# Two Light-dependent Conductances in *Lima* Rhabdomeric Photoreceptors

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ABSTRACT Light-dependent membrane currents were recorded from solitary *Lima* photoreceptors with the whole-cell clamp technique. Light stimulation from a holding voltage near the cell's resting potential evokes a transient inward current graded with light intensity, accompanied by an increase in membrane conductance. While the photocurrent elicited by dim flashes decays smoothly, at higher stimulus intensities two kinetically distinct components become visible. Superfusion with TEA or intracellular perfusion with Cs do not eliminate this phenomenon, indicating that it is not due to the activation of the Ca-sensitive K channels that are present in these cells. The relative amplitude of the late component vs. the early peak of the light response is significantly more pronounced at −60 mV than at −40 mV. At low light intensities the reversal potential of the photocurrent is around 0 mV, but with brighter lights no single reversal potential is found; rather, a biphasic response with an inward and an outward component can be seen within a certain range of membrane voltages. Light adaptation through repetitive stimulation with bright flashes diminishes the amplitude of the early but not the late phase of the photocurrent. These observations can be accounted for by postulating two separate light-dependent conductances with different ionic selectivity, kinetics, and light sensitivity. The light response is also shown to interact with some of the voltage-sensitive conductances: activation of the Ca current by a brief conditioning prepulse is capable of attenuating the photocurrent evoked by a subsequent test flash. Thus, Ca channels in these cells may not only shape the photoresponse, but also participate in the process of light adaptation.

## INTRODUCTION

In the first article (Nasi, 1991*a*), the light response of enzymatically dissociated *Lima* photoreceptors was examined with intracellular voltage recording and current clamp methods. Its complex time course indicated the presence of multiple ionic mechanisms modulated by light and by changes in membrane voltage. Voltage-dependent conductances were subsequently characterized by means of the whole-cell clamp technique (Nasi, 1991*b*). Two of the most prominent components that could be identified are a Ca current ($I_{Ca}$) and a Ca-activated K current ($I_{K(Ca)}$). These

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conductances can account for the photoreceptors' ability to produce regenerative responses, and probably play an important role in shaping the light response. In this report, light-dependent currents were examined under voltage clamp to characterize them in isolation from voltage-dependent factors. Some of these results have been presented in preliminary form (Nasi, 1990).

## METHODS

The protocol for obtaining solitary photoreceptors has been described previously (Nasi, 1991*a*). Macroscopic currents were recorded with the whole-cell clamp method, as discussed in Nasi (1991*b*). Patch electrodes were filled with an intracellular solution containing 200 mM K-aspartate, 100 mM KCl, 6 mM $Na_2ATP$, 9 mM $MgCl_2$, 1 mM EGTA, 300 mM sucrose, and 10 mM HEPES, buffered to pH 7.3 (KOH). In some experiments $Cs^+$ ions replaced $K^+$ in order to block outward currents through K channels. Electrode resistance measured in sea water ranged from 2 to 4 MΩ. Artificial sea water (ASW) contained 480 mM NaCl, 10 mM KCl, 49 mM $MgCl_2$, 10 mM $CaCl_2$, and 10 mM HEPES, pH 7.8 (NaOH). When the K channel blocker tetraethylammonium bromide (TEA) was used, it replaced NaCl on an equimolar basis.

All experiments were conducted at room temperature (22–24°C). Cells were visualized by means of a Newvicon-tube TV camera (model 1550; Panasonic) attached to a side port of an ICM-405 inverted microscope (Carl Zeiss, Inc., Thornwood, NY), while illuminated with dim, red light through a long-pass filter (50% transmission cut-off at 650 nm; Ditric Optics Inc., Hudson, MA). Upon formation of a tight seal, the electrode potential was set to the holding voltage (−50 or −60 mV) and the membrane patch was ruptured. Several minutes of dark adaptation were allowed to elapse before the beginning of an experiment.

The same optical bench described in the first article of this series was used for photostimulation. White light was used in all experiments because the stimulus intensity required for some of the measurements was high enough to preclude the use of a narrow-band interference filter. The intensity of the unattenuated beam of light was measured with a radiometer (United Detector Technology, Hawthorne, CA). To estimate the effective photon flux, the amplitude of the photocurrent elicited by monochromatic light stimulation through a 500-nm, 10-nm half-width filter (Ditric Optics Inc.) was matched to the response evoked by a moderately dim flash of white light (−3.5 log attenuation). The calculated equivalent photon flux for the unattenuated beam of white light was $3 \times 10^{16}$ photons · $cm^2/s$.

The voltage-clamp pulses, the operation of the shutter, and the trigger signals to initiate data collection by the computer were controlled by a multi-channel programmable stimulator. Data were low-pass filtered and digitized as previously described (Nasi, 1991*b*) and stored on Bernoulli cartridges (Iomega Corp., South Roy, UT) for subsequent analysis. Linear subtraction of leakage currents was performed digitally when voltage stimulation was involved (Nasi, 1991*b*).

## RESULTS

### *Basic Features of the Light Response under Whole-Cell Clamp*

After several minutes of dark adaptation many cells start producing discrete waves of inward current, with a rate of 0.5–2/s. Their frequency can be increased by dim, sustained illumination (not shown). The amplitude of these fluctuations varies between 10 and 30 pA. Considering the range of input resistance values typically measured in these cells (300 MΩ–1 GΩ), a current of such magnitude would generate depolarizations quantitatively consistent with the size of the quantum bumps that

were recorded in unclamped photoreceptors (Nasi, 1991*a*). Presentation of a brighter flash of light evokes a larger, phasic, inward current. Accurate voltage clamping requires electrodes with considerably larger tips than the ones used for membrane voltage recordings (2–4 vs. 8–15 MΩ, measured in sea water), which can result in faster wash-out of intracellular constituents. Therefore, it was important to determine the time window during which the cell's phototransducing machinery remained viable. Fig. 1 *A* shows several superimposed photoresponse records. The trace with the slightly faster kinetics was evoked by a flash (100 ms duration, −4.1 log attenuation) during the course of a light intensity series. The remaining four responses were elicited by a repetitive stimulus of the same intensity, presented every

