

# Adaptation in the Ventral Eye of *Limulus* is Functionally Independent of the Photochemical Cycle, Membrane Potential, and Membrane Resistance

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**ABSTRACT** The early receptor potential (ERP), membrane potential, membrane resistance, and sensitivity were measured during light and/or dark adaptation in the ventral eye of *Limulus*. After a bright flash, the ERP amplitude recovered with a time constant of 100 ms, whereas the sensitivity recovered with an initial time constant of 20 s. When a strong adapting light was turned off, the recovery of membrane potential and of membrane resistance had time-courses similar to each other, and both recovered more rapidly than the sensitivity. The receptor depolarization was compared during dark adaptation after strong illumination and during light adaptation with weaker illumination; at equal sensitivities the cell was more depolarized during light adaptation than during dark adaptation. Finally, the waveforms of responses to flashes were compared during dark adaptation after strong illumination and during light adaptation with weaker illumination. At equal sensitivities (equal amplitude responses for identical flashes), the responses during light adaptation had faster time-courses than the responses during dark adaptation. Thus neither the photochemical cycle nor the membrane potential nor the membrane resistance is related to sensitivity changes during dark adaptation in the photoreceptors of the ventral eye. By elimination, these results imply that there are (unknown) intermediate process(es) responsible for adaptation interposed between the photochemical cycle and the electrical properties of the photoreceptor.

## INTRODUCTION

Light adaptation is the decrease in visual sensitivity during background illumination. Dark adaptation is the subsequent recovery of sensitivity when the background illumination is decreased or turned off. In both vertebrate and invertebrate eyes, dark adaptation may be related either to the photochemical cycle (Dowling, 1963; Rushton, 1965; Donner and Reuter, 1967;

Hamdorf et al., 1971) or to receptor polarization (Glantz, 1972; Grabowski et al., 1972). Likewise, light adaptation in both vertebrates and invertebrates may be related either to receptor polarization (Fuortes and Hodgkin, 1964; Boynton and Whitten, 1970) or to some other factor such as the concentration of intracellular calcium (Yoshikami and Hagins, 1971; Hagins, 1972; Lisman and Brown, 1972).

With so many proposed bases for adaptation in so many preparations, it seemed desirable to study these bases in one and the same photoreceptor. In this paper we report the first such study: we present for the first time the kinetics of dark adaptation in the ventral photoreceptors of *Limulus*, and we show that adaptation in these photoreceptors is independent of both the photochemical cycle and the membrane electrical properties. By elimination, we conclude that there are unknown, intermediate process(es) responsible for adaptation which are interposed between the photochemical cycle and the membrane electrical properties of the photoreceptor.

#### MATERIALS AND METHODS

##### A. *Biological Preparation*

*Limulus* (carapace diameter 6–8 inches) were obtained from the Marine Biological Laboratory, Woods Hole, Mass. Clark, Millechia, and Mauro (1969) have previously described the ventral photoreceptors of *Limulus*. Their evidence indicates that this preparation consists of isolated primary photoreceptors, located on the lateral olfactory nerves. In the present experiments, the nerves were removed from the animal, desheathed, and placed in a chamber containing artificial seawater. The connective tissue remaining on the cell bodies was softened with 1% Pronase (Calbiochem, Los Angeles, Calif.) in buffered seawater (pH 7.4) for 1 min. The data presented in this paper were obtained from 26 different cells.

##### B. *Artificial Seawater and Perfusion Media*

The artificial seawater concentrations were 435 mM NaCl, 10 mM KCl, 10 mM CaCl<sub>2</sub>, 20 mM Mg Cl<sub>2</sub>, and 25 mM MgSO<sub>4</sub>. The isotonic KCl consisted of 500 mM KCl and 10 mM CaCl<sub>2</sub>. Both solutions were buffered to pH 7.4 using Tris-OH and HCl. All experiments were carried out at about 20°C.

##### C. *Optical Stimulation*

The optical bench has been described previously (DeVoe et al., 1969). Briefly, 530 nm light was generated by a Bausch & Lomb High Intensity monochromator (Bausch & Lomb, Inc., Rochester, N. Y.) with a xenon light source. This beam passed through circular neutral density wedges (Eastman Kodak Company, Rochester, N. Y., Type M) and a Uniblitz shutter (Velmex, Inc., Holcomb, N. Y.). A beam splitter combined this beam with a beam from a tungsten-iodide lamp, which was used as the adapting light. The combined light beams were focused on a 0.5 mm diameter, fiber optic bundle (American Optical Corp., Buffalo, N. Y., type ULGM). The other end

of the fiber optic bundle was placed in the seawater at a distance of about 300  $\mu\text{m}$  from the cells to be stimulated. During the experiments, the preparation could be observed with a water immersion microscope. In experiments where the early receptor potential (ERP) was measured, the cells were stimulated by an intense flash (lasting approximately 1 ms) from a strobe light (Honeywell Auto/Strobonar 332, Honeywell, Inc., Minneapolis, Minn.) delivered to the preparation through the water immersion microscope. Where double flashes were delivered to the preparation, a beam splitter was placed in the tube of the microscope and a second strobe light was used.

