Light-Induced Changes of Sensitivity in *Limulus* Ventral Photoreceptors

J. E. LISMAN and J. E. BROWN

From the Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138, the Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee 37232, and the Marine Biological Laboratory, Woods Hole, Massachusetts 02543. Dr. Lisman's present address is the Department of Biology, Brandeis University, Waltham, Massachusetts 02154.

ABSTRACT   The responses of *Limulus* ventral photoreceptors to brief test flashes and to longer adapting lights were measured under voltage clamp conditions. When the cell was dark adapted, there was a range of energy of the test flashes over which the peak amplitude of the responses (light-induced currents) was directly proportional to the flash energy. This was also true when test flashes were superposed on adapting stimuli but the proportionality constant (termed peak current/photon) was reduced. The peak current/photon was attenuated more by brighter adapting stimuli than by less bright adapting stimuli. The peak current/photon is a measure of the sensitivity of the conductance-increase mechanism underlying the light response of the photoreceptor. The response elicited by an adapting stimulus had a large initial transient which declined to a smaller plateau. The peak current/photon decreased sharply during the declining phase of the transient and was relatively stable during the plateau. This indicates that the onset of light adaptation is delayed with respect to the onset of the response to the adapting stimulus. If the adaptational state just before the onset of each of a series of adapting stimuli was constant, the amplitude of the transient was a nearly linear function of intensity. When the total intensity was rapidly doubled (or halved) during a plateau response, the total current approximately doubled (or halved). We argue that the transition from transient to plateau, light-elicited changes of threshold, and the nonlinear function relating the plateau response to stimulus intensity all reflect changes of the responsiveness of the conductance-increase mechanism.

INTRODUCTION

The ventral eye of *Limulus* contains large isolated photoreceptor cells which have proved suitable for electrophysiological analysis of the transduction process. The plasma membrane of these cells has a microvillous structure (Clark et al., 1969) and is believed to contain the visual pigment. Intracellular recordings have shown that light elicits a positive-going receptor potential in ventral photoreceptors (Millecchia et al., 1966). This depolarizing re-

ceptor potential is generated by a light-induced increase in membrane conductance, with the consequent current carried mainly by an inflow of sodium ions (Millecchia and Mauro, 1969 a, b; Brown and Mote, 1974).

Both in Limulus lateral and ventral eyes, dim illumination evokes small discrete depolarizations termed "discrete waves," each of which is thought to arise from the absorption of a single photon (Fuortes and Yeandle, 1964; Borsellino and Fuortes, 1968). As the intensity is increased, the frequency of the discrete waves increases linearly; at bright intensities, the discrete waves summate to form a smooth response (Dodge et al., 1968). The response waveform to a long, bright stimulus is characterized by a large initial transient which declines to a smaller plateau. The amplitude of the plateau phase is a nonlinear (nearly logarithmic) function of intensity (MacNichol, 1958; Millecchia et al., 1966) over a wide range of stimulus intensities.

The adaptational state of Limulus photoreceptors changes when the level of illumination is changed (Hartline and McDonald, 1947). Light adaptation is defined as the decrease in sensitivity that results from illumination. Dark adaptation is the recovery of sensitivity that occurs after the cell is returned to darkness. In Limulus photoreceptors, complete dark adaptation may take many minutes (Benolken, 1962; Fein and DeVoe, 1973). It has been argued (Dodge et al., 1968) that light adaptation occurs because the voltage change produced per absorbed photon is reduced by illumination, and that this accounts for the nonlinear relation between the amplitude of the plateau phase and light intensity. Dodge et al. (1968) inferred from this reduction of the voltage response that the conductance change per absorbed photon is reduced.

In the present study we have measured the light-induced current by using a voltage clamp technique. With membrane voltage held constant, the current induced by a brief flash is directly proportional to the light-induced change in a membrane conductance. An adapting stimulus is shown to reduce the current (hence the conductance change) induced by a test flash. The time-course of this reduction during an adapting stimulus is compared to the waveform of the response to the adapting stimulus itself. A preliminary report of some of these experiments appeared previously (Lisman and Brown, 1970).

METHODS

The recording and voltage clamp circuitry was described in a previous paper (Lisman and Brown, 1971). In all experiments reported here, the membrane voltage was clamped to the resting potential of the dark-adapted cell (−45 to −60 mV). For averaging light-evoked currents, the output of the current to voltage transducer (10 mV/nA) was connected to the input of a Digital Equipment Corporation (Marlboro, Mass.) Lab 8 computer, programmed for signal averaging. In most of the experiments, the cell was continuously perfused with artificial seawater (Brown and Lisman, 1972).