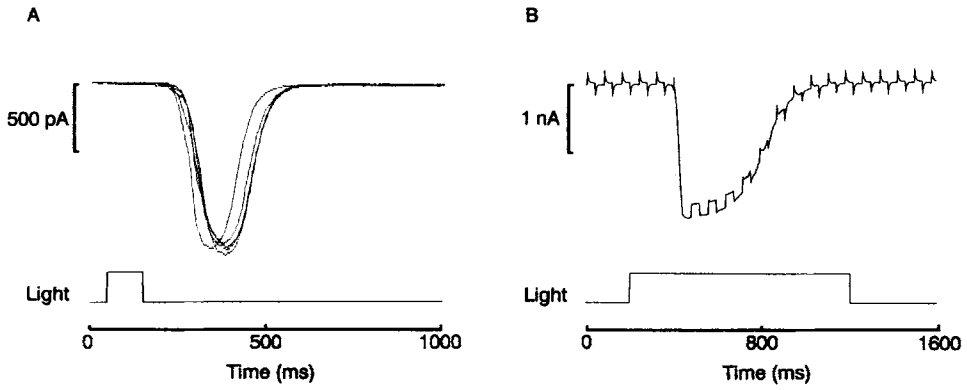


FIGURE 1. (*A*) Superimposed photocurrent records, evoked by repetitive stimulation with a dim flash of light (−4.1 log). The trace exhibiting the shorter latency was recorded first, and 12 min later the other four responses were elicited by flashes of light of the same intensity, delivered once a minute. The amplitude of the photocurrent is maintained virtually unchanged during this period of time. (*B*) Membrane conductance changes in a voltage-clamped photoreceptor. The cell was held at −60 mV and a rectangular voltage command step, 10 mV in amplitude, was superimposed on the holding potential. Presentation of a 1-s, −3.2 log step of light gives rise to an inward current accompanied by an increase of membrane conductance, reflected by the larger current steps in response to the voltage perturbation. The photocurrent decays to baseline during the sustained stimulus, with a concomitant increase in input resistance.

60 s, beginning 12 min after the first trace was recorded. The photocurrents have nearly identical amplitudes, and only the time course has become somewhat slower. In general, responses appeared to be stable over a period of 10–30 min, after which a progressive, irreversible run-down occurred. This is significantly more limited than the interval available for voltage recordings of the light response using finer patch pipettes (Nasi, 1991*a*). Voltage-dependent currents, however, usually persisted for a long period of time after the photocurrent had deteriorated.

The light response in *Lima* photoreceptors is accompanied by an increase in membrane conductance, consistent with other invertebrate rhabdomeric visual cells (Millecchia and Mauro, 1969; Brown et al., 1970). The current record presented in

Fig. 1 *B* was obtained by voltage-clamping a cell at −60 mV, with a repetitive voltage step superimposed on the steady holding potential (10 mV amplitude, 4 Hz frequency, 0.4 duty cycle). As a 1-s step of light of moderate intensity (−3.2 log) was delivered, the membrane current perturbations in the photocurrent trace increased in size by a factor of nearly 7 at the peak of the response, corresponding to an increase in membrane conductance from 2.8 nS under resting conditions to 19 nS. A similar conductance increase was measured in three other cells. The figure also illustrates the fact that the photocurrent under voltage clamp is phasic, and decays to baseline even in the presence of a sustained step of light, with a concomitant increase in the input resistance. This observation is consistent with the transient time course of the light-induced changes in membrane voltage reported previously (Nasi, 1991*a*), and extends those results by showing that the repolarization during a prolonged step of light is not simply due to the activation of voltage- and Ca-dependent K channels (Nasi, 1991*b*).

### *Light Intensity Effects*

The amplitude and the time course of the photocurrent were examined as a function of the intensity of stimulating light. Fig. 2, *A* and *B*, shows typical light intensity series for two different cells. At low light levels the response to a 100-ms flash is quite slow, with latency on the order of 300 ms, and the current has a bumpy appearance, probably reflecting the superposition of a small number of discrete waves. As the stimulus intensity is raised, the light-evoked inward current grows larger and smoother, usually reaching saturation in ~2 log units above threshold. The relation of normalized current vs. log light intensity is plotted in Fig. 2 *C* for both cells. The kinetics of the photocurrent becomes faster as a function of light intensity, and the time to the peak of the response can decrease below 100 ms (see *D* of the same figure). A feature that was consistently observed in all cells tested with bright lights ($n$ = 12) is the change in the time course of the late phase of the photocurrent. This consists of the appearance of a distinct inflection point shortly after the early transient, marking the beginning of a prominent hump or a tail. The fact that this secondary component becomes conspicuous in a range where the early photocurrent peak approaches saturation argues against the possibility that the phenomenon is an artifact of voltage clamping (such as uncompensated series resistance), since it does not correlate with a large increase in membrane current.

It is conceivable that the observed complex kinetics of the light response is due to the presence of separate mechanisms. A simple conjecture is that the inflection in the current records reflects the activation of a delayed, outwardly directed transient that competes with the inward photocurrent, as schematically depicted in Fig. 3 *A*. Alternatively, two staggered waves of inward current could be envisioned, as in Fig. 3 *B*. The first possibility is rather plausible, in view of the prominent Ca-dependent K conductance that was characterized in these cells (Nasi, 1991*b*), and the probable existence of mechanisms providing for an increase in intracellular Ca following photostimulation, via either influx through the plasma membrane (Brown and Blinks, 1974; Connor and Alkon, 1984) or release from intracellular stores (Brown and Blinks, 1974; Lisman, 1976; Levy and Fein, 1985).