#### D. *Electrical Recording*

Glass micropipettes were made from partition tubing of borosilicate glass (Friedrich & Dimmock, Inc., Millville, N. J.); these usually filled in less than a minute after injection with 2 M KCl. The electrodes used in these experiments had resistances of 6–10 M $\Omega$ , measured in seawater. The electrodes were connected to a unity gain, negative capacity amplifier which had an active bridge for measurement of membrane resistance changes. Responses were recorded on film with a Grass C-4 camera (Grass Instrument Co., Quincy, Mass.).

#### E. *Calibrations*

For measurement of dark adaptation, the test stimulus was a 40 ms, 530 nm flash. The steady intensity was calibrated at this wavelength using a PIN-10 UV photodiode (United Detector Technology, Santa Monica, Calif.) calibrated by the manufacturer in the photovoltaic mode. The diode was positioned at the end of the fiber optic in place of the photoreceptors. The number of photons incident on the photoreceptor in the most intense 40 ms, 530 nm flash available was calculated to be  $7 \times 10^9$  photons, assuming the size of the photoreceptor to be  $60 \times 100 \mu\text{m}$  (Clark et al., 1969). Using the response of the photoreceptor for comparison, it was then found that the strobe and tungsten-iodide lamps delivered to the photoreceptor the equivalent of  $2.8 \times 10^{11}$  530 nm photons/1 ms flash and  $1.4 \times 10^{12}$  530 nm photons/s, respectively.

Two separate lines of evidence indicate that each strobe flash activates the majority of pigment molecules in the photoreceptor. First, the calibrations indicate that each strobe flash delivers the equivalent of 1 photon of 530 nm for every  $2 \text{ \AA}^2$  ( $2.8 \times 10^{11}$  photons per cell of cross sectional area  $60 \times 100 \mu\text{m}$  or  $6 \times 10^{11} \text{ \AA}^2$ ). Assuming the photopigment in ventral eye cells of *Limulus* have a molar extinction similar to rhodopsin (40,600: Wald and Brown, 1953), then each pigment molecule has an absorption cross section of  $1.5 \text{ \AA}^2$ . If there is no self-screening within the photoreceptor, then the average pigment molecule absorbs 0.75 photons ( $1.5 \text{ \AA}^2 \div 2.0 \text{ \AA}^2$ ) per flash.

Secondly, in two experiments, we found that attenuating the strobe flash by 1 log unit caused the ERP to decrease by 50 and 65 %, respectively. According to the analysis of Cone (1964), which related the ERP amplitude to the flash intensity, this would indicate that more than one photon was absorbed per pigment molecule in the unattenuated flash.

#### F. *Definitions*

**THRESHOLD** The number of photons in a flash of light which elicits a criterion response of the late receptor potential. In these experiments, the flash duration was

40 ms and the criterion response was peak amplitude of 4 mV. For all levels of threshold studied in this paper, 40 ms was below the integration time of the photoreceptor. As the time-course of threshold recovery was found to be independent of the criterion chosen, 4 mV was chosen for convenience.

**SENSITIVITY** The reciprocal of threshold.

**LIGHT ADAPTATION** The decrease in sensitivity (increase in threshold) which occurs *while* a light (background illumination; ambient illumination) is shining upon the photoreceptor.

**DARK ADAPTATION** The increase in sensitivity (decrease in threshold) which occurs after the ambient light is decreased or turned off. This is a temporal process which occurs in the dark or at the new lower level of ambient illumination.

## RESULTS

### A. Photochemistry and Dark Adaptation

The photochemical cycle of the visual pigment in the ventral eye of *Limulus* was followed by measuring the amplitude of the early receptor potential (ERP), since pigment absorptions in these photoreceptors are small (1–3%: Murray, 1966) and absorption changes would be difficult to measure. The ERP is elicited by intense flashes (Brown and Murakami, 1964; Smith and Brown, 1966) and is an indicator of the photochemical cycle in the ventral photoreceptors of *Limulus*, since the action spectra for both the R<sub>1</sub> and R<sub>2</sub> components of the ERP as well as action spectrum of the late receptor potential (Brown, Murray, and Smith, 1967) are similar to the absorption difference spectrum of the pigment within the photoreceptor (Murray, 1966). Furthermore, in the rat, the amplitude of the ERP has been shown to be proportional to the number of unbleached pigment molecules activated by a flash (Cone, 1964; Cone and Cobbs, 1969).

