Two Light-Induced Processes in the Photoreceptor Cells of *Limulus* Ventral Eye

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ABSTRACT The dark-adapted current-voltage (I-V) curve of a ventral photoreceptor cell of *Limulus*, measured by a voltage-clamp technique, has a high slope-resistance region more negative than resting voltage, a lower slope-resistance region between resting voltage and zero, and a negative slope-resistance region more positive than 0 v. With illumination, we find no unique voltage at which there is no light-induced current. At the termination of illumination, the I-V curve changes quickly, then recovers very slowly to a dark-adapted configuration. The voltage-clamp currents during and after illumination can be interpreted to arise from two separate processes. One process (*fast*) changes quickly with change in illumination, has a reversal potential at +20 mv, and has an I-V curve with positive slope resistance at all voltages. These properties are consistent with a light-induced change in membrane conductance to sodium ions. The other process (*slow*) changes slowly with changes in illumination, generates light-activated current at +20 mv, and has an I-V curve with a large region of negative slope resistance. The mechanism of this process cannot as yet be identified.

INTRODUCTION

Photoreceptor cells in many arthropods respond to light with a change in membrane voltage, the receptor potential; the amplitude of this receptor potential is graded with the intensity of the light (Hartline et al., 1952; Tomita 1956; Naka, 1961; Eguchi, 1965; Fulpius and Baumann, 1969). In the eyes of two such arthropods (the barnacle, *Balanus* sp. and the ventral rudimentary eye of *Limulus*), the photoreceptor cells are large enough to be impaled with more than one microelectrode; this has allowed these cells to be studied using voltage-clamp and current-clamp techniques. Thus, in both systems, it is possible to measure the current-voltage (I-V) relations of the cell membrane over a wide range of membrane voltages, and to study the changes in the I-V relations produced by light and by replacement or addition of ions (or drugs)

to the external bath. By such experiments, Brown et al. (1970), working with the barnacle eye, and two groups (Smith et al., 1968 and Millecchia and Mauro, 1969 *a*, *b*) studying the *Limulus* ventral eye, have sought to elucidate the membrane mechanisms by which receptor potentials are produced.

In the barnacle photoreceptor, Brown et al. (1970) have shown that: (a) there is a reversal potential for the light response (*b*); the reversal potential changes with changes in the concentration of external sodium; (*c*) the steady-state I-V curve of the light-activated process has a positive slope at all voltages; and (*d*) the instantaneous I-V curves of the light-activated process are linear and intersect a unique reversal potential, independent of holding voltage. To account for these data, Brown et al. (1970) proposed that light initiates a conductance increase, primarily to sodium ions, which allows the sodium ions to flow down their concentration gradient into the cell; this influx of net positive charge depolarizes the membrane.

Similarly, Millecchia and Mauro (1969 *a*, *b*) have argued that a conductance increase mechanism produces the receptor potential in *Limulus* ventral photoreceptors. They found that the reversal potential for the light-induced current, measured by a voltage-clamp technique, changes with the concentration of external Na^+. They furthermore showed that the I-V curve of the light-activated process, measured up to 2.5 sec after the onset of the light, had a positive slope at all voltages studied. They proposed that a change of membrane conductance to sodium ions produces the receptor potential in the ventral eye photoreceptors.

In contrast, Smith, Stell, and Brown (1968) presented evidence which indicated that the I-V curve of the light-activated process has a region of negative slope resistance. Partially on the basis of this evidence, they argued against a permeability increase mechanism. Instead they proposed that a light-modulated electrogenic pump generates the receptor potential.

We have found that the above difference in the data between the two studies on *Limulus* ventral eye photoreceptors arises almost entirely from differences in experimental protocols. Furthermore, we report a new observation: there is a slow change in the I-V curves in the dark as the cell dark-adapts after exposure to a bright light. We argue that the process underlying this slow change of the I-V curve is fundamentally different from a faster process, which occurs rapidly with changes in light intensity. We suggest that the data (ours, as well as those of Smith et al., 1968 and Millecchia and Mauro, 1969 *b*) can best be interpreted in terms of two light-induced processes, the faster of these two processes being best described by a conductance increase model. A preliminary report of some of our data has appeared (Lisman and Brown, 1970).

METHODS

Glass micropipettes filled with 3 M KCl were used for both current-passing and voltage-measuring electrodes. The electrode resistances were between 7 and 15 MΩ,

measured in sea water. We used field effect transistor (F.E.T.) input, capacitance compensated, unity gain preamplifiers which had a settling time of less than 20 μsec. The circuit of the voltage clamp was similar to those described by others (Millecchia and Mauro, 1969 *b*). The feedback amplifier used in the voltage clamp was a Tektronix 132 power amplifier driven by a Tektronix 1A7 (Tektronix, Inc., Beaverton, Ore.). Using this voltage clamp, the time required to clamp the membrane to a steady voltage was 3 msec or less. Current was measured with a current-to-voltage transducer built with an Analog Devices 501 F.E.T. operational amplifier (Analog Devices Inc., Cambridge, Mass.). The voltage signal representing the clamping current was smoothed with a single-stage RC filter. The time constant of this filter was 0.5–5 msec; the larger values for the time constant were used only with very slowly changing command voltages. The command potentials were triangular voltages (from a Wavetek 111 waveform generator, Wavetek, San Diego, Calif.) or voltage pulses (from a Tektronix 161 pulse generator). I–V curves were made directly on an oscilloscope by using the horizontal axis for membrane voltage and the vertical axis for clamp current.