In order to superimpose two independent stimuli, we combined light beams from two tungsten sources with a beam splitter. When measured, the intensities of the unattenuated beams are given in the figure captions. In experiments using monochromatic light (530-nm narrow band interference filter), the beams were calibrated with the monochromatic filters in place. Neutral density filters were calibrated with a spectrophotometer. Light intensity was measured with a United Detector Technology (Santa Monica, Calif.) PIN-10 photodiode in the unbiased mode. Intensities were always measured after the beam passed through infrared cutoff filters. The measured light intensities were only approximations of the true intensities at the cell surface. We have not corrected for the attenuation (0.3 $\pm$ 0.1 log units) of the light beam due to its passage through the nerve bundle on which the photoreceptors lie. Further ambiguity ($\pm$0.15 log units) was introduced because of variability in the distance between the condenser lens and the cell surface. We thus estimate that the light intensity at the cell surface was lower than the measured values by 0.05–0.55 log units.

In order to avoid problems of electrical coupling between receptor cells, we were careful to select cells which appeared isolated. A cell was impaled with two microelectrodes, each of which was manipulated independently to maximize the receptor potentials recorded. Cells were studied only if both microelectrodes recorded equal responses. Except where otherwise indicated, the experiments were performed on cells which produced large "discrete waves" (5–10 mV, not including the regenerative event [Millecchia and Mauro, 1969 a; Dowling, 1968]). Many cells never produced discrete waves of this size, even when fully dark adapted. Although we did not study the problem systematically, it seemed that in cells that produced large discrete waves, the responses to stimuli of fixed size remained stable longer. In this paper we use the word "response" to mean light-activated current except where voltage response is clearly specified.

RESULTS

Responses of Dark-Adapted Cells to Brief Flashes

Cells were penetrated with two microelectrodes and allowed to dark adapt. During dark adaptation the spontaneously occurring discrete waves (Fuortes and Yeandle, 1964) increased in size. After about 20 min there was no further increase in the size of the discrete waves and the cell was taken to be dark adapted. By convention, inward (positive) membrane current is plotted downward in the figures. Fig. 1 A shows typical responses of such a cell to brief flashes near threshold. We define threshold as the flash energy at which the response of a dark-adapted cell was approximately one discrete wave, on the average. Near threshold the number of discrete waves per stimulus varied, as did their latency and amplitude. To determine how the discrete waves summed, we averaged responses at two different low energies. Fig. 1 B and C shows that by doubling the energy, the average response was doubled, but that the waveshape of the average response was nearly unchanged. This is consistent with the idea that the discrete waves are evoked independently
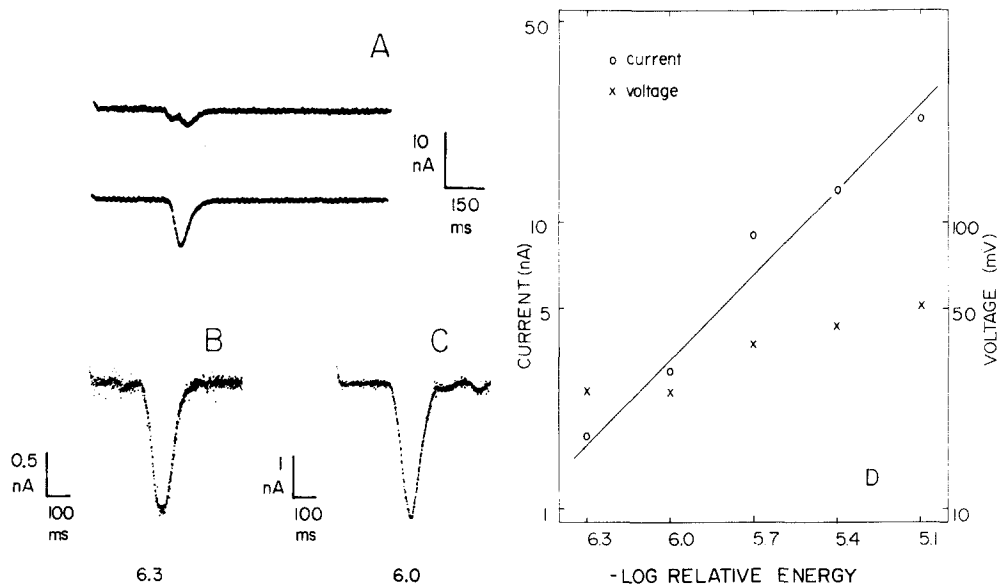
FIGURE 1.   Current induced by summation of discrete waves. All data are taken from the same cell. Inward positive current is downward. (A) Current necessary to voltage clamp discrete waves evoked by 10-ms dim flashes (6.0 log units of attenuation). In the upper trace there are two discrete waves, in the lower trace one discrete wave. (B) Computer average of currents evoked by 32 flashes (10 ms, 6.3 log units of attenuation). The interstimulus interval was 15 s. The flash evoked approximately one discrete wave on the average. (C) Average current induced by 10-ms flashes (6.0 log units of attenuation) twice the energy of the flashes in B. Average current is double that in B (note doubling of calibration), but has nearly identical waveform. 32 responses were averaged. Interstimulus interval was 15 s. (D) Log-log plot of average current (O's) and average voltage (X's) as a function of energy. Each point is the average of eight responses. The line drawn through the O's has a slope of 1. The voltage responses show comparatively little change with energy (see text for explanation). The interstimulus interval was 15 s.