Fig. 1 shows the log relative threshold of the late receptor potential of 12 cells during dark adaptation after a single flash from a strobe light at time zero. The data were fitted by eye as the sum of two exponentials, the faster of which had a time constant of 20 s.

Fig. 2 shows the ERP's that resulted from double flash experiments. The late receptor potential was eliminated by bathing the cells in isotonic KCl. This was because the present experiments could not be performed if the late receptor potential were present, since the response to the second flash would be masked by the late receptor potential. The ERP was not eliminated by this procedure, since the ERP has been shown to be resistant to a wide variety of agents that eliminate the late receptor potential (Brindley and Gardner-Medwin, 1966; Arden et al., 1968).

A flash elicits an ERP that is biphasic and mainly hyperpolarizing (downward). Figs. 2 A and B are control responses elicited by each of the two strobe

lamps. Figs. 2 I and J are the result of a double flash experiment with a 2 s spacing between the flashes. No data are presented beyond 2 s, as it was found that the second ERP showed no change in size or shape beyond 2 s. If a second flash is delivered at different times after the first flash, the results shown in Fig. 2 C through 2 H are obtained. In this paper we are concerned only with the recovery of the ERP and not with any of the photochemical

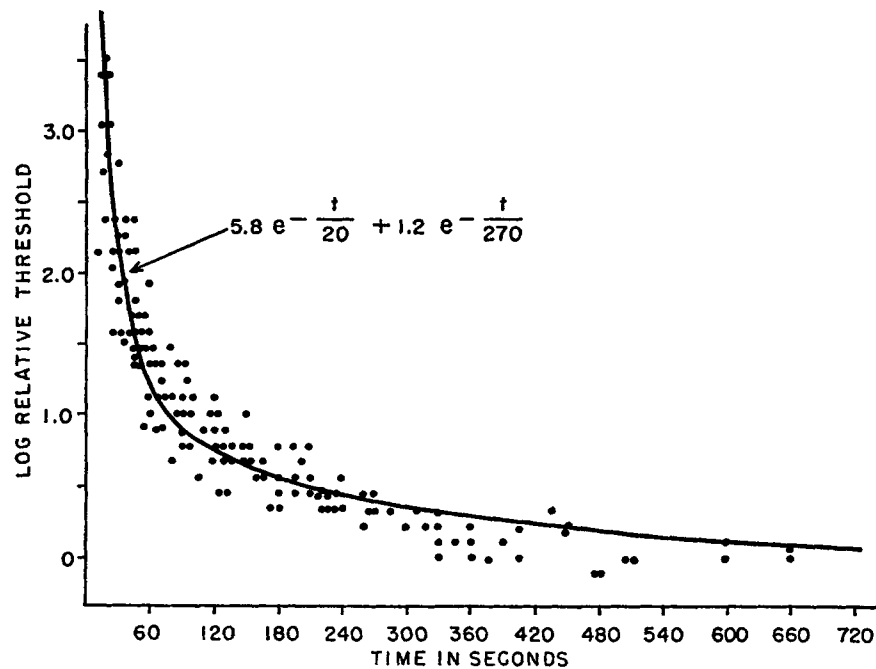


FIGURE 1. Log relative thresholds of the late receptor potential after single intense flashes from a strobe light (see Methods) at time zero. Composite data of 12 cells from 9 experiments. Since these cells had absolute thresholds that differed by as much as 2 log units, the data are presented in the form of relative thresholds. Each cell was dark-adapted before the flash was delivered. The smooth curve was fitted by eye to give a rough estimate of the time constants (in seconds) involved.

intermediates that are produced by light. Therefore the changes in shape of the ERP elicited by the second flash will not be discussed (Fein, 1972). Fig. 3 shows the composite results of three experiments on six cells. The recovery of the  $R_2$  component (downward component) of the ERP is shown as a function of time. The time constant for the ERP recovery is about 100 ms, which can be compared with the 20 s time constant for the initial threshold recovery shown in Fig. 1. Because the threshold recovers more than 100 times slower than the ERP, it seems reasonable to conclude that the sensitivity changes take place at a stage of transduction after the photochemical cycle.