RESULTS

We can routinely find cells having highly nonlinear current-voltage (I-V) curves in the dark-adapted state, as described by Smith, Stell, and Brown (1968). Fig. 1 A shows the I-V relations made by the current-clamp technique in which ramps of current are applied to the cell. Resting potential is typically −45 to −60 mv in cells impaled with two microelectrodes. Near resting potential, the I-V curve has a region of changing slope resistance; at 10 mv more negative than resting potential, the slope resistance is 8–30 MΩ, while at 10 mv more positive than resting potential, the slope resistance is 1–4 MΩ. Near zero voltage, there is a second region of changing slope resistance. As the amplitude of depolarizing current is increased, the membrane voltage becomes unstable and rapidly shifts from near zero to a large inside positive potential (+30 to +80 mv). If the depolarizing current is then decreased, the voltage decreases proportionally. As the current is decreased still further, the voltage again becomes unstable and rapidly shifts down toward 0 v. There is thus a region on the I-V curve just positive of 0 v in which it is impossible to stabilize the voltage using the current-clamp technique. This region appears as a region of negative slope resistance when studied with the voltage-clamp technique.

I-V Curves by Voltage Clamp in the Dark-Adapted State

The I-V curve obtained by voltage clamp of a dark-adapted cell is shown in Fig. 1 B. We used a triangular wave as the command voltage ("ramp clamp"). Fig. 1 C shows the superposition of the voltage- and current-clamp curves for the same cell. The two curves are identical except in the "unstable" region

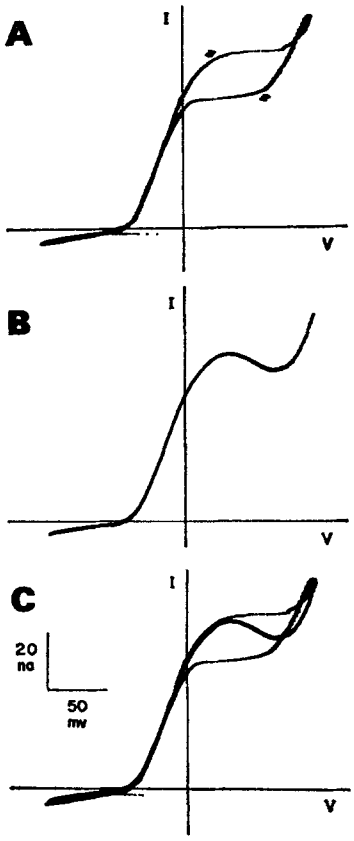

FIGURE 1

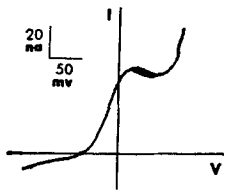

FIGURE 2

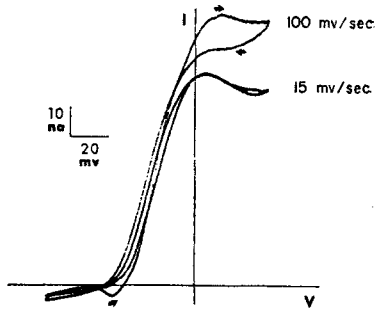

FIGURE 3

FIGURE 1. (A), I-V curve of a dark-adapted *Limulus* ventral eye photoreceptor cell made with current-clamp technique. There is a region of changing slope resistance around resting potential (−50 mv). In a voltage range just positive to 0 v there is a region of instability in which it is impossible to maintain the voltage with the current-clamp technique. Also notice the spikelike potential near resting voltage evoked upon the release of hyperpolarizing current. (B), I-V curve for the same dark-adapted cell made with voltage-clamp technique. The region of instability seen in (A) is here a region of negative slope resistance. Command voltage was a rising ramp with $dv/dt = +10$ mv/sec. (C), (A) and (B) superposed for comparison. The curves are nearly identical except in region of "instability" seen in (A).

FIGURE 2. I-V curve of a dark-adapted cell made with voltage-clamp technique. Voltage was slowly retraced in the region of negative slope resistance (trace thickened). Ramp rate was +8 mv/sec.

FIGURE 3. Voltage clamp I-V curves from a dark-adapted cell made with triangular command voltages with different dv/dt. Where the voltage became more positive than zero, the I-V curve made with the faster of the two ramps (100 mv/sec) has a much smaller negative resistance region. This curve does have a small negative resistance region near resting potential arising from the electrically excitable, spikelike event evoked by the rapid positive-going ramp voltage.

where the ramp clamp shows negative slope resistance on an upgoing (or downgoing) ramp.

This negative resistance region is part of the steady-state I-V curve of the cell. This is shown in Fig. 2, in which the trace thickening in the negative resistance region indicates where the command voltage was halted and then retraced.

The shape of the I-V relation varied with ramp rate (dv/dt) as is evident in Fig. 3. At rates faster than 80 mv/sec, the I-V relation has little, if any, negative slope resistance for voltages more positive than zero. For steady-state measurements, we felt that a ramp rate of 8 mv/sec was operationally reasonable because slowing the ramp rate still further seemed to have no significant effect on the shape of the I-V relation. Even at 8 mv/sec, however, the upgoing and downgoing ramps showed hysteresis for voltages between 0 and −40 mv. In order to eliminate this hysteresis, we had to use very slow ramps (1 mv/sec) which required several minutes for the tracing of one I-V relation.