The potential involvement of a K current is easily tested by conducting the measurements in the presence of suitable channel blockers. Previous voltage-clamp experiments have shown that cell superfusion with TEA is quite effective in abolishing the outward current through Ca-activated K channels (Nasi, 1991*b*). In Fig. 4 a bright flash of light (−1.5 log) is presented as a photoreceptor cell is bathed in ASW (*A*), or after 10 min of exposure to 20 mM TEA (*B*). In both cases the stimulus produces a response with a similar, biphasic time course, clearly displaying both the early peak

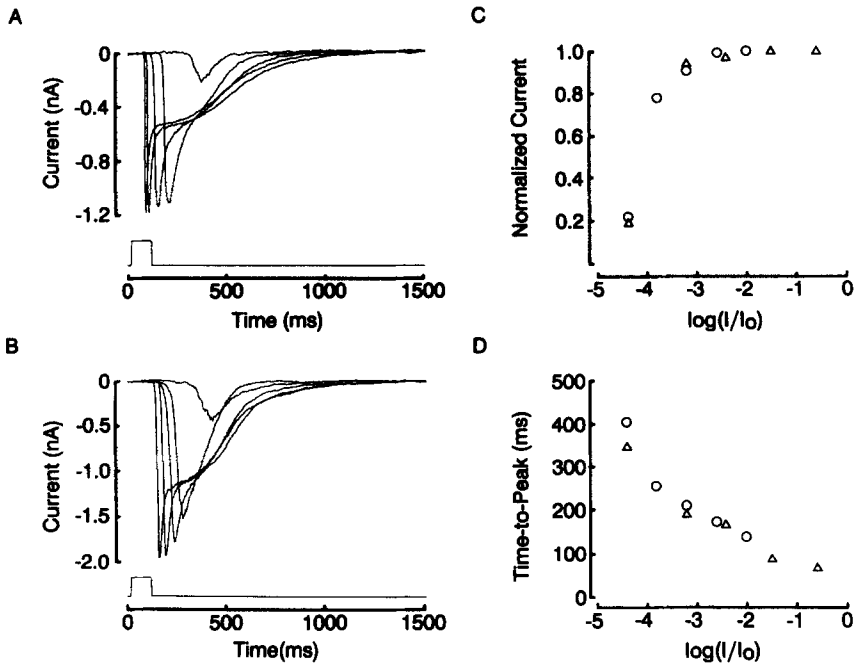


FIGURE 2. Light intensity series for two different cells (*A* and *B*). The holding potential was −60 mV, and stimulating flashes were attenuated by −4.4, −3.2, −2.4, −1.5, and −0.6 log for cell A, and −4.4, −3.8, −3.2, −2.6 and −2.0 log for cell B. In both cases a distinct late hump appears in the inward light-evoked current at stimulus intensities in the saturating range for the early transient. (*C*) Plot of the normalized peak current vs. light intensity for the two cells, showing a dynamic range of ~2 log units under dark-adapted conditions (cell A, *triangles;* cell B, *circles*). (*D*) Time to peak for the light response as a function of stimulus intensity, providing a measure of the progressive acceleration of the photocurrent kinetics with brighter lights.

and the late component. The survival of the late tail in the presence of TEA was confirmed in five other cells.

Another treatment that is drastically effective in eliminating not only $I_{K(Ca)}$, but all outward currents in this cell type, is internal perfusion with Cs (Nasi, 1991*b*). In Fig. 5 *A* a cell was voltage clamped with a patch electrode containing 300 mM Cs. Stimulation with a bright flash of light elicits a fast peak of inward current, followed by a slower wave. Fig. 5 *B* shows that under these conditions all outward currents are

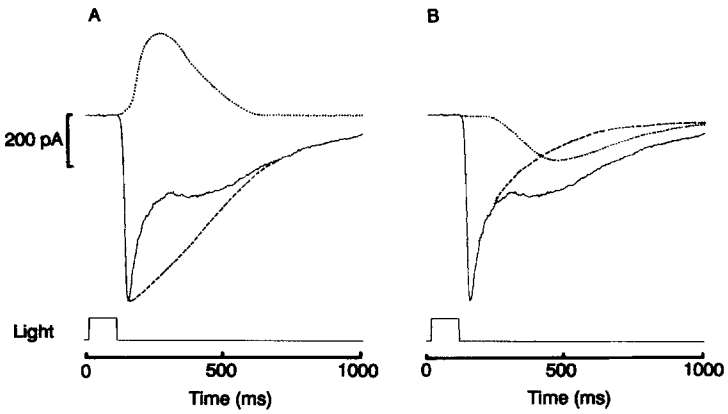


FIGURE 3. Two possible schemes to account for the complex kinetics of the light response at high stimulus intensities, in terms of the activation of separated processes. In both panels an actual response record elicited by a saturating flash is shown (*solid line;* holding potential −60 mV, light attenuation −1.2 log), displaying the early transient and late component of the response. In *A* this complex time course is represented as the superposition of a smooth inward current and an outward transient component (*dashed and dotted lines*). (*B*) Same photocurrent decomposed into two staggered waves of inward current.

suppressed when a depolarizing voltage clamp step to +15 mV is administered in the dark, and only the Ca current remains visible. Similar results were obtained in another cell. These observations confirm that the complex kinetics of the photocurrent cannot be due to the activation of $I_{K(Ca)}$. The temporal separation of the two components in the record shown in Fig. 5 *A* is especially evident, possibly because this cell was partially light adapted (see also Fig. 9 *B*). Taking advantage of this fact, the time course of the conductance changes during the light response was measured with a repetitive voltage-step perturbation. Fig. 5 *C* shows that after the early

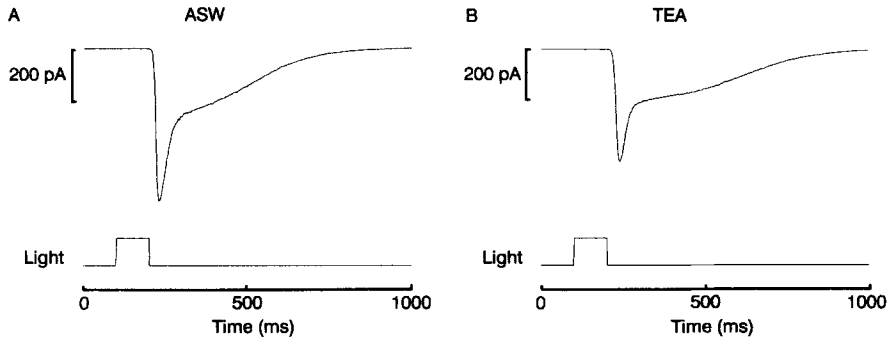


FIGURE 4. Response of a photoreceptor to a −1.5 log light in normal sea water (*A*), and after 10 min of superfusion with 20 mM TEA (*B*). The response decreased in amplitude as the cell began to desensitize, but the biphasic time course is clearly preserved, with a very distinct early transient and a late tail.

transient the membrane conductance is low (almost back to prestimulus levels), but it increases again as the second wave of inward current develops.