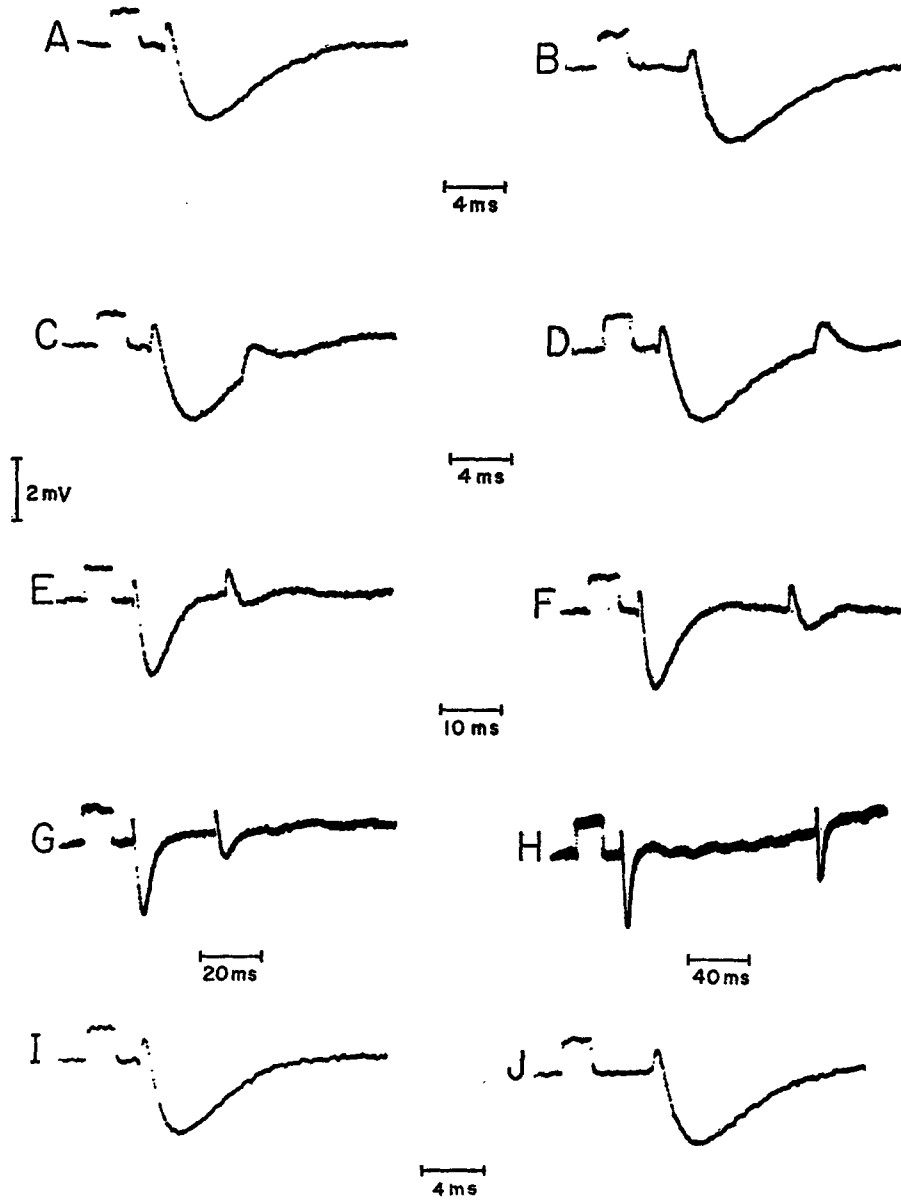


FIGURE 2. Intracellular, early receptor potentials recorded in response to double flashes, each flash coming from a different strobe light. (A) and (B), controls for each strobe light. The strobe that produced the response in (B) always delivered the second flash in the double flashes. (C)-(H), responses to double flashes. (I) and (J), response to two flashes spaced 2 s apart. Downward deflections correspond to membrane hyperpolarization.

These experiments on the time constant of ERP recovery are consistent with earlier experiments of Hillman et al. (1972),<sup>1</sup> who found that the ERP of the ventral eye recovered with an 80 ms time constant at 24°C. However, these workers used less intense flashes of longer duration and they did not measure the time constant of the recovery of threshold under their conditions of stimulation.