The current-voltage relation of a cell can also be constructed from the values of current measured when step changes in membrane voltage are made by a series of rectangular command-voltage pulses. Fig. 4 A shows the current as a function of time for several amplitudes of voltage pulses. The holding potential was the resting potential of the cell. Fig. 4 B shows the reconstruction of the I-V relation for the three sets of points taken 0.15, 1.0, and 5 sec after the onset of the command-voltage pulse. The three curves superimpose at all points more negative than −20 mv. At voltages more positive than −20 mv, the 0.15 sec curve is approximately the linear extension of the less positive region of the curve. The 1.0 sec curve steepens and becomes vertical in a voltage range on the positive side of zero, whereas in the same range the 5 sec curve has a region of negative slope resistance.

The negative resistance region of the I-V curve can be measured with the pulse-clamp technique in a different way. If holding potential is placed at 0 v, and stepwise command-voltage pulses are used, the negative resistance region can be determined. With holding voltage at 0 v, no large surge of current is needed to clamp the voltage, at least for small excursions. The results of such an experiment are seen in Fig. 4 C superimposed on a ramp-clamp I-V curve.

In many penetrations of cells in the ventral eye, the I-V relations measured do not correspond to those shown in Figs. 1–4. They do not have the high resistance region in the voltage range more negative than resting potential and have little, if any, negative resistance region for voltages more positive than zero; usually such cells are relatively insensitive to light. Rarely, these cells may have large resting potentials and sensitivities to light comparable to those of cells having highly nonlinear I-V curves. Nevertheless, we feel that such cells with relatively (low resistance) linear I-V curves are damaged cells.

Occasionally, we have seen the transition of the I-V relation of a penetrated cell from a highly nonlinear curve to a relatively linear one, but we have never observed the opposite.

Action of Light on the Voltage-Clamp I-V Curves

Fig. 5 A shows two I-V curves, one made in the dark-adapted eye, the other in the steady-state light (to be referred to as *dark-adapted* and *light*). We use

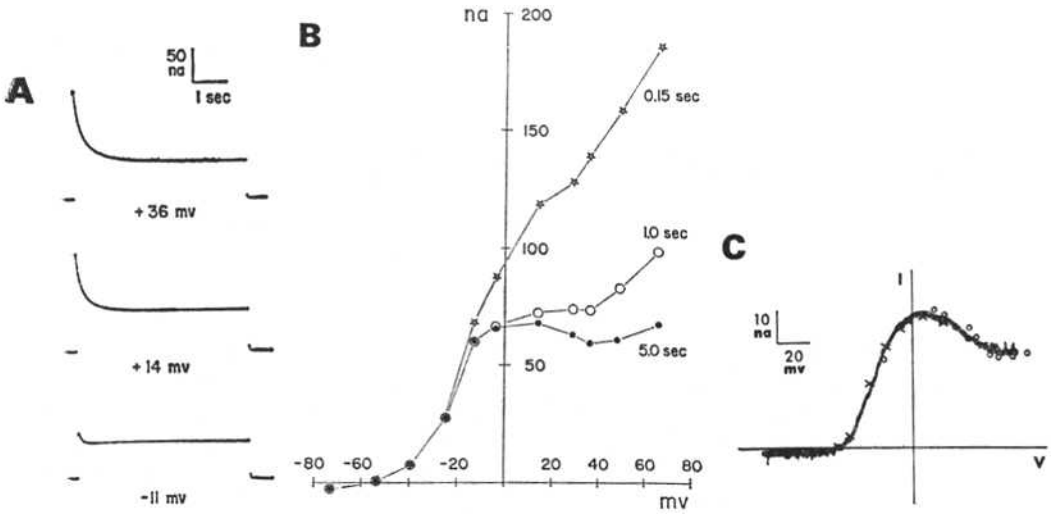

FIGURE 4. (A) Examples of clamping currents required for voltage clamping a dark-adapted cell to different amplitude command-voltage pulses. The holding voltage was maintained at resting potential. The value of each command-voltage pulse is given below the corresponding current trace. (B) Clamp current as a function of clamping potential at three times after onset of the command-voltage pulse (0.15 sec, stars; 1.0 sec, open circles; 5 sec, closed circles). The three curves superpose at voltages below −20 mv. At more positive voltages, the 150 msec curve is an approximately linear extension of the curve at lower voltages. The 1 sec curve has a region of very high resistance for voltages more positive than 0 v, whereas the 5 sec curve has negative slope resistance for such voltages. (C) Similarity of I-V curve for a dark-adapted cell made by voltage clamp using a +8 mv/sec ramp and 2.5-sec pulses as command voltages. For the pulse-command voltages, holding voltage was kept at 0 v. At this holding voltage, even with short command pulses, the steady-state I-V curve of the dark-adapted cell could be determined. The ×'s and circles were measured on two successive series of trials. Currents were measured at the end of the 2.5-sec command-voltage pulses.

the word "dark-adapted" to mean that the shape of the I-V curves no longer changes with increasing time in the dark. This condition is not necessarily the same as that implied by the usual definition of dark-adapted, which refers to a state of maximum sensitivity to light. In ventral eye photoreceptors, the time necessary to reach the dark-adapted state after an illumination seems to be the same by either definition within a factor of two. By "steady-state light" we mean that the light pulse is sufficiently long such that the light-