*Voltage Sensitivity of Early and Late Components*

The preservation of the biphasic time course of the light response in the presence of TEA or intracellular Cs indicates that an outward current is not involved. However,

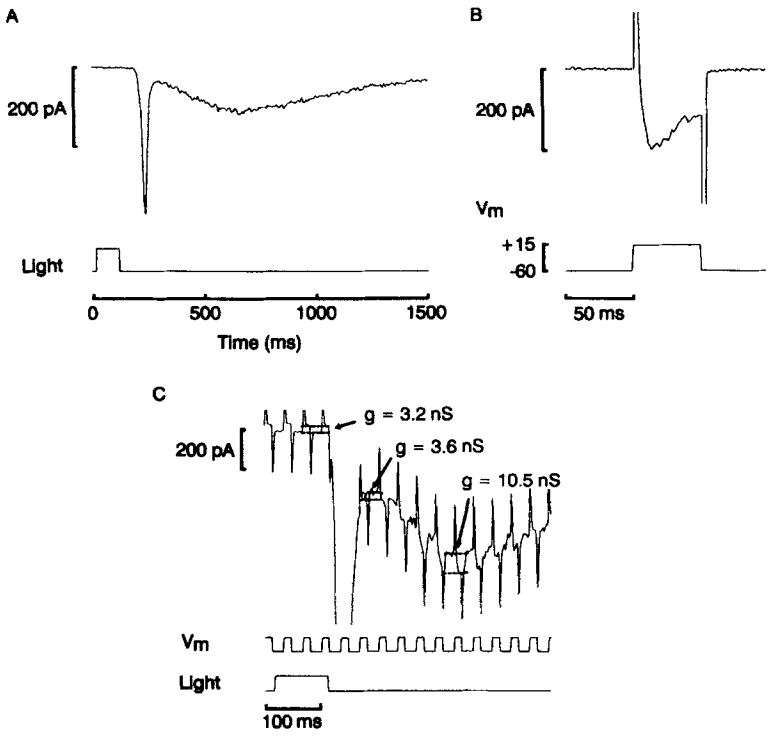


FIGURE 5. (*A*) Photocurrent evoked by a bright flash of light (−2.0 log) in a cell internally perfused with 300 mM Cs. The kinetic separation of the two components is particularly pronounced. (*B*) Effect of administration of a 50-ms pulse from −60 mV to +15 mV in the same cell, showing that blockage of outward currents is complete. (*C*) Expanded view of the photocurrent elicited by a light stimulus (−0.6 log attenuation) in the same cell, while small repetitive voltage steps were superimposed on the steady holding potential. After the end of the early inward transient the membrane conductance is low, and it gradually increases as the late wave develops.

such results provide no direct argument for the existence of two separate ionic mechanisms (as suggested in Fig. 3 *B*) rather than a single conductance exhibiting complex kinetics. Different conductances are unlikely to share identical ionic selectivity, and hence should be differentially affected by changes in membrane potential. To test such a possibility, experiments were conducted to compare the responses elicited

by identical light stimuli delivered at two different holding potentials, chosen within a range where voltage-dependent conductances are not activated.

In Fig. 6 a photoreceptor cell was stimulated with light stimuli of increasing intensity, as the membrane voltage was alternately clamped at −60 mV (top left) or −40 mV (top right). As the flashes are made brighter, a tail in the photocurrent becomes quite pronounced when the membrane potential is −60 mV, but it is barely visible at −40 mV. The comparison of the photocurrent kinetics in response to the three most intense flashes (−3.8, −3.2, and −2.0 log) is more striking if the

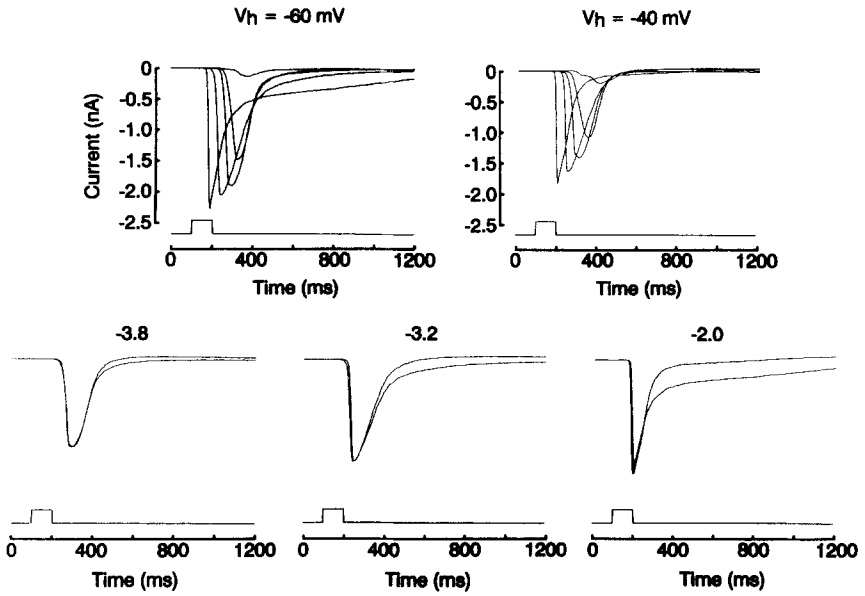


FIGURE 6. Effect of the holding potential on the kinetics of the photocurrent at different light intensities. The membrane voltage was switched between −60 and −40 mV (no active currents are elicited within this range), and a series of flashes of increasing intensity was administered (−5.0, −4.4, −3.8, −3.2, and −2.0 log). Each stimulus intensity was repeated at the two voltages. A late tail becomes apparent with brighter lights when the holding potential is −60 mV, but is barely visible at −40 mV. In the bottom part of the figure, current traces in response to the three brightest lights were normalized and superimposed. The comparison underscores the difference in the late but not the early part of the photoresponse, and clearly illustrates how this difference depends on stimulus intensity.

corresponding pairs of records are normalized and superimposed (bottom part of the figure). It is evident that the time course of the early component is nearly indistinguishable at the two holding voltages. By contrast, the late tail is substantially larger at −60 mV than at −40 mV, the difference becoming progressively more pronounced the higher the stimulus intensity. For the two dimmest flashes (−5.0 and −4.4 log) the normalized response pairs were nearly superimposable (not shown). Another cell was tested in a similar way, and yielded comparable results. This

observation is consistent with the existence of separate ionic mechanisms contributing to the kinetic complexity of the light response.