and sum linearly (Adolf, 1964; Borsellino and Fuortes, 1968; Dodge et al., 1968). The nearly exact agreement with linearity shown in Fig. 1 B and C is fortuitous; by Poisson statistics, for the number of discrete waves averaged, the accuracy cannot be greater than 20%. The experiment illustrated in Fig. 1 B and C was repeated in two other cells; the numbers of responses averaged were $n = 150$ (or $n = 200$) at each energy. The responses were recorded 1/15 s, alternating groups of 10 between the two energies. For these two cells, doubling the energy at threshold doubled the average response within 5%.

Borsellino and Fuortes (1968) showed by intracellular recordings from the *Limulus* lateral eye that the average voltage responses to brief flashes varied linearly with energy over a small intensity range (from one to four absorp-

tions per rhabdome). Fig. 1 D demonstrates that in the energy range just above threshold, the average light-induced current was a linear function of energy, but that the average voltage response was not. The voltage responses were a poor reflection of the underlying light-activated conductance changes because single discrete waves often initiated a regenerative event (Dowling, 1968; Millecchia and Mauro, 1969 a), the size of which was not graded with energy. The regenerative event is excited by a change in membrane voltage rather than directly by light; it can be evoked by positive-going voltage changes produced either by light or by pulses of current passed through a microelectrode. In some cells small depolarizations failed to evoke regenerative responses; presumably this was true of the cells used in the experiments of Borsellino and Fuortes.

We examined the range of energies over which there was a linear relationship between peak light-induced current and energy. Fig. 2 A shows the responses to single brief flashes presented to a dark-adapted cell. Near threshold, the responses varied greatly (the linearity in this region of the curve was established as in Fig. 1). The responses to brighter flashes had much less relative variability. For stimuli 2.4 or more log units above threshold, the latency of the peak current decreased and the falling phase of the response was more rapid. After responses to these brighter stimuli there was a marked decrease in the size of the spontaneous discrete waves. In these cases, we were careful to wait for complete dark adaptation to occur (up to 10 min) before presenting another stimulus. The graph in Fig. 2 B is a plot of peak current versus flash energy for the responses in Fig. 2 A, and shows that the last response falling in the linear range was evoked by a flash about 100 times brighter than that necessary to evoke one discrete wave on the average. Brighter lights caused comparatively little increase in peak current.

Responses of Light-Adapted Cells to Brief Flashes

The responses to brief test flashes were examined during plateau responses to prolonged adapting lights. In the presence of a background light a test flash induced an average current that was directly proportional to flash energy over some range of energies (Fig. 3 A). Similarly, before the onset of the adapting light, the size of the response was linearly related to the energy of the test flash, but the cell was more sensitive. Thus, the effect of the adapting light was to reduce the responses to all test flashes by the same fraction (i.e. the proportionality constant, relating peak current to test flash energy, was reduced).

The reduction of the proportionality constant was graded with intensity of the adapting stimuli (i.e. the higher the intensity, the smaller the proportionality constant) (Fig. 3 B). This proportionality constant, given in
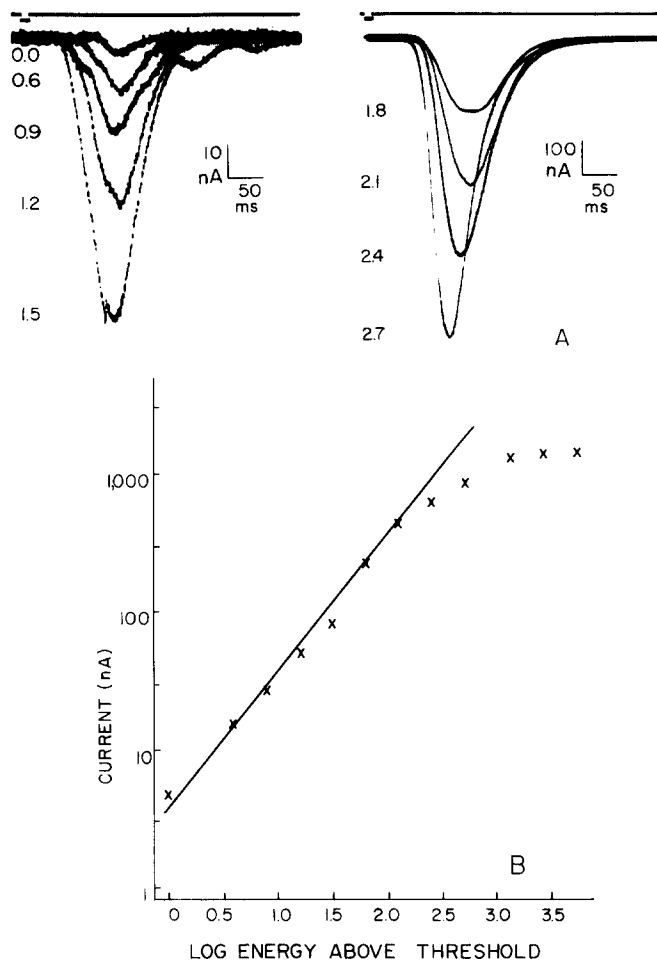
FIGURE 2. Current induced by brief flashes. In a dark-adapted cell, light-induced current increases linearly with stimulus energy over a wide intensity range. (A) Single responses at different energies. The log energy relative to threshold (flash which evokes one discrete wave on the average) is marked near the peak of each response. The cell was dark adapted between stimuli. At energies up to 2.1 log units above threshold, the responses have nearly the same shape. Duration of each flash was 12 ms. The upper trace is the output of a stimulus monitor. (B) Log-log plot of response vs. energy curve; part of the data is shown in A. The line has a slope of 1. The linear range extends from threshold to about 100 times threshold.