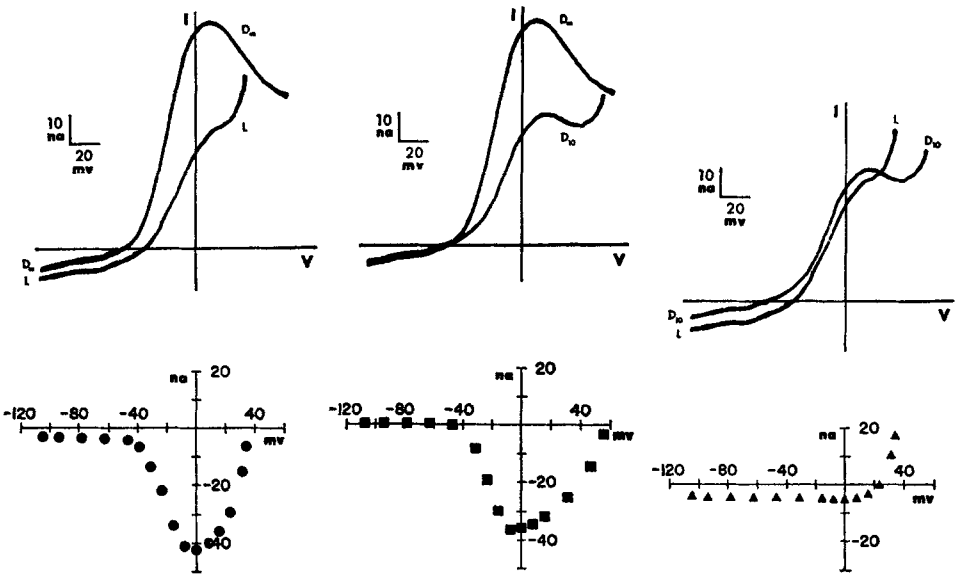

FIGURE 5 FIGURE 6 FIGURE 7

FIGURE 5. (A) I-V curves made in steady-state light (*light*), marked *L*, and made in the dark-adapted state (*dark-adapted*), marked D_∞ In a voltage region just positive to resting potential the *light* curve has a higher slope resistance than the *dark-adapted* curve. (B) The difference curve constructed by subtracting the *dark-adapted* curve from the *light* curve. There is a region of negative slope resistance in a voltage region more positive than resting potential. The curve is the sum of the currents (for each voltage) graphed in Figs. 6 B and 7 B.

FIGURE 6. (A) I–V curves made in the dark, 10 sec after termination of light (*dark after*), marked D_{10}, and made in the dark-adapted state (*dark-adapted*), marked D_∞ The *dark after* curve has higher slope resistance than the *dark-adapted* curve between −50 and +5 mv. At voltages below −50 mv the curves superpose to within 2 na. (B) The difference curve constructed by subtracting the *dark-adapted* curve from the *dark after* curve. It has negative slope resistance from −50 to −10 mv.

FIGURE 7. (A) I–V curves made in steady-state light (*light*), marked *L*, and made 10 sec after termination of light (*dark after*), marked D_{10}. The *light* curve has the same or lower slope resistance than the *dark after* curve at all voltages. The two curves cross at about +25 mv. (B) The difference curve constructed by subtracting the *dark after* curve from *light* curves. It has positive slope at all voltages and reversal potential at about +25 mv.

initiated current has come to a fixed value and that successive I-V curves do not differ from each other.

As reported by Millecchia and Mauro (1969 *a*), we confirm that the action of light produces a depolarization graded with light intensity (for example in Fig. 5 A, 15 mv for a light five log units above threshold). The *light* I-V curve has a greater slope resistance than the *dark-adapted* curve in a region just positive to resting potential. This observation confirms a similar finding

made by Smith, Stell, and Brown (1968) using a current-clamp technique. For membrane voltage more positive than zero, the *light* curve has a lower slope resistance than the *dark-adapted* curve; extrapolations of the two curves cross at about +40 mv. The I-V curve in the dark, made 10 sec after turning off the light (the *dark after* curve), has a shape different from that in the dark-adapted state. For comparison, we show the two figures together in Fig. 6 A. Below resting potential, the two curves approximately superpose. In a large voltage range above resting potential, the *dark after* curve has a greater slope resistance than the *dark-adapted* curve. In a period 5–10 min after illumination is removed, the shape of the I-V curve returns gradually to that of the dark-adapted state; the time required for this return depends on the intensity of the preceding illumination. During this recovery, there is little, if any, change in the membrane potential. The primary decay of the depolarizing receptor potential occurs in less than a second after the light is turned off. This is followed by a small residual depolarization of only a few millivolts which decays to resting voltage within 2 min.

We have recorded a noticeable hyperpolarization immediately subsequent to the end of illumination from ventral eye photoreceptor impaled with one microelectrode (similar to that recorded from lateral eye retinular cells; Benolken, 1961). With the cell impaled with two micropipettes, as in this voltage-clamp study, we measured an after hyperpolarization only rarely.

To examine more easily the change between the I-V curves made at two different times, one can graph the differences between the two curves. Such a graph ("difference curve") is constructed by subtracting the current in one curve from the current in the other, at each voltage. The subtracting itself is model independent: the difference (ΔI) indicates a change in the net current flowing through the membrane, but in no way presupposes whether the pathway for this current is active or passive, nor does it assume any mechanism underlying changes in the current pathways.

To reexamine the effects of light on the I-V relations of the ventral eye photoreceptors, we can divide the transition from the steady-state light to the dark-adapted state into two periods: (*a*) from steady-state light to 10 sec after the light is turned off (the time at which the *dark after* curve is taken), (*b*) from 10 sec after the end of illumination to the dark-adapted state.

Fig. 7 B shows the change in the I-V curve during the first period. This difference curve was constructed by subtracting the *dark after* curve from the *light* curve. The slope conductance is either zero or positive at all voltages and there is a reversal potential ($\Delta I = 0$) at about +25 mv. At voltages more positive than reversal potential, the slope conductance is considerably greater than for voltages more negative than reversal potential.

