to a flash was markedly degraded under those conditions. This suggests that some other aspects of the full-fledged photoresponse—including perhaps a Ca spike—might have been affected by the removal of extracellular Ca. Superfusion with 0 Na ASW nearly abolished quantum bumps in another cell tested under similar conditions. The resistance of quantum bumps to removal of Ca from the external solution was confirmed in two other photoreceptors. A supranormal production of quantum bumps, as in Fig. 7, *C* and *D*, sometimes developed during prolonged experiments and could be related to a similar phenom-

enon described in *Limulus* ventral photoreceptors internally dialyzed with solutions lacking nucleotides (Stern and Lisman, 1982; Stern et al., 1985).

Membrane depolarization by constant current injection reduces the size of the early component of the light response and concomitantly increases the size of the dip. Fig. 8 *A* shows one of the two instances in which it was possible to reverse the depolarizing phase of the response. Most attempts were unsuccessful because a region of marked instability was encountered when the membrane was depolarized above −20 mV. The first component appears to have reversed sign when the cell was depolarized to +14 mV. The dip, on the other hand, vanished with membrane hyperpolarization to −80 mV. Clear reversal of the dip could not be obtained, in part

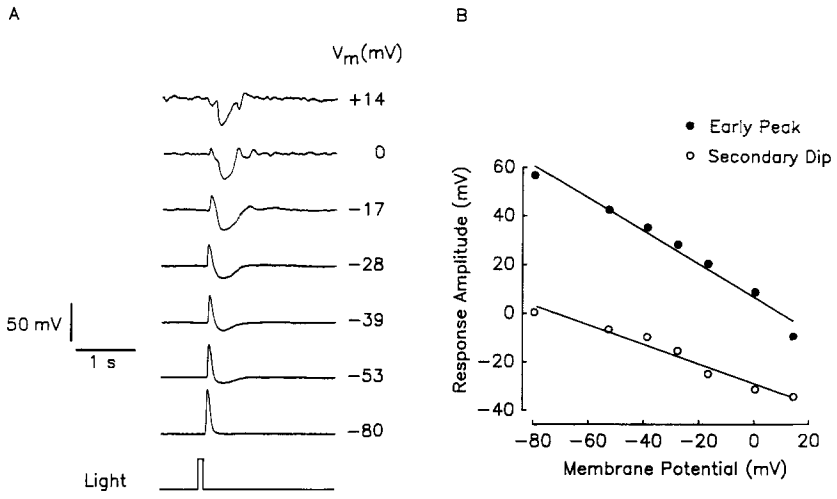

FIGURE 8. Effect of membrane potential changes on the light response. (*A*) A photoreceptor cell was hyperpolarized or depolarized by injecting steady current through the recording electrode (resting potential −53 mV), and stimulated with a 100-ms flash (−2.4 log). As the voltage is made more positive, the amplitude of the early depolarization decreases, and the size of the dip increases. At +14 mV the early phase of the response appears to reverse sign. (*B*) Amplitude of the light response as a function of initial membrane potential, plotted separately for the two components.

because larger hyperpolarizations usually proved detrimental to the cells and were avoided. Fig. 8 *B* plots the amplitude of the two phases of the response as a function of membrane potential. The lines were fitted to the two sets of data points by the method of least squares.

The amplitude of the hyperpolarizing phase of the light response was found to be related to light intensity, growing larger well beyond the point at which the early depolarization reached saturation (n = 5). Fig. 9 *A* shows a typical example (see also Fig. 3). In addition, this component appeared to be prominent if a cell was stimulated while still light-adapted from a previous flash. In Fig. 9 *B*, bright stimuli were presented in close succession, one every 15 s. Under these conditions the

depolarizing component of the response grew progressively smaller, while the hyperpolarizing dip increased, eventually resulting in a purely hyperpolarizing receptor potential. The induction of the hyperpolarizing response is not simply a consequence of the depolarizing shift of membrane potential shortly after stimulation: if constant current was injected in order to recover the prestimulus resting potential, the response to subsequent test flashes was still hyperpolarizing (Fig. 9 *B*, right-hand side). On the other hand, if a sufficient period of dark adaptation was