units of peak light-induced current per photon incident on the photoreceptor, is termed the peak current/photon.[1]

[1] The duration of the test flashes used (10–35 ms) was shorter than the integration time of the photoreceptor response (the interval during which intensity × time is approximately constant for eliciting a constant response). Therefore, the important variable is flash energy (intensity × time) which is directly related to the number of photons in the flash.
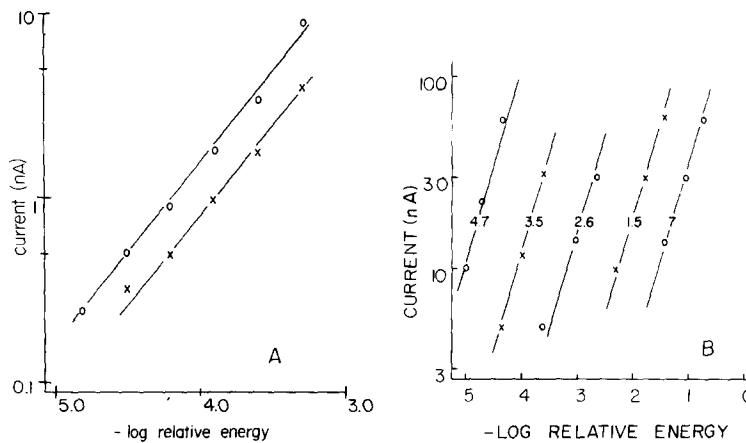
FIGURE 3.   Effect of background illumination on responses to test flashes. (A) The average current (X's) produced by test flashes (10 ms) superposed on adapting stimuli of a fixed intensity varies linearly with the test flash energy. The responses (O's) to the same test flashes given just before the adapting stimuli are larger by a constant factor. The lines have a slope of 1. Each point is the average of the responses to 16 test flashes. Absolute energies were not measured. (B) The response vs. energy curves for brief (25 ms) flashes superposed on the plateau phase of the response to 700-ms adapting stimuli, recorded from a different cell than A. For each different intensity of the adapting stimuli, the log attenuation is given on the corresponding graph. The lines drawn through data recorded at each level of background intensity have a slope of one. Both the test beam (1 × 10^{-6} W-s/cm^2) and the adapting beam (0.6 × 10^{-5} W/cm^2) were monochromatic. Adapting stimuli were given every 3.5 s.

Time-Course of the Decrease in Peak Current/Photon

We sought to determine whether the linear relationship between test flash response and energy found during the plateau phase also obtained during the rest of the response to the adapting stimulus. The response waveform to an adapting stimulus has an initial transient followed by a smaller plateau (Figs. 6 and 8). In order to measure the responses to test flashes superposed on this waveform, it ideally should be noise-free and slower than the response to the test flash. The noisiness of the responses to light tends to decrease with increasing intensity (Dodge et al., 1968). Increasing intensity, however, leads to a large increase in the amplitude of the transient, with much less effect on the plateau (see Fig. 7). As a result, the waveform tends to have a fast decline from the peak transient current to the plateau. If the adapting stimulus is given at high repetition rates the decline is slowed because the amplitude of the transient phase is reduced more than the plateau (Millecchia and Mauro, 1969 a). Thus, bright adapting stimuli given at high repetition rates elicit responses having both low noise and a slowly varying waveform.