Fig. 6 B shows the change in I-V curve during the second period. This difference curve was constructed by subtracting the *dark after* curve from

the *dark-adapted* curve. At voltages below resting potential ΔI is approximately zero. At voltages above resting potential, the curve has a negative slope over a large voltage range. From cell to cell there is a marked variability of the positive voltage at which this curve begins to have positive slope, but the voltage is always greater than -10 mv. At some voltage significantly more positive, e.g. at about $+50$ mv, the difference curve crosses the zero current axis. Investigation of this region of the curve is difficult because the voltage must be made so positive that it causes partial membrane breakdown, as evidenced by the fact that the I-V curves constructed with upgoing, then downgoing ramps no longer coincide.

Figs. 6 B and 7 B, taken together, indicate that the light produces two distinctively different types of change in the I-V curves. These two types have been separated by their differing time-courses, as is further described below.

The time resolution of the technique of making I-V curves with ramps is limited by the duration of a single command ramp (usually 15 sec). Better temporal resolution can be achieved by recording the current necessary to hold the transmembrane potential at a fixed value before, during, and after the illumination. A set of such current traces, taken at different holding potentials, defines the I-V relation as a function of time.

To generate these current traces, the following procedure was used. The cell was clamped to a given potential for over a minute. The command pulse had a greatly slowed rise time. When the current necessary to clamp the cell to a given potential became nearly steady, the light was turned on for 30 sec. About 30 sec after the light was turned off, the voltage was brought back to its resting value. This sequence of manipulations was repeated once every 3 min and thus there was only partial dark-adaptation between stimuli.

Fig. 8 shows a set of current traces for a ventral eye photoreceptor measured by this procedure. When the cell is clamped to -80 mv, the time-course of the light-induced current is similar to the time-course of the depolarizing response to light when the cell is not voltage clamped. There is an initial transient upon illumination, a plateau, and a fast decay of the maintained component back to resting level when the light is turned off. When the cell is clamped to a voltage just positive to resting potential, an additional component appears: a slow decay of current back towards the dark-adapted level after the light is turned off. At clamping voltages of about $+20$ mv, the rapid changes of clamping current elicited by the onset and termination of the light become very small, leaving only a relatively slow change in current at the onset of light and an even slower decay in current after the termination of the light. When the cell is clamped to a still more positive potential, the fast transients at the onset and removal of illumination reverse in sign, but the slow components do not.

The difference in current between any two times can be determined directly from data such as that seen in Fig. 8 for several values of holding voltage. We picked two times, one just before and the other just after the transient which occurred when the light was turned off. We measured the change in current between the two times for each current trace and plotted this data in Fig. 9 (triangles). The shape of the curve is similar to that for the *light* minus

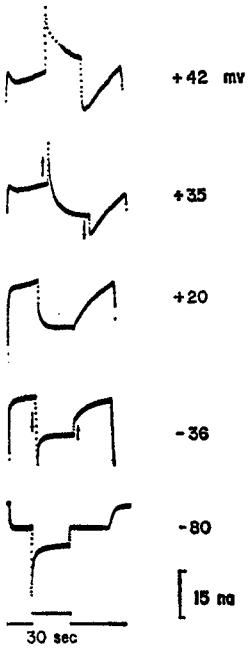

FIGURE 8

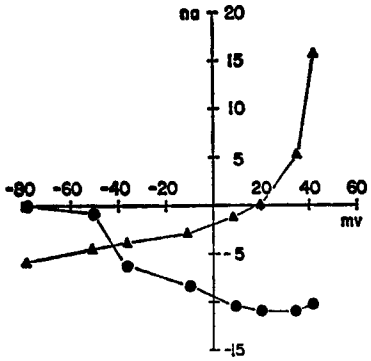

FIGURE 9

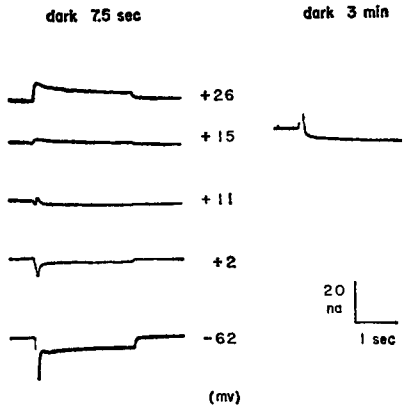

FIGURE 10

FIGURE 8. Examples of clamping current before, during, and after 30-sec light pulses at holding voltages marked beside each trace. A schematic interpretation of these currents as the sum of two processes is shown in Fig. 11.

FIGURE 9. The change in current during the fast transient following the termination of light at different clamping voltages (triangles). The curve has positive slope at all voltages. The change in current during the first 25 sec of recovery after the termination of the light *versus* clamping voltage is shown by the circles. The curve has negative slope from −60 to +40 mv. Both curves are constructed from data like that shown in Fig. 8.

FIGURE 10. Light-induced current measured at different holding voltages (marked in the center column). Resting voltage was −62 mv. The responses on the left were elicited by 2.5-sec flashes separated by 7.5 sec of darkness (i.e., 1 flash/10 sec). Reversal voltage was about +13 mv. The response on the right was measured at a holding voltage of +15 mv after allowing a 3 min interval of darkness; there is a prominent, slow component which was not reversed in sign. The fast component of light-induced current is always found to be larger after dark-adaptation.

dark after difference curves but always has positive slope conductance. With greater time resolution, this rapid change in clamp current can be seen to occur in less than 200 msec.