Additional evidence was sought by examining the reversal potential of the photocurrent. The protocol involved stepping the membrane voltage from the holding potential to any given level, and presenting a brief light stimulus after sufficient time had elapsed for the voltage-dependent currents to reach a reasonably steady level. A 10-mV hyperpolarizing step without photostimulation was also administered, and was used for digital subtraction of leakage currents. If dim lights preferentially activate the early component of the photoresponse, it should be possible to find a reversal potential when a low intensity flash is used. This prediction is borne out by the results shown in Fig. 7 *A*, where a photoreceptor was held at progressively more depolarized voltages, and the photocurrent evoked by a dim

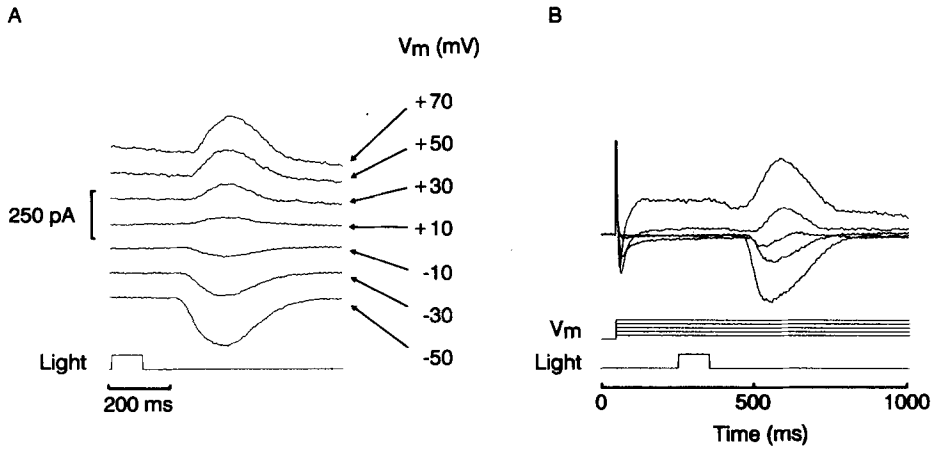


FIGURE 7. (*A*) Reversal of the photocurrent elicited by a dim light as the cell membrane was progressively depolarized. The light response reverses smoothly in the region between 0 and +10 mV. Traces have been displaced vertically for clarity. (*B*) Reversal of the light response in the same cell after the stimulus intensity was increased by 0.6 log units. In this case, the response shows no clear reversal potential. Rather, it acquires an biphasic time course around 0 mV, with an early inward component and a late outward component ($V_h$ = −60 mV. Steps to −40, −20, 0, +20, and +40 mV).

stimulus reversed sign between 0 and +10 mV. The same cell was then tested with a flash 0.6 log units more intense. In this case no single reversal potential was observed (Fig. 7 *B*); rather, at 0 mV there was a visible, inwardly directed, early component followed by a small outward hump. Further examples of photocurrents with no single reversal potential were found in eight other cells using brighter flashes of light. Two such examples are shown in Fig. 8. The appearance of the biphasic response in the range between 0 and +20 mV is particularly evident in the panels on the right-hand side of the figure, where the current traces from the left side have been enlarged and superimposed. It is difficult to estimate the exact location of the individual reversal voltages because neither the extent of overlap of the two response components nor their relative magnitude can be determined with any degree of certainty.

*Effects of Light Adaptation*

The lower light sensitivity of the late component of the photocurrent could also imply a lower susceptibility to light adaptation. In such a case it should be possible to differentially adapt the two phases of the photoresponse. To that end the photocurrent was examined first under dark-adapted conditions, and then with repetitive stimulation using bright flashes. Fig. 9 *A* shows the light response of a cell clamped at −60 mV, stimulated with flashes of increasing intensity. The brightest stimulus evoked the typical pattern, consisting of the fast inward peak and the slower secondary tail. At that point maximum intensity flashes were presented repetitively,

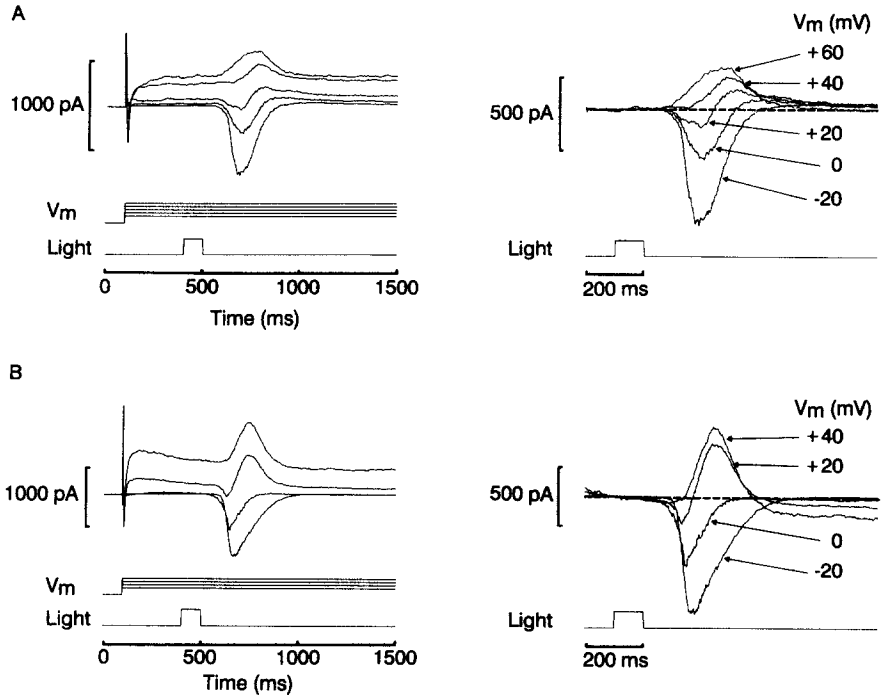


FIGURE 8. Example of markedly biphasic photocurrents obtained with a reversal paradigm in two different cells (*A* and *B*). A near-saturating light intensity was used (−2.9 and −3.2 log, respectively). The right-hand panels show an expanded view of the photocurrents, which were superimposed with respect to the baseline level just before the start of the response.