Using this procedure we measured response vs. energy curves for test

flashes given at four different times relative to the adapting stimulus (Fig. 4). The response vs. energy curves are approximately linear in each case. This indicates that values of peak current/photon can be defined throughout the response to an adapting stimulus. Fig. 4 also illustrates that the responses to test flashes superposed on the transient are nearly identical in magnitude to those evoked by the same size stimuli given just before the adapting light. Apparently the reduction in peak current/photon does not occur immediately at the onset of the adapting light, but occurs with a delay. During the falling phase of the transient the peak current/photon decreases; it reaches a minimum during the plateau phase.
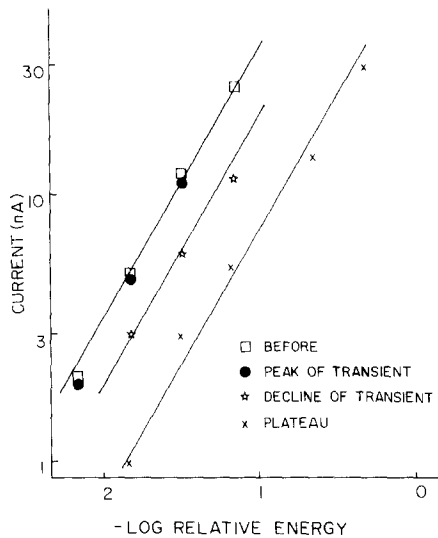

FIGURE 4

FIGURE 5

FIGURE 4. Response vs. energy curves for test flashes (35 ms) given at various times during the responses to adapting stimuli (1.1 s). The lines (fitted by eye) have a slope of one. The response to a given size test flash was (a) approximately the same when given before the adapting stimulus (□) and (b) when superposed on the transient phase of the response to the adapting stimulus (●); was somewhat smaller when superposed on the declining edge of the transient phase, 270 ms after peak of the transient (☆), and was smallest when superposed on the plateau (X). The adapting light was 4×10^{-7} W/cm^2 and the energy of an unattenuated flash was 3.5×10^{-7} W-s/cm^2; both were monochromatic.
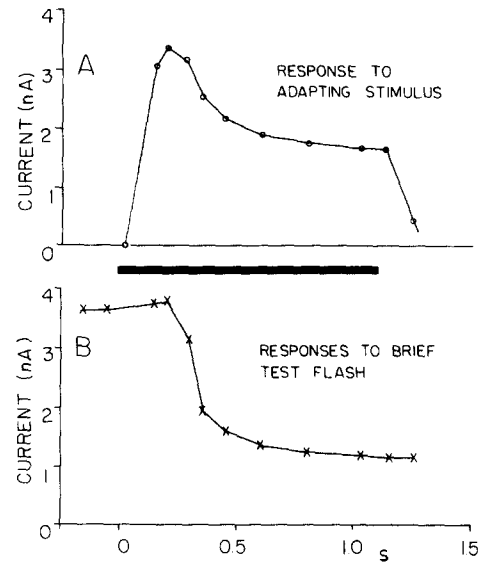
FIGURE 5. Changes in amplitude of responses to test flashes during an adapting stimulus. (A) The response (at discrete times) to the adapting stimulus used in B. (B) Amplitude of the responses to brief (35 ms) test flashes given at different times during the response to the adapting stimulus. The amplitudes of the test-flash responses (X's) decline most rapidly during the falling phase of the transient. Test stimulus energy was 5.6×10^{-7} W-s/cm^2 monochromatic light. An adapting stimulus (0.35×10^{-5} W/cm^2 monochromatic light) was given every 2.2 s. The dark bar represents the duration of the adapting stimulus. Zero time marks the onset of the adapting stimulus.

To follow more precisely the time-course of the changes in peak current/ photon, test flashes of fixed energy were given at many times during an adapting stimulus (Fig. 5). The greatest changes in responsiveness to the test flashes always occurred during the transition from transient to plateau. The responsiveness to the test flashes changed little during the plateau response. Thus, the reduction in peak current/photon qualitatively paralleled the decline of the response to the adapting stimulus during the transition from transient to plateau.

The response to a test flash during the peak of the transient was sometimes slightly larger than the response before the adapting stimulus (Fig. 5 B). This 5–20% increase was seen in four of the nine cells studied (see also Fig. 6). Four other cells showed no such effect (see Fig. 4), whereas in one cell the test response was somewhat smaller at the peak of the transient.

Another way to measure the responses to test flashes superposed on adapting stimuli is to overcome the noisiness of responses to dim illumination by averaging the signals. A noisy response to a single dim adapting stimulus is shown in Fig. 6 A; the response had transient and plateau phases. The records in Fig. 6 B, C, and D are computer-averaged responses. In Fig. 6 B the test flash preceded the adapting stimulus and evoked an average response of 1.6 nA. Fig. 6 C is the superposition of two traces: one trace is the average response to the adapting light alone, and the second trace is the average response when the response to the test flash fell on the peak of the transient. The difference between the two traces is the average response increment evoked by the test flashes and is approximately 2.0 nA. Thus in the first 200 ms of the response to this adapting light there was no decrease in the average response to the test flashes. Test flashes were also given near the end of the adapting stimulus. Fig. 6 D shows the superposition of two such average responses. In one, the test flash occurred before the adapting light was turned off, in the other the test flash followed the adapting light. The average responses were the same just before and just after the termination of the adapting light (see also Fig. 5) but were smaller than the average response to equally bright test flashes superimposed on the transient phase. These results obtained with dim adapting lights are similar to those obtained with brighter adapting stimuli (Fig. 4).