The change in I-V relation in the first 30 sec after the transient at the termination of light can also be constructed from data such as that in Fig. 8. Such an I-V curve is shown in Fig. 9 (circles). It has a shape similar to the *dark after* minus *dark-adapted* curves. That is, it has a region of negative slope between resting voltage and 0 v and very small values of ΔI for voltages more negative than resting voltage.

Millecchia and Mauro (1969 *b*) reported that there was a unique positive voltage (about +20 mv) at which the light-induced current changed sign. In their pulse-clamp experiments, they gave repetitive flashes of 2.5 sec duration every 10 sec (personal communication). We have replicated their result (Fig. 10). We gave 2.5-sec flashes every 10 sec; using resting potential of the cell as holding voltage, we clamped every fourth response to a command-voltage pulse with a slowed rising edge. We slowed the rise time of the command-voltage pulse with a two-stage RC low-pass filter. This procedure avoided eliciting the large, slowly decaying surge of positive current seen when fast-rising command-voltage pulses more positive than 0 v are used (Fig. 4 A and Millecchia and Mauro, 1969 *b*).

In Fig. 10, we see that, when the cell is kept light-adapted with repetitive flashes, well-defined reversal voltage can be measured. However, we often find that there is a small biphasic current at the onset of illumination, even with the cell clamped at reversal voltage. If we interrupt the repetitive flashes for 3 min and then measure the light-induced current at a voltage slightly positive to reversal voltage determined for the responses to repetitive flashes, we find, as in Fig. 8, that there is an additional component to the light response (Fig. 10), which has not reversed in sign.

DISCUSSION

Models with One Light-Induced Process

Millecchia and Mauro (1969 *b*) have proposed that the light-induced changes in the I-V curves of the ventral eye can be explained solely on the basis of a conductance change model in which the equivalent electromotive force (emf) for the light-activated path is independent of light, voltage, and time. A consequence of this hypothesis is the existence of a unique "reversal potential." If the membrane is clamped to this unique voltage there should be no light-activated current. Fig. 8 shows that for 30-sec light flashes, there is no voltage at which there is no light-induced current. Also, in Fig. 10, we see that for short flashes of light there is no voltage at which there is no light-induced current, if the cell is allowed to dark-adapt between flashes. We thus conclude that the hypothesis of a single light-induced process involving changes

of ionic conductances in series with a fixed, equivalent emf fails to explain our data.

Another simple, conductance change model can be made as follows: the membrane conductances to more than one ion change in the light. Each ionic conductance change (Δg_i) is in series with an emf equal to the equilibrium voltage for its ion (E_i); these pathways, one for each ion, are electrically in parallel. An equivalent single conductance change (G_L) in series with an equivalent emf (E_L) can be calculated for this circuit by Kirchhoff's Laws:

$$G_L = \sum \Delta g_i \quad \text{and} \quad E_L = \frac{\sum \Delta g_i \cdot E_i}{\sum \Delta g_i}.$$

In two cases, our data contravene this model: (*a*) if only one ion is involved then $E_L = E_i$ or (*b*) if the voltage, light, and time dependencies of the Δg_i's are such that E_L remains constant, then a unique reversal voltage would be predicted. If the Δg_i's have differing functional dependencies on voltage, light, or time, then both G_L and E_L will change during the light response. We refer to such a system as having more than one "process."

Model with Two Light-Induced Processes

Figs. 6 and 7 show that in the transition from the illuminated steady state to the dark-adapted state, the membrane undergoes two very different types of changes in its I-V curve. When a long light pulse is terminated, the membrane potential returns quickly to a value within a few millivolts of the voltage preceding the light pulse. Fig. 7 B shows the change in the I-V curve during this transition; the difference curve has either zero slope or positive slope at all voltages and a reversal potential at +20 mv. Perhaps our most important observation is that the I-V curve taken 15 sec after the light is turned off differs greatly from the I-V curve taken 5 min after the light is turned off. Fig. 6 B shows the difference curve representing the change in the I-V curve which occurs in the dark as the cell dark-adapts. This curve has a negative slope from -50 to -10 mv. There are thus two types of changes in the I-V curve during the transition from the illuminated state to the dark-adapted state; these have been separated here on the basis of their time-courses. One type of change occurs within the first 15 sec after the light is turned off; the other occurs during a prolonged recovery period after the light is terminated. The pulse-clamp data gives a more accurate measure of the kinetics of the faster type of I-V curve change. Fig. 8 shows that there is a very fast change in current when the light is turned off. With greater time resolution, the fast change is seen to occur in less than 200 msec. Fig. 9 (triangles) shows the voltage dependence of the fast change in current immediately following the termination of light, taken from the data, part of which is shown in Fig. 8. This curve is equivalent to a difference curve, and is similar in some aspects

of its shape to the *light* minus *dark after* difference curve. Also, in both Fig. 9 (circles) and a *light* minus *dark after* difference curve such as in Fig. 6 B, there is both a region of negative slope resistance between resting voltage and 0 v and a region of very small ΔI for voltages more negative than resting voltage. In addition, curves measured in both ways tend to bend back toward zero at very positive voltages. Typically, the extrapolations of the curves cross zero at voltages more positive than +50 mv.

Using Fig. 8, it is also possible to examine the change in the I-V curve during the transition from the dark-adapted state to the illuminated steady state. Fig. 8 shows that, at the onset of the light, there is a fast change of current at every voltage except at +20 mv; this is the same voltage at which there is no fast change of current following the termination of light. This indicates that the process underlying the I-V curve change (with reversal potential at +20 mv) occurs rapidly at both the onset and the termination of light. If indeed this process has a reversal potential at +20 mv independent of time, then the current trace at +20 mv clamping potential indicates the kinetics of the slower type of I-V curve change. The magnitude of the deviation of the current from the value in the dark-adapted state is a qualitative measure of the activation of the underlying process. The trace shows that the buildup of this process is itself slow, though not nearly so slow as the decay.