*B. Potential, Resistance, and Threshold of the Late Receptor Potential during Dark Adaptation*

If instead of the photochemical cycle, the membrane potential or membrane conductance were determining the sensitivity during dark (and light) adap-

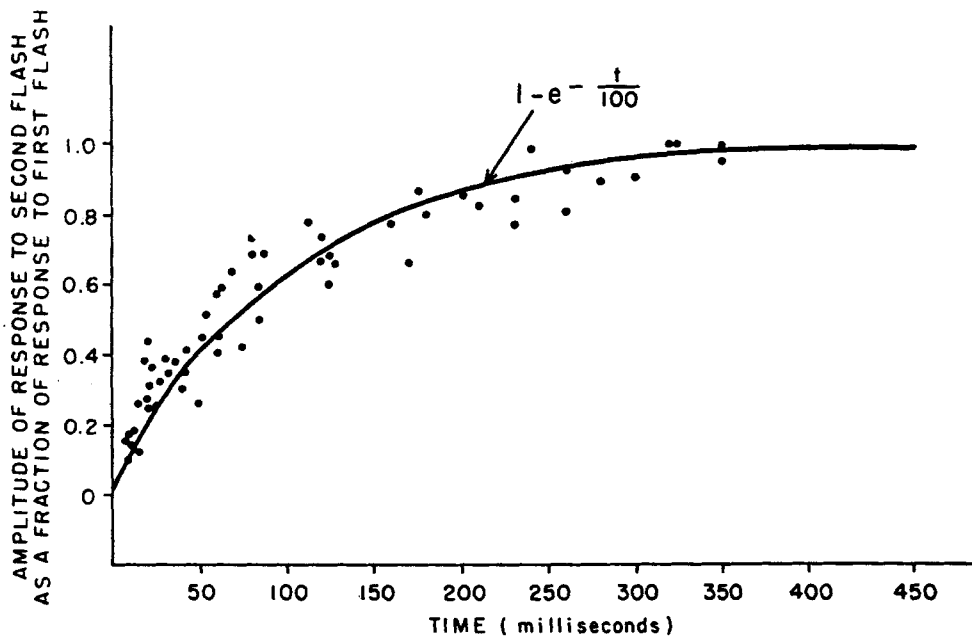


FIGURE 3. Recovery of the hyperpolarizing ( $R_2$ ) component (see Fig. 2) of the ERP. Composite data of six cells from three experiments. The curve was fitted by eye to give a rough estimate of the time constant (in milliseconds) involved in the recovery process.

tation, the time-course of the sensitivity change during dark adaptation should parallel the time-course of the membrane potential or membrane conductance. In Fig. 4, the log threshold, membrane potential, and membrane resistance are shown for the time immediately before and after turning off an adapting light which causes a 2 log unit decrease in threshold. Figs. 4 *a* and *b*

<sup>1</sup>Hillman, P., F. A. Dodge, S. Hochstein, B. W. Knight, and B. Minke. 1972. Rapid dark recovery of the invertebrate early receptor potential. Submitted for publication.