every 90 s (Fig. 9 *B*). As expected, the early peak grew progressively smaller with successive stimuli as the cell desensitized. By contrast, the late component did not appear to diminish in amplitude, and the last response in the series consisted of the slow wave of inward current with little contamination from the fast transient. A similar effect was documented in another cell.

*Modulation of the Photoresponse by the Ca Current*

The experiments described above were carried out under controlled, steady membrane potential to examine the light-dependent mechanisms in isolation from other

conductances that respond to voltage changes alone. A relevant question, however, concerns whether such voltage-sensitive channels interact in some way with the light-activated conductances. Of particular interest is the presence of a conspicuous, voltage-dependent Ca conductance in solitary *Lima* photoreceptors, which was examined in the preceding paper (Nasi, 1991*b*). Membrane depolarization in response to light can be well above threshold for these channels (Nasi, 1991*a*, *b*), and activation of the Ca current may result in a significant intracellular accumulation of Ca ions. Since it is widely thought that rises in intracellular Ca mediate light adaptation in invertebrate photoreceptors (Brown and Blinks, 1974; Brown and Lisman, 1975; Lisman and Brown, 1975; Fein and Charlton, 1977), it is conceivable that a voltage-triggered Ca transient may modulate the cell's responsiveness to light. To evaluate this possibility, cells were tested with flashes of light, either delivered

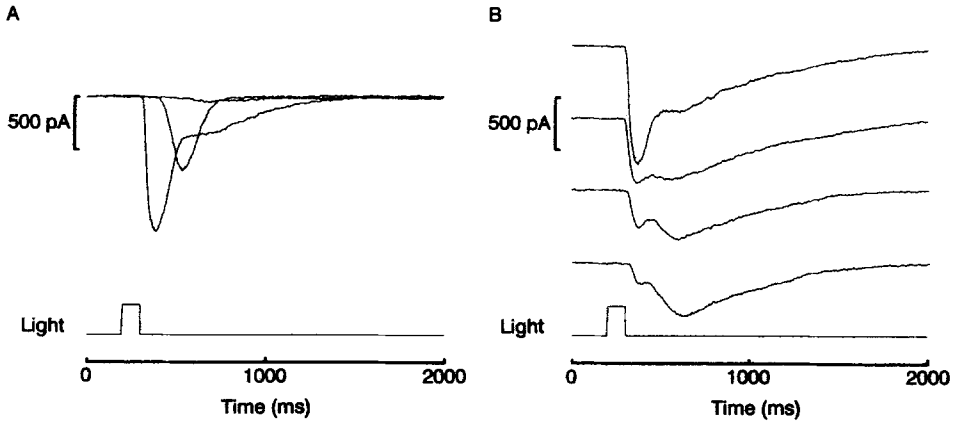


FIGURE 9. Effect of light adaptation via repetitive stimulation with bright flashes. In *A* the cell was voltage clamped at −60 mV and stimulated with 100-ms flashes of increasing intensity (−4.4, −2.4, and −0.9 log). Notice the appearance of the characteristic late tail for the brightest light. Subsequently, unattenuated steps of light (0 log) were presented repetitively every 90 s (*B*). The first stimulus evoked the typical biphasic response, but with subsequent stimuli the early transient progressively decreased, whereas the amplitude of the late wave was maintained.

alone or preceded by a brief (100 ms) depolarization to a voltage that strongly activates Ca channels.

The results of such an experiment are shown in Fig. 10, and demonstrate that the conditioning prepulse causes a measurable reduction in the amplitude of the photocurrent, accompanied by a slight acceleration of its kinetics. These changes in the response are clearly reminiscent of the effects of light adaptation. Similar results were obtained in four other cells, using conditioning depolarizations from +20 to +40 mV, with a pulse duration between 50 and 100 ms. The prepulse-induced decrease of the light response ranged from 10 to 30%. This effect also obtains with internal perfusion with Cs, which completely abolishes outward currents, leaving only the Ca current functional (not shown). Increasing the time between the prepulse and the test flash from 100 ms to 1 s showed little or no sign of recovery, probably

because such an interval is not sufficiently long for the accumulated Ca to dissipate. In neurons, the decay of Ca transients after activation of voltage-sensitive Ca channels can require several seconds (Gorman and Thomas, 1978; Gorman et al., 1984).

## DISCUSSION

### *Presence of at Least Two Light-sensitive Conductances*

Light-dependent currents of *Lima* photoreceptors voltage clamped at a steady potential exhibited a complex kinetics as the intensity of stimulating light was increased, revealing an early transient followed by a delayed hump or a tail. Several considerations suggest that this is due to the presence of two separate ionic

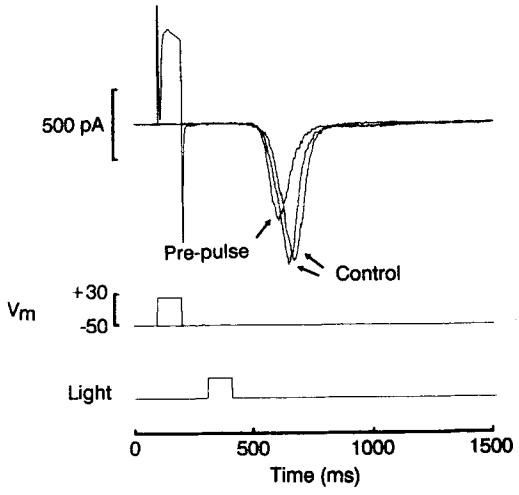


FIGURE 10. Reduction of the light-induced current resulting from administration of a depolarizing conditioning prepulse. The cell was voltage clamped at −50 mV and a 100-ms light stimulus (−3.2 log) was presented (control). A second test flash was then delivered, this time preceded by a 100-ms depolarizing step to +30 mV, a potential that causes near-maximum activation of voltage-dependent Ca channels. The amplitude of the photocurrent following the conditioning voltage step is significantly reduced, and the kinetics is faster. Finally, a control test flash without prepulse was again presented, producing a response similar to the one elicited by the initial trial. Current traces were not leak-corrected. The cell was bathed in ASW, with no K blockers added.