We propose that the simplest representation of these data has two light-induced membrane processes. One process, which we call the *fast process*, has the following properties.

(*a*) it turns on and off rapidly with the onset and termination of the light;

(*b*) it has a reversal potential at about +20 mv; and

(*c*) its I-V curve has positive slope resistance at all voltages.

The other process we call the *slow process*. The distinguishing properties of the *slow process* are:

(*a*) it turns on slowly and turns off even more slowly with the onset and termination of the light;

(*b*) its I-V curve has negative slope resistance over much of the voltage range; and

(*c*) it accounts for less than 2 na of light-activated current at voltages more negative than resting potential.

We thus interpret each trace in Fig. 8 as the summation of the currents from two different processes as indicated diagramatically in Fig. 11.

In the above discussion, we use the word "process" to signify any mechanism capable of influencing the net flow of charged particles across the cell membrane. A list of such processes would include conductance changes, changes in the net current of electrogenic pumps, changes in concentration, pressure, or osmotic gradients, etc.

It should be pointed out that, at least in principle, our data could be

described by a single process model, in which some noncurrent-carrying agent could modify the voltage dependence of a single process. Such a single process could not, however, be a conductance change. That this is so can be seen from Figs. 6 B and 7 B; the slow and fast changes have different reversal potentials. But the reversal potential of a single process conductance-change mechanism cannot be modified without changing an ionic concentration; by our definition, such a change in concentration would itself be a second process.

In our measurments of the *slow process*, both long-duration clamping pulses and long-duration illumination periods were used. Under such conditions, it might be argued that the failure to find a unique value for reversal potential occurred because the equilibrium potential of some ion (or ions)

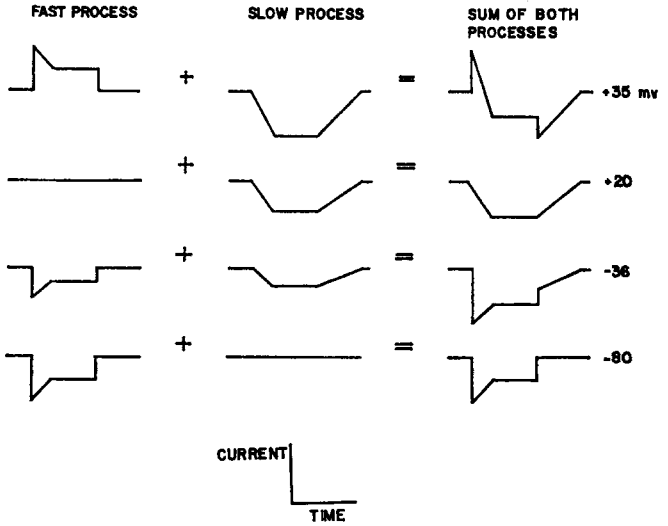

FIGURE 11. A schematic drawing of our interpretation of the current traces in Fig. 8 as the sum of the *fast* and *slow processes*.

had been changed significantly. If the concentration gradient of the ion (or ions) which carries membrane current during the light response were changing, the reversal potential for the light response would also be changing. If this were true, then the rapid change in light-induced current at the end of a long-duration stimulus would be expected to have a reversal potential which depended on the duration of the flash. Also, the rapid changes in current at the beginning and the end of the flash would be expected to have different reversal potentials. To the contrary, we find that the reversal potential is the same for the fast components of the light-induced current at both the onset and removal of the stimulus, and is independent of the duration of illumination (up to our longest flash). We conclude that the *slow process* is not simply a change in the equilibrium potential of a single ion whose light-induced influx generates the depolarizing receptor potential.

Millecchia and Mauro (1969 *b*) state that the extracellular concentration of no ion other than Na^+ contributes to the emf for the fast changes of light-induced current. If we accept the hypothesis that the *fast process* is a conductance increase to the sodium ion (whose equilibrium potential is close to +20 mv), it is unlikely that the entry of additional sodium ions during the light response obligatorily preceeds the appearance of the *slow process*. Fig. 8 shows that when the membrane is clamped to +42 mv, the fast component of the light-induced current is outward across the membrane. Nevertheless, the slow component of the light-induced current is present, in the inward direction. Hence, in terms of the Na^+-conductance–increase hypothesis, the *slow process* can be induced despite the outward flow of sodium ions.

Alternatively, it is possible that some proportion of the light-induced current is carried by a second ionic species whose Nernst equilibrium potential is more positive than +42 mv. We cannot eliminate the possibility that a small influx of the second ion obligatorily preceeds the generation of the *slow process*.

The I-V Curves of the Two Processes

In systems with two simultaneously active processes (as for instance the Na^+ and K^+ conductances of squid), it is possible to describe the time-dependent I-V curve of each process, provided that it is possible to study at least one of the processes in isolation. Since we have not been able to do this for ventral eye photoreceptors, it is impossible to determine unambiguously the I-V curve of either of our postulated processes.

If, however, we assume that there is negligible change of the *slow process* during the 200 msec transient after the light is turned off, then we can assert that Fig. 9 (triangles) represents the I-V curve of the *fast process*. Since the *fast process* has positive slope at all voltages, the *slow process* must account for all of the negative slope in the *dark after* minus *dark-adapted* difference curve and thus must itself have negative slope from −50 to +0 mv.