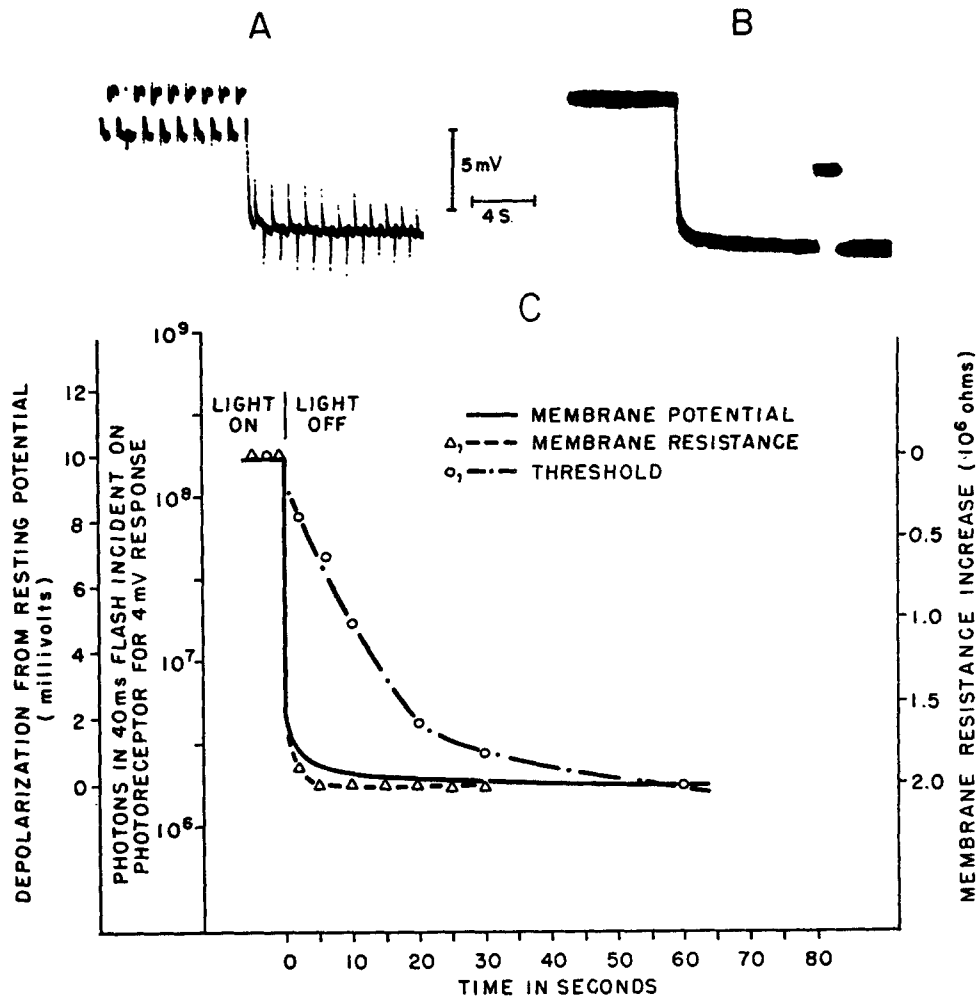


FIGURE 4. (A), bridge measurement before and after an adapting light is turned off. A depolarizing bridge current of 1 nA, on for  $\frac{1}{2}$  s, off for  $\frac{1}{2}$  s was used. The bridge was initially balanced in the dark; the unbalance represents a decrease in resistance. 1 mV of unbalance corresponds to a resistance decrease of 1 M $\Omega$ . (B), membrane potential before and after adapting light is turned off. The calibration pulse is 5 mV. (C), change in membrane resistance, membrane potential, and threshold when adapting light is turned off. The adapting light was the equivalent of  $1.4 \times 10^9$  photons/s at 530 nm (see Calibrations). The adapting light was on for 15 s before being turned off.

are oscilloscope records of the resistance bridge and the potential measurements, respectively. In Fig. 4 a, the bridge was originally balanced when the photoreceptor was in the dark, and so was off balance during illumination. There was a large transient change in the membrane potential and membrane resistance when the light was turned off, but there was no similar change



in the log threshold. It can be seen that when the adapting light was turned off, the recovery of membrane potential and membrane resistance had a time-course similar to each other. On the other hand, the decay of log threshold did not exhibit the large initial transient change observed in both the membrane potential and membrane resistance, when the light was turned off. The data in Fig. 4 *c* show that there is not the parallel recovery of membrane potential with log threshold that was seen by Grabowski et al. (1972) in the axolotl. Similar results were found in five other cells.

It might be argued that the log threshold is not the appropriate function of the threshold to be comparing to the membrane potential. Rather, the threshold itself or some other function of the threshold might be related to the membrane potential. However, if it is assumed that the instantaneous value of the membrane potential is controlling sensitivity during background illumination and dark adaptation, then the following would be expected: At any given threshold determined either during background illumination or during dark adaptation, the cell should have the same membrane potential. That is, there should be a single unique relation between membrane potential and sensitivity, if the sensitivity is being controlled by the membrane potential. In Fig. 5, the membrane depolarizations during dark and light adaptation are compared for the same cell as in Fig. 4. The data for dark adaptation were obtained from Fig. 4; at different times in Fig. 4 the threshold and membrane depolarization were measured and plotted in Fig. 5. The data for light adaptation were obtained by measuring the threshold and membrane depolarization, for different adapting lights. These results, and similar ones from three other cells show that a cell can have different membrane depolarizations while having identical thresholds. Therefore, the instantaneous value of the membrane potential cannot be controlling the threshold.

The membrane depolarization instead of the membrane potential is plotted in Figs. 4 and 5. This was done because it took more than 1 h to obtain the data for these figures, and during this time the membrane potential was not stable but slowly changed, sometimes as much as an absolute change of 15 mV. However one of the cells we recorded from maintained a stable membrane potential (less than 1 mV drift) during the course of the experiment. The results for this cell were completely similar to the results in Figs. 4 and 5. These slow changes in membrane potential provide additional evidence that the sensitivity does not depend on the membrane potential; for when a cell spontaneously depolarized by 10 mV the sensitivity did not decrease by 2 log units (see Fig. 4) but decreased by less than 0.3 log units. Likewise, extrinsic currents passed through the cell membrane via the intracellular electrode had only small effects on response amplitude. That is, when the two cells tested were either light-adapted or dark-adapted, extrinsic currents that caused the membrane potential to change by 10 mV caused the sensitivity to change by less than 0.3 log units. Calculations based on the voltage-clamp studies of Millechia and Mauro (1969)

indicate that 10 mV depolarizations cause approximately 0.4 log unit changes in sensitivity.