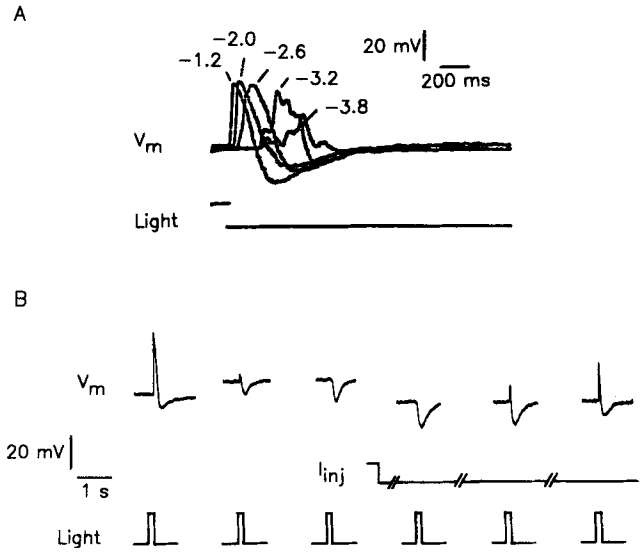

FIGURE 9. (*A*) Changes in the amplitude and kinetics of the early depolarization and the hyperpolarizing dip as a function of light intensity. The latency for both components decreases as the stimulating flash is made brighter, and the size of the dip increases even after the early depolarization has reached saturation. (*B*) Effect of light adaptation on the photoresponse. A dark-adapted cell was stimulated with repeated flashes of moderate intensity every 15 s. The first one (−1.8 log) elicits a normal-looking biphasic response, while with subsequent stimuli (0 log) the early depolarization selectively decreases in such a way that the photoresponse eventually becomes a pure hyperpolarization. The effect is not a consequence of the changes in membrane potential: if baseline membrane voltage is restored by injection of hyperpolarizing current, the light response still remains hyperpolarizing. Interposing a 1-min interval between flashes (last two traces) leads to a recovery of the original shape of the photoresponse.

allowed to elapse, the biphasic response was recovered (last trace in Fig. 9 *B*). Similar results were obtained in three other cells.

DISCUSSION

The results obtained demonstrate that physiologically viable isolated photoreceptor cells from the eye of the mollusk *Lima scabra* can be obtained by an enzymatic dissociation procedure. These cells remain healthy for several hours and are suitable for electrophysiological recording with fire-polished patch electrodes. Under condi-

tions of dark adaptation they produce discrete waves in the absence of photostimulation. Their shape, amplitude, duration, and frequency are similar to the quantum bumps that have been described in other invertebrates, such as *Limulus* lateral and ventral photoreceptors (Yeandle, 1958; Millecchia and Mauro, 1969), as well as *Hermissenda* photoreceptors (Takeda, 1982). The skewed shape of the amplitude histogram is reminiscent of the distribution observed in *Hermissenda* (Takeda, 1982), and may reflect the presence of two distinct classes of waves (Adolph, 1964; Yeandle and Spiegler, 1973). In such a case they probably overlap considerably, since no discrete peaks were evident in the histograms, and separation may thus be difficult. Dim continuous illumination causes an increase in the rate of quantum bumps, proportional to the light flux (Yeandle, 1958; Adolph, 1964; Lillywhite, 1977). At higher light intensities, however, the frequency of discrete waves falls significantly below such linear relation, as it occurs in *Limulus* retinular cells (Adolph, 1964). It is possible, however, that temporal overlap of quantum bumps (and the consequent difficulty of accurately counting them) may in part account for the apparent departure from linearity. The effect of dim light is not only to increase the rate of quantum bumps, but also to increase their mean amplitude. In darkness, large amplitude waves are usually infrequent, whereas in the presence of background illumination they tend to become predominant. A similar phenomenon was examined by Yeandle and Spiegler (1973) in *Limulus* photoreceptors.

Flashes of more intense stimulating light evoke a complex pattern of voltage changes, consisting of a phasic depolarization followed by a hyperpolarizing dip. If the light is sufficiently intense, a second depolarizing wave is produced, which is accompanied by action potentials. The almost all-or-none appearance of the initial depolarizing phase of the response at higher stimulus intensities probably reflects the triggering of a regenerative response once a sufficient number of overlapping quantum bumps depolarize the membrane to threshold. This conjecture receives support both from the observation that passive depolarization produced by current injection is capable of eliciting an action potential in darkness, and also from the fact that spikes often accompany the late depolarizing wave of the response. The presence of voltage-dependent conductances in *Lima* photoreceptors is consistent with the fact that they encode information about light in the form of action potentials which propagate along the circumpallial nerve without the mediation of any second-order cell (Mpitsos, 1973). It is nevertheless noteworthy to find such mechanisms functional in these cells, which are nearly always axotomized as a result of the dissociation procedure.