Although the I-V curve of the *slow process* does have a large region of negative slope, this does not necessarily indicate that it arises from a conductance-decrease mechanism. At least several of the other kinds of processes (e.g. electrogenic ion pumps, changes in ionic concentration) possibly could produce such an I-V curve with a region of negative slope. Furthermore, it is possible that more than one process contributes to the phenomenon that we have called the *slow process*.

Further Properties of the Fast Process

The *fast process* appears to be primarily responsible for the observed voltage response elicited by light. Its time-course correlates with the voltage response, and its positive reversal potential and positive slope conductance indicate that it can provide the driving force for a depolarizing voltage change.

It appears likely that Millecchia and Mauro (1969 *b*), in their study of the light-activated currents, were studying what we call the *fast process*, in relative isolation. In their pulse-clamp experiments, they gave light stimuli of 2.5 sec duration, once every 10 sec (personal communication). This interstimulus interval is short in comparison to the time constant of the *slow process* recovery. They were thus comparing clamping currents during light stimulation with clamping currents in the dark, characteristic of a light-adapted cell. Hence, their curves for the light-activated process are similar to our steady-state *light* minus *dark after* difference curve but are very different from the comparisons of illuminated steady state and dark-adapted state made by Smith, Stell, and Brown (1968).

Millecchia and Mauro propose that the mechanism underlying these rapid, light-activated currents (i.e., our *fast process*) is a conductance change, primarily to sodium ions. At the present, we feel that this is the best hypothesis for the mechanism underlying the *fast process*.

Further Properties of the Slow Process

During the recovery following illumination there is little, if any, after-potential despite the large slope resistance increase observed in the I-V relation. This means that the *slow process* does not provide the driving force for a voltage change. This does not mean that changes in the *slow process* can have no effect on the magnitude of the light-induced voltage responses. For example, let us accept the hypothesis that the rapid changes in the light response result primarily from a light-activated increase in membrane conductance to sodium ions; the driving force on the sodium ions (E_{Na}) remains fixed (Millecchia and Mauro, 1969 *a*, *b*). Thus, light induces an inward flow of current carried by sodium ions. The amplitude of the rapid voltage changes will depend on the size of the effective load resistance through which the light-induced current flows out of the cell. The *slow process* acts as if it increases the effective load resistance of the cell. Hence a given size of light-induced Na^+ conductance increase will produce relatively more voltage change in the presence of the *slow process* than in its absence.

For example, in Fig. 7, the *slow process* would affect the amplitude of the steady phase of the (unclamped) receptor potential by as much as 50%. If there were no *slow process*, the light-induced current of the *fast process* (about 5 na) would cause a voltage drop across the dark-adapted I-V curve (D_∞ in Fig. 6 A) of about +12 mv. When the dark I-V curve has been changed by the *slow process* (see D_{10} in Fig. 6 A), the same 5 na of *fast process* current causes a voltage drop of about +20 mv.

Since the *slow process* decays with a time-course comparable in length to that of the dark-adaptation of the sensitivity to light, it is at least possible that the two may be related. At present we have no evidence on this correlation. It is clear that the *slow process* itself cannot produce a decrease in the sensitiv-

ity to light necessary to account for the decreased amplitude of response to a fixed size stimulus when the cell is light-adapted. On the contrary, the increase in effective load resistance of the membrane involved in the *slow process* would tend to augment the light response, as argued above.

Current-Voltage Curves

Our I-V curves of dark-adapted ventral eye photoreceptors confirm the findings of Smith, Stell, and Brown (1968), obtained by a current-clamp technique. As shown in Fig. 1, the current-voltage curves made with current ramps (2.5 na/sec), voltage ramps (5 mv/sec), and voltage pulses (for times greater than 5 sec after the voltage step) are mutually consistent. We feel that the absence of a negative resistance region in Millecchia and Mauro's (1969 *a*) curves is due to the fact that their so called "steady-state" measurements made 2.5 sec after the voltage step were not truly steady state. At 2.5 sec after the onset of a voltage pulse (with holding voltage at resting potential), the currents are still changing.

The negative resistance region (more positive than 0 v) of the dark-adapted I-V curve does not underlie any regenerative event which occurs in the normal (unclamped) response of the cell. The only time the membrane voltage would become sufficiently positive is during the transient phase of the receptor potential. The transient phase has a duration only as great as 0.5 sec, whereas the negative resistance takes several seconds to develop after the membrane voltage has been made more positive (Fig. 4 B). Therefore there is no effective negative resistance (more positive than zero) during the transient phase of the receptor potential.

In comparing the I-V curves of the cell membrane taken when dark-adapted with those taken when stimulated by light, Smith, Stell, and Brown (1968) found that, for voltages just positive to resting potential, the slope resistance of the I-V curve taken in the light was greater than in the dark. Fig. 5 confirms their finding. On this evidence, Smith, Stell, and Brown discounted a conductance-increase mechanism. Hagiwara (personal communication) has since pointed out that, to the contrary, the I-V curve of a conductance increase pathway can have a region of negative slope resistance. Thus a light-activated conductance pathway having this property could account for the slope resistance increase in the light observed by Smith, Stell, and Brown.

Considering our new data, the results of Smith, Stell, and Brown (1968) can be explained more simply in terms of a two-process model. They compared the steady-state curve in the light to the dark-adapted curve. Thus, we conclude that they were studying the sum of both the *fast* and the *slow processes*. Since in a region just above resting potential the slope resistance increase due to the *slow process* is greater than the slope resistance decrease

due to the *fast process*, the net effect of light, in the steady state, is to produce the slope resistance increase actually observed.

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