It should be noted that the transient elicited by the adapting stimulus in Fig. 6 B (4.7 nA) is smaller than the transient evoked by the same stimulus in Fig. 6 C (5.5 nA). Part of the difference can be attributed to light adaptation caused by the test flash which was given just before the adapting stimulus in Fig. 6 B, but not in Fig. 6 C. The responses to test flashes were in the linear range; nevertheless the responsiveness of the cell could be affected at times after the peak of the response to a flash.

During the transition from transient to plateau, responses to test flashes
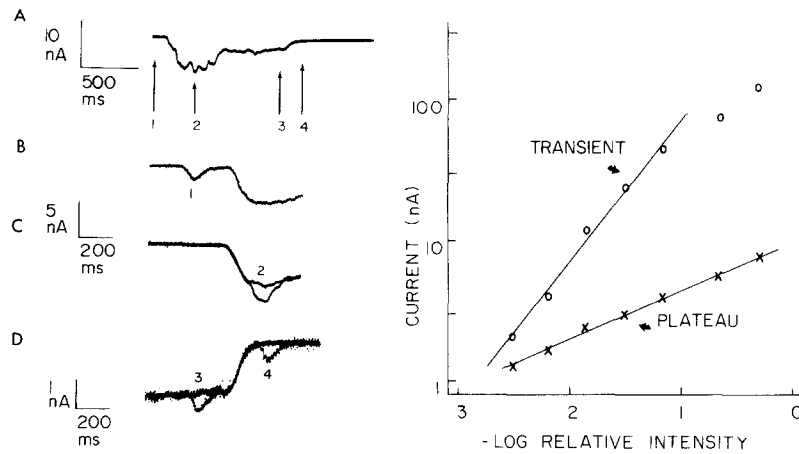
FIGURE 6   FIGURE 7

FIGURE 6. The average response produced by a test flash (10 ms) of fixed energy at different times relative to an adapting light. B, C, and D are the averages of eight responses. (A) Single response to a dim adapting stimulus. There is an initial transient, followed by a smaller plateau phase. The arrows (1–4) mark the different times at which we gave test flashes in B, C, and D. (B) Average response to a test flash given just before the adapting light (arrow 1). (C) Two superimposed traces showing the average current during the transient phase (arrow 2) with and without superposed test flash. The increment produced by the test flash is slightly larger than the response to the same intensity test flash given just before the adapting light in B. (D) Two superimposed average responses. In one, the test flash was given during the plateau just before the termination of the adapting light (arrow 3). In the other, the test flash was given just after the termination of the adapting light (arrow 4). The responses to the test flash were nearly the same in the two conditions, but both were smaller than the responses to the same flash intensity in B and C.

FIGURE 7. Amplitude of transient and plateau response as a function of intensity. The state of adaptation just before (2.2 s) the stimulus was constant as judged by the response to conditioning stimuli. In the lower intensity range, the amplitude of the transient (O's) was a linear function of intensity. Line through O's has slope of 1. Plateau amplitude (X's) is a much weaker function of intensity (line through X's has slope of 0.34 on log-log coordinates). Stimulus duration was 1.1 s; intensity of unattenuated beam was 2.5×10^{-3} W/cm². The conditioning stimulus duration was 1.1 s (beam attenuated by 2.8 log units).

were reduced by factors of 3.2 in Fig. 5 and 3.7 in Fig. 6. The reduction of the responses to the adapting stimuli (the ratio of peak transient current to plateau current) can also be computed for the same experiments and were 2.0 and 3.2, respectively. Thus in both cases the ratio of transient to plateau was somewhat less than the reduction of the responses to test flashes. This result was obtained from all the cells examined.

That changes in peak current/photon occur after the peak of the transient (Figs. 4, 5, 6) suggests that the amplitude of the transient should depend

linearly on the intensity of the adapting light, provided that the state of adaptation is always the same at the onset of the adapting stimulus. This condition was achieved by repetitively stimulating the cell to maintain it in a constant state of light adaptation. When the responses to the repetitive stimuli reached a fixed amplitude, an adapting stimulus was given. The peak transient responses to the adapting stimuli, plotted in Fig. 7, were directly proportional to intensity except at higher intensities. For another cell, the maximum slope on log-log coordinates was closer to 0.9.