C. *Differences in Time-Courses of Response Between Light and Dark Adaptation*

In the results presented so far, there has been no single measured parameter, such as membrane potential or ERP amplitude (photopigment concentra-

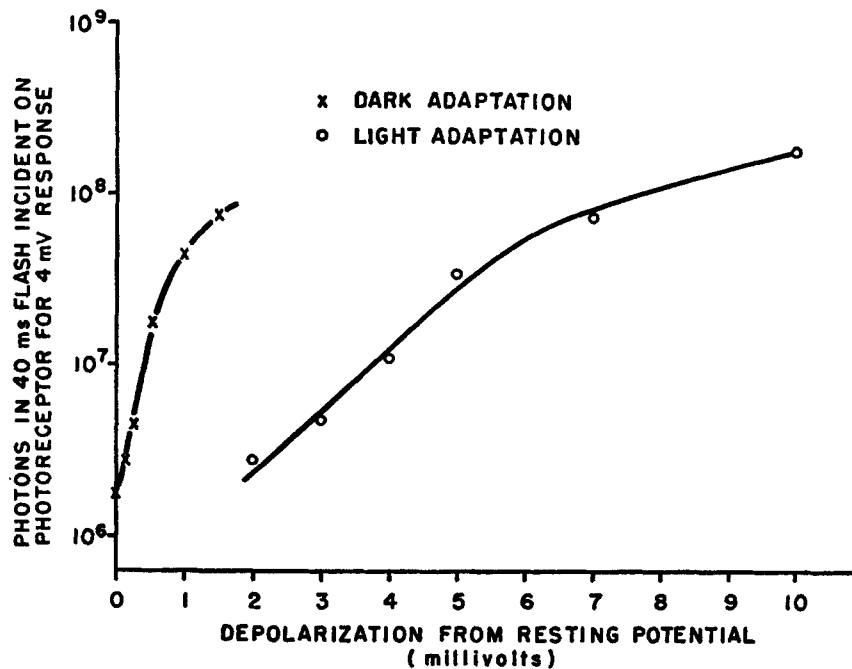


FIGURE 5. Comparison of membrane depolarizations during light adaptation and dark adaptation. The dark adaptation data were obtained from Fig. 4 C. The light adaptation data were obtained by noting the threshold and membrane depolarization while different adapting lights were illuminating the cell. The most intense adapting light used corresponds to the adapting light used before dark adaptation in Fig. 4 ( $I = 1.4 \times 10^9$  equivalent photons/s at 530 nm). The other adapting lights correspond to intensities of  $4.4 \times 10^8$ ,  $1.4 \times 10^8$ ,  $4.4 \times 10^7$ ,  $1.4 \times 10^7$ , and  $4.4 \times 10^6$  equivalent photons/s at 530 nm (see Calibrations).

tion) which was related to the threshold changes during light and/or dark adaptation. This might mean only that the appropriate, single parameter was not being measured, or that there was no such single parameter.

If there were some one, unique parameter which alone set the sensitivity of the cell during either light or dark adaptation, then all aspects of the late receptor potential of the photoreceptor should be the same regardless of what caused that given sensitivity. The experiment shown in Fig. 6 was designed to