On the basis of ion substitution experiments it appears that the primary ion involved in the generation of the photoresponse is Na (but certainly not the only one, since Na removal never abolishes the light response completely). Unambiguous determination of the ionic selectivity of the light-sensitive conductance would entail measuring the reversal potential under different ionic conditions (see Brown and Mote, 1974). Such a goal proved elusive with current-clamp techniques because of the instability of the membrane potential above -20 mV, and the detrimental effects of maintaining the cells depolarized for more than a few seconds. Reversal potential measurements under voltage clamp are presented in the third paper of this series.

The elimination of Ca ions from the bath also has an inhibitory effect on the photoresponse, although probably through a less direct mechanism, since it does not adversely affect the light-induced production of quantum bumps. Possible explanations include the reduction of voltage-dependent conductances that may normally contribute to the shape of the photoresponse. This issue is addressed in a more systematic way in the following article, using the whole-cell patch-clamp technique (Hamill et al., 1981; Marty and Neher, 1983). Other mechanisms are also likely to be implicated in the effects of Ca removal: for example, in *Limulus* ventral photoreceptors 0 Ca solutions markedly degrade the time course of the light response by introducing a large variability in the latency of the underlying discrete waves (Lisman, 1976). In addition, repeated photostimulation in 0 Ca ASW leads to a progressive decline in the response amplitude and sensitivity (Bolsover and Brown, 1985).

A prominent feature of the light response of *Lima* photoreceptor cells is the transient hyperpolarizing phase, which is reminiscent of the dip that has been observed in the photoresponse of *Hermissenda* (Detwiler, 1976) and *Balanus* (Hanani and Shaw, 1977). The dip is usually small in dark-adapted cells stimulated with dim or moderately bright light, but becomes conspicuous at higher stimulus intensities, growing larger and faster well beyond the point where the depolarizing potential reaches saturation. Since the hyperpolarizing transient is reduced (but usually not entirely abolished) in 0 Ca ASW (e.g., Fig. 4 *D*), it can be hypothesized that it is due to the activation of a Ca-dependent K conductance (Meech and Strumwasser, 1970; Meech and Standen, 1975). If such is the case, the source of the Ca ought to be in part extracellular, with influx occurring either through a voltage-dependent Ca conductance (see the following paper), or perhaps through the light-sensitive conductance. Stimulation under light-adapted conditions progressively increases the magnitude of the dip, relative to the early depolarizing wave, to the point that light stimulation will evoke only a hyperpolarization. A similar phenomenon has been observed in the eye of *Hermissenda* (Dennis, 1967; Detwiler, 1976) even when axons were severed in order to reduce the potential confounding of cell–cell interactions (Detwiler, 1976).

The response of visual cells has been known to undergo profound changes as a function of the intensity of stimulating light (Fuortes and Hodgkin, 1964; Penn and Hagins, 1974) and the state of adaptation (Fuortes and Hodgkin, 1964; Baylor and Hodgkin, 1974). The effects concern both the amplitude and the time course of the photoresponse. Accounts have been proposed in terms of the cascade of biochemical reactions that intervene between quantum absorption by photopigment molecules and conductance changes at the plasma membrane (for example, Baylor et al., 1974*a, b*). Within such a context, however, it is usually assumed that a single conductance is implicated that is directly controlled by light-activated mechanisms. Such a simplification in the present case may be unwarranted, and the possibility of parallel light-dependent effector mechanisms suggests itself. Multiple light-controlled processes were first suggested by Lisman and Brown (1971) in *Limulus* ventral photoreceptors. The observations reported by Detwiler (1976) in *Hermissenda* also provide evidence for the existence of separate conductance changes controlled by light. Rigorous evaluation of such a possibility requires (*a*) characterizing those ionic mechanisms that can be activated independently of photostimulation, and (*b*)

analyzing the photocurrent under voltage clamp. These topics will be the subject of the two reports that follow.

A puzzling feature that has been apparent throughout the course of this investigation is the uniformity (both in terms of morphology and responsiveness to light stimulation) of the numerous cells examined. In particular, no dark-adapted photoreceptor was found to respond to light with a hyperpolarizing receptor potential. Clearly, none of the photoreceptors studied fits the description of the distal cells reported by others (Bell and Mpitsos, 1968; McReynolds, 1976; Cornwall and Gorman, 1983). A tentative explanation could be that since the ciliary hyperpolarizing photoreceptors are presumably located in a different supporting structure, conditions for dissociating them may be quite different. For example, one could speculate that such cells are more fragile and easily destroyed during mechanical trituration, or that a more extensive enzymatic incubation is required to free them from surrounding tissue. Alternative dissociation protocols will be explored in an attempt to obtain solitary ciliary photoreceptors.

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