Some cells were studied that did not meet either or both of our criteria of acceptability: large discrete waves and isopotential responses recorded by the two microelectrodes. In such cells, we often observed that doubling test flash intensity more than doubled the response (the response-intensity curve plotted on log-log coordinates had a slope greater than one). Furthermore, the response to test flashes superposed on the transient phase of the response to an adapting light could be as much as 50% greater than the response seen just before the adapting stimulus. These results could be taken as evidence for cooperative transduction phenomena. However, we presume that in these cells some assumption required for meaningful interpretation of voltage clamp data did not hold.

Responses to Step Changes in Intensity

When the total light intensity is rapidly doubled (or halved) the light-induced current transiently doubles (or halves) approximately (Fig. 8), except at high intensities. Averaged data from many trials on two cells shows that at the two lower background intensities, changing the stimulus by a factor of two elicited a change in current of a factor of 1.9 $\pm$ 0.1, whereas at the brightest background intensity, the average change in current was by a factor of 1.7 $\pm$ 0.1. These data indicate that the magnitude of the response to step increments or decrements of intensity will be within 20% of the plateau current before the step multiplied by the proportional change of light intensity.

DISCUSSION

Changes in the Sensitivity of the Conductance Increase Mechanism

In general, more than one membrane process can contribute to membrane current in the ventral photoreceptors of *Limulus*. The best understood of these processes is a light-induced change in membrane conductance that has a reversal potential at approximately +15 mV (independent of stimulus intensity and duration) and results in the influx of sodium ions (Millecchia and Mauro, 1969 *b*; Lisman and Brown, 1971; Brown and Mote, 1974). This process responds rapidly (within 200 ms) to changes in illumination, and will be referred to as the conductance-increase mechanism (called the "fast

process" by Lisman and Brown, 1971). Membrane current can result from two other processes that respond more slowly to changes in illumination and may persist for minutes after termination of a stimulus. One of these, the electrogenic sodium pump, produces the outward current that causes the hyperpolarization often seen after termination of a bright stimulus (Brown and Lisman, 1972). The other, termed the "slow process" (Lisman and Brown, 1971) is of unknown mechanism and produces voltage-dependent inward currents. The inward currents generated by the slow process increase slowly at the onet of a stimulus and decay even more slowly after the end of the stimulus. It is unlikely that either the electrogenic sodium pump or the slow process currents contribute significantly to the observed responses to brief test flashes. This is because at voltages near resting potential, both processes produce currents that are relatively small (at most, a few nano-amperes). We therefore think that the observed responses to test flashes are an accurate reflection of the time variation of a single process, the conductance-increase mechanism. However, when long or bright stimuli are used the responses may have a small slow process component (see below).

At all background intensities, and at all times during a prolonged stimulus, there was a range of test-flash energies over which the peak amplitude of the light-induced current varied approximately linearly with energy. Therefore, it is possible to define an instantaneous value of the peak current/photon that relates response amplitude to flash energy. Because the responses to brief flashes are generated by activation of mainly the conductance-increase mechanism, the light-induced conductance is directly proportional to the light-induced current measured at a constant voltage. The peak current/ photon is therefore a measure of the sensitivity of the conductance-increase mechanism. Figs. 3, 4, 5, and 6 demonstrate that light causes a reduction in sensitivity of the conductance-increase mechanism in the absence of any change of membrane voltage. A process by which this reduction of sensitivity might occur is the subject of a companion paper (Lisman and Brown, 1975).

A linear relationship between the response to test flashes and test flash energy seems incompatible with recent results of Srebro and Behbehani (1974). They found a nonlinear summation of the responses to two dim flashes having a very short (15 ms) interflash interval. Our findings is in agreement with the linear response versus intensity relation reported in squid photoreceptors (Hagins, 1965). Also, the biphasic wave-form of the light-induced current (of the discrete waves) reported by Srebro and Behbe-hani (1974) is at variance with the monophasic waveform reported here (Fig. 1) and observed by others (R. Millecchia, personal communication).

Delayed Changes in Peak Current/Photon

The reduction in peak current/photon caused by an adapting stimulus occurs with a delay after the onset of the response to the adapting light (Figs. 4, 5,

and 6). This reduction of peak current/photon persists after the adapting stimulus is terminated and even after the conductance increase produced by the adapting stimulus has decayed (Figs. 5, 6).

The delayed reduction of peak current/photon during a stimulus suggests an explanation of why the response to a long bright stimulus usually has two phases, the transient and the plateau. Up to the peak of the transient phase there is relatively little change in peak current/photon (Figs. 4, 5, 6); the peak current/photon decreases during the transition from transient to plateau (Fig. 5). However, since the reduction of current from transient to plateau is less than the simultaneous reduction in the responses to test flashes, the ratio of transient to plateau cannot be used as a quantitative indicator of light adaptation. At least part of this quantitative difference might be explained if a small fraction of the plateau current is due to the slow process. The slow process turns on slowly during a long stimulus and therefore contributes more current to the plateau than to the transient. For this reason, the plateau current due to the conductance-increase mechanism would be smaller than the total measured plateau current, and the ratio of transient to plateau currents (of the conductance-increase mechanism alone) would be larger than the ratio computed from the total measured currents.