mechanisms activated by light, and rule out the possible involvement of the Ca-dependent K conductance present in these cells: (*a*) The Ca sensitivity of $I_{K(Ca)}$ at voltages near the cell resting potential is modest (Barrett et al., 1982), and so is the driving force on K ions. It is difficult to envision a large current contributed by Ca-activated K channels under such conditions. (*b*) Cell superfusion with TEA did not eliminate the complex kinetics at high stimulus intensities (Fig. 4), nor did internal perfusion with Cs (Fig. 5 *A*), although these treatments are effective in suppressing a Ca-activated K current elicited by voltage-clamp steps. (*c*) The biphasic time course becomes more pronounced with hyperpolarization (Fig. 6), whereas the contribution of a K current should be reduced at more negative voltages.

The differential voltage sensitivity of the early and late phases of the photoresponse also argues in favor of the existence of two separate light-dependent ionic

mechanisms. Further support for this notion is provided by the observation that in a reversal paradigm using dim light stimulation (thus preferentially activating the early component of the response) a null potential can be found (Fig. 7 *A*). With brighter stimuli, however, the photocurrent acquires a striking S-shaped time course within a certain voltage range (Fig. 8). Finally, the two phases of the light response appear to be differentially susceptible to light adaptation, and it is possible to evoke the late component under conditions that cause a substantial reduction in the amplitude of the early peak.

The identification of a separate, late component in the photocurrent gives no a priori reason to attribute it to a different light-controlled conductance, and the possible involvement of other ion transport mechanisms must be considered. In particular, many cell types possess a Na/Ca exchange mechanism activated by high levels of intracellular Ca (DiPolo, 1973), with a stoichiometry of ~3Na:1Ca (Yau and Nakatani, 1984; Rasgado-Flores and Blaustein, 1987). The operation of this antiporter is electrogenic, contributing a net inward current in the forward mode (i.e., the Ca extrusion mode; see Yau and Nakatani, 1984; Kimura et al., 1987). The Na/Ca exchanger plays an important role in regulating Ca levels in vertebrate photoreceptors (Yau and Nakatani, 1984), and its existence has also been demonstrated in invertebrate visual cells (Minke and Armon, 1984; O'Day and Gray-Keller, 1989).

Intense light stimulation of invertebrate photoreceptors is known to result in a substantial rise in internal Ca (Brown and Blinks, 1974; Levy and Fein, 1985), and could conceivably lead to the activation of the electrogenic exchanger. There are reasons to doubt, however, that such a mechanism can account for the second component of the light response in *Lima* photoreceptors. In the first place, the size of the late wave of current can be quite large, usually several hundred pA. Even considering a conservative figure of 100 pA and an upper bound estimate of the cell surface area of $60–70 \times 10^{-6}$ cm$^2$, based on capacitance measurements (Nasi, 1991*a*), the peak transport rate would be ~150 pmol/cm$^2$ · s. In comparison, the maximum Ca extrusion rate for the Na/Ca exchanger measured under controlled conditions in dialyzed squid axons is ~3 pmol/cm$^2$ · s (DiPolo, 1973), and even the higher value of 16 pmol/cm$^2$ · s estimated by O'Day and Gray-Keller (1989) in ventral photoreceptors of *Limulus* is still too low (by one order of magnitude). It is unlikely that the plasma membrane of *Lima* cells would contain such an anomalous high density of the exchanger. An additional consideration is the issue of voltage dependence: the late component of the photocurrent appeared to be rather sensitive to manipulations of the membrane potential, its amplitude changing up to several fold with a 20-mV voltage change (see Fig. 6). The voltage dependence of the Na/Ca exchanger, on the other hand, is quite modest, of the order of 8–32% for a 25-mV step in membrane potential (DiPolo et al., 1985; see also Allen and Baker, 1986). Finally, in the few cases where kinetic separation of the two waves appeared to be quite unambiguous (e.g., Fig. 5), input resistance measurements indicated that the secondary component was accompanied by an increase in membrane conductance. The contribution of an electrogenic exchange mechanism to the total light response should not be ruled out, though. Further experiments will be necessary with substitutions of Na ions with Li or guanidinium. The limited survival time of the cells under the present recording

conditions makes such tests difficult. If applicable, the newly developed "perforated patch" technique (Horn and Marty, 1988), which circumvents the washout problems associated with conventional whole-cell recording, may be a promising approach to alleviating such difficulties in future experiments.

Taken together, the available pieces of evidence suggest that separate light-controlled conductances with different kinetics, ion-selectivity, light-sensitivity, and susceptibility to light adaptation are present in *Lima* rhabdomeric photoreceptors. Lisman and Brown (1971) invoked a fast and a slow light-dependent process to account for the effects of membrane voltage on the photocurrent evoked by prolonged steps of light in *Limulus* ventral photoreceptors. The slower mechanism had an onset and a decay kinetics in the time scale of many seconds, and displayed a negative slope resistance over the entire range of voltages examined. Leonard and Lisman (1981) later characterized it as a decrease of a voltage-sensitive K conductance, which is produced by light stimulation via a Ca-dependent mechanism (Chinn and Lisman, 1984). As such, the phenomenon is of a different nature than the comparatively rapid, light-induced late wave described in this report, in terms of both time course and voltage dependence. Maaz et al. (1981) have also argued that separate mechanisms with different susceptibilities to light adaptation may account for the shape of the response of *Limulus* cells to brief flashes.