Fuortes and Hodgkin (1964) observed that the voltage on the rising edge of the response to a long stimulus was a linear function of intensity but that linearity failed at later times during the response. On the basis of this and other observations, they constructed a model of the voltage responses attributing the decline from transient to plateau to some form of delayed reduction of sensitivity. Our interpretation of the response waveform is thus similar to theirs.

The delayed changes in peak current/photon both at the onset and termination of a stimulus suggests an explanation of another response pattern. The responses to long stimuli are usually characterized by two phases: a transient and plateau. However, if a stimulus is turned on shortly after it has been turned off, there is practically no transient phase of the voltage response (Millecchia and Mauro, 1969 *a*). Rather, the voltage rises rapidly to a level close to that of the previous plateau phase. If the peak current/photon is unchanged during the brief period of darkness, turning on the stimulus again should lead to an initial light-induced conductance equal to the light-induced conductance during the previous plateau.

The initial response to an increment or decrement of light can be predicted approximately from the plateau current measured just before the increment or decrement (Fig. 8), assuming that the instantaneous relationship between total current and light is linear. Dodge et al. (1968) proposed that the plateau response of *Limulus* lateral eye photoreceptors is generated by the linear summation of discrete waves whose frequency depends on light intensity. The plateau responses are a nonlinear function of light intensity because as

the light intensity is raised the amplitude of the discrete waves is reduced. The ventral photoreceptor recordings in Fig. 8 are consistent with this notion if the size of the summating unitary events does not change instantaneously upon a change of illumination, but changes after a delay.

Fig. 8 shows that, to a first approximation, the total current is proportional to light intensity for short times after a step change of light intensity. The proportionality constant has units of current per unit intensity and depends on the history of previous illumination. Peak current/photon is an analogous proportionality constant for the linear relationship between the responses evoked by brief flashes and flash energy. The two constants cannot be used interchangeably to describe the responsiveness of the cell since they are dimensionally different. What is needed is a mathematical treatment which describes both the responses to brief flashes and the responses to longer stimuli (as for example the Fuortes-Hodgkin model), but the development of such a model is outside the scope of the present study. However some general terminology for describing the responsiveness of the cell would be useful. For this we suggest the term conductance-change per unit light intensity, henceforth abbreviated, Δg/intensity.
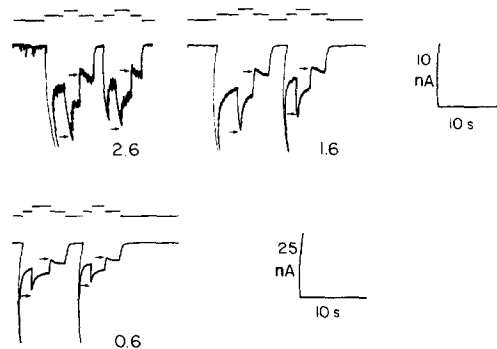

FIGURE 8. Changes in total current in response to doubling (or halving) the total intensity, at three different intensity ranges (log attenuation of the beam at onset of stimulus is given under each trace). Upper lines indicate pattern of light stimulation. Initially, the adapting stimulus was turned on. When the large transient phase of the light-induced current had decayed approximately to a plateau, the intensity was suddenly doubled and the response transiently increased. When a new plateau was reached, the illumination was halved. The current dropped rapidly, but then began to increase. At that point, the stimulus was turned off. Shortly thereafter the same sequence was repeated. The arrows indicate the predicted magnitude of the current if the total current doubled (halved) when the total intensity was doubled (halved). Measurements were made from the bottom edge of the trace width. Intensity changes were achieved by rapidly inserting or removing neutral density filters from the beam. Intensity at zero attenuation of the monochromatic beam was 4×10^{-5} W/cm^2. Responses were recorded on a curvilinear polygraph (Grass Instrument Co., Quincy, Mass.).

Our results suggest that three different aspects of the responses to light in *Limulus* ventral photoreceptors are interrelated reflections of light-induced changes in Δg/intensity. (*a*) The energy of a brief flash necessary to elicit a response of criterion amplitude is raised by an adapting stimulus. (*b*) The plateau phase of the response to an adapting stimulus is a nonlinear function of the stimulus intensity. (*c*) The response to a long stimulus declines from a large initial transient phase to a smaller plateau phase. We would predict that if Δg/intensity could be stabilized, the voltage-clamp currents induced by long stimuli would vary linearly with intensity at all times during the responses, there would be no decline from transient to plateau, and light-induced changes in sensitivity would not occur.

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