Under certain conditions, a long-lasting tail (called prolonged depolarizing afterpotential, or PDA) has been observed in the photoresponse of many invertebrate visual cells, such as the UV-sensitive photoreceptors of the median ocellus of *Limulus* (Nolte and Brown, 1972), and the photoreceptors of *Balanus* (Hochstein et al., 1973). This phenomenon appears to be related to the presence of long-lived photoproducts of rhodopsin, and can be induced by intense stimulation causing a net shift in pigment state (when the absorption spectra of the photopigment and that of the photoproduct are different), but not by spectrally "neutral" stimuli (for a comprehensive review, see Hillman et al., 1983). The membrane conductance changes underlying the PDA are apparently the same as those responsible for the late receptor potential (Nolte and Brown, 1972; Hochstein et al., 1973; Brown and Cornwall, 1975), and can last for extended periods of time (tens of seconds to hours). While in this report no attempt was made to investigate wavelength-dependent effects, the second component of the photocurrent of *Lima* cells does not share many similarities with the PDA: (*a*) the late wave can be elicited by white light, which should be a neutral stimulus in a cell not previously subjected to chromatic adaptation; (*b*) its duration is only on the order of a few hundred milliseconds; and (*c*) the underlying ionic mechanisms are clearly different from those of the early phase of the light response.

The behavior of the photocurrent in *Lima* is more akin to the complex light-evoked voltage changes that have been observed in photoreceptors of *Hermissenda*, which also display distinct components (Detwiler, 1976). Rigorous characterization of the properties of the two light-induced conductances in *Lima* would entail examining them in isolation. This goal may prove difficult using macroscopic electrophysiological recording because of the temporal overlap and the lack of specific pharmacological blockers. A more promising strategy, currently being pursued, is patch clamp recording of single-channel currents. This technique may not only circumvent the

difficulties of separating different ionic mechanisms, but should also make it possible to investigate potential activators and modulators of light-sensitive channels. Recordings of unitary light-sensitive currents in both invertebrates and vertebrates have revealed the existence of at least two populations of channel events (Bacigalupo and Lisman, 1983; Zimmerman and Baylor, 1986). The presence of parallel effector mechanisms in visual cells could thus be relatively widespread.

The kinetics and light sensitivity of the late component of the photocurrent, along with the effects of membrane voltage, indicate that it underlies the slow depolarizing wave that can be elicited by bright stimuli in unclamped photoreceptors (Nasi, 1991*a*). The hyperpolarizing dip in the voltage response, on the other hand, was previously attributed chiefly to the activation of a Ca-sensitive K conductance (Nasi, 1991*b*). It can be tentatively suggested, therefore, that the progressive enhancement of such a hyperpolarizing phase induced by stimuli presented in rapid sequence may be due to an accumulation of intracellular Ca. Further studies will be required to clarify the nature of the mechanisms involved.

### *Possible Involvement of Voltage-activated Calcium Channels in the Visual Process*

Voltage-dependent conductances of *Lima* photoreceptors play an important role in shaping the light response and initiating the action potentials that propagate centripetally along the pallial nerve. In addition, the results presented in Fig. 10 indicate that the Ca current may also participate in the modulation of visual excitation. In *Limulus* ventral photoreceptors it has been established that an increase of intracellular free Ca mediates the process of light adaptation (Brown and Lisman, 1975; Lisman and Brown, 1975; Fein and Charlton, 1977). This mechanism involves primarily the release of Ca from intracellular stores (Brown and Blinks, 1974; Lisman, 1976; Levy and Fein, 1985). A voltage-dependent conductance permeable to $Ca^{2+}$ ions has been described in *Limulus* photoreceptors (Lisman et al., 1982), and membrane depolarization has also been shown to produce a desensitization of the light response (O'Day et al., 1982). Such an effect, however, is significant only with prolonged voltage-clamp steps ($>1$ s) administered under dark-adapted conditions, whereas it contributes little if the cell is light adapted, and therefore its intracellular Ca concentration is already high (Brown et al., 1977). The conditioning pulses used in the present experiments, on the other hand, were quite short (50–100 ms), comparable to the duration of the action potentials generated by *Lima* photoreceptors (Nasi, 1991*a*), although somewhat larger in amplitude. Therefore, in these cells such a mechanism is probably functional under normal physiological conditions, particularly in view of the fact that bright stimuli can elicit a burst usually containing five to eight action potentials. One reason why in *Lima* Ca entry through the plasma membrane may contribute more significantly to desensitization is the small size of the photoreceptors, providing a more favorable surface-to-volume ratio for Ca influx to substantially modify cytosolic Ca levels.

A rough estimate of the increase in intracellular Ca can be obtained by integration of a representative Ca current record over a 100-ms time interval. For a peak amplitude of 1 nA and a decay time constant of 30 ms (closely approximating a typical Ca current in these cells; see Nasi, 1991*b*), calculations yield a total Ca influx of $\sim 15 \times 10^{-17}$ mol. The volume of an average photoreceptor can be estimated at

$3.25 \times 10^{-6}$ mm$^3$ (corresponding to a prolate spheroid with axis length of 25 and 10 μm, respectively). About 85–95% of the incoming Ca is presumed to be rapidly buffered because of reversible binding to intracellular sites (Blaustein and Hodgkin, 1969; Nasi and Tillotson, 1985). Taking a midpoint estimate of 90% for the fraction of Ca instantaneously buffered, this yields an average 4.5-μM increase in cytosolic free Ca (assuming that metabolically driven Ca uptake and extrusion mechanisms operate on a much slower time scale, and thus contribute little at early times after Ca influx). Clearly, the above argument is an oversimplification, in view of the facts that (*a*) $[Ca]_i$ will not be uniformly distributed in the cytosol in time scale of seconds after voltage stimulation (Gorman et al., 1984); (*b*) the distribution of Ca channels in the plasma membrane may also not be homogeneous; and (*c*) the spatial location of the relevant target site were Ca induces its desensitizing effects is unknown. The resulting figure is nevertheless noteworthy, being of the same order of magnitude as the values of $\Delta[Ca]_i$ reported to accompany light adaptation in *Limulus* (Brown et al., 1977; Levy and Fein, 1985).

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