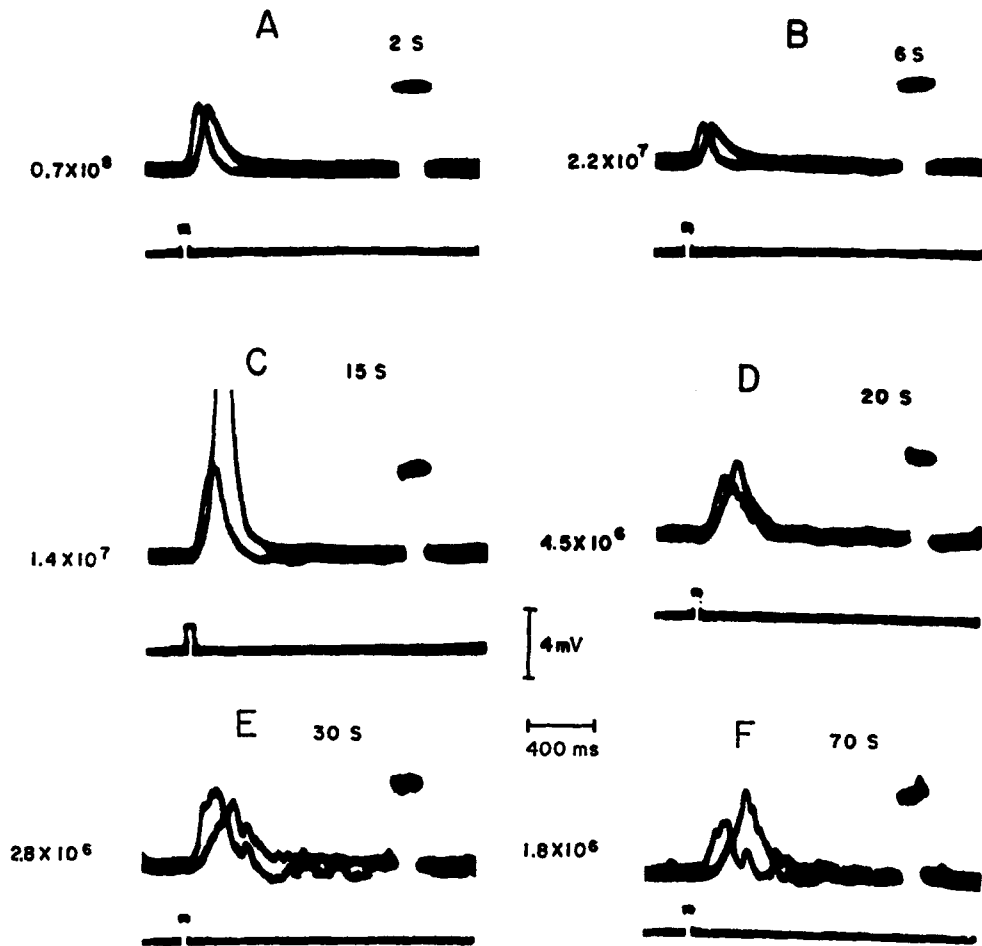


FIGURE 6. Comparison of response waveforms, during light adaptation and dark adaptation, to identical 40-ms flashes. In (A) through (F) the times correspond to times after the adapting light is turned off. The number before each pair of response waveforms is the number of photons in the stimulus (see Calibrations), the calibration pulse is 5 mV, the lower trace gives the time-course of the stimulus flash. In each case the earlier (faster) response was recorded with ambient illumination and the later response during dark adaptation. The steady adapting lights for the earlier responses were, in (A),  $4.4 \times 10^8$ ; (B),  $1.4 \times 10^8$ ; (C),  $4.4 \times 10^7$ ; (D),  $1.4 \times 10^7$ ; (E) and (F),  $4.4 \times 10^6$  equivalent photons/s at 530 nm (see Calibrations). The illumination from which the cell was dark adapting, for the slower responses, was  $1.4 \times 10^9$  equivalent photons/s at 530 nm. Same cell as used in Figs. 4 and 5 and as part of Fig. 1.

test the applicability of this model to the ventral eye of *Limulus*. The idea was to compare the responses of the late receptor potential to identical flashes during both light adaptation and dark adaptation under conditions that would produce about the same sensitivity. In each part of Fig. 6, two re-

sponses are shown to these identical flashes, whose quantal contents are given to the left of each pair of traces. The important point in Fig. 6 is that the two responses in each part were obtained under different conditions of adaptation. The slower responses with the longer latencies are responses that were obtained during the dark adaptation shown in Fig. 4. The times (in seconds) given in each part of Fig. 6 correspond to times during the dark adaptation of Fig. 4. It is clear in Fig. 6 that as time progresses, the threshold decreases (it takes fewer quanta to elicit an approximately 4 mV response) and it is this decrease in the threshold that was plotted in Fig. 4. The responses with the shorter latencies were obtained when different adapting lights were illuminating the photoreceptor. All these adapting lights were less intense than the one (in Fig. 4) from which the cell was dark adapting and were chosen to give sensitivities about equal to those of the same cell when dark adapting.

In each part of Fig. 6, the responses to identical flashes are compared, and except for Fig. 6 C, the amplitudes of the responses are approximately equal. Therefore, the responses are being compared at approximately equal sensitivities (except for Fig. 6 C). It is clear that when the sensitivity is decreased by the presence of an adapting light, the response has a different time-course than when the sensitivity is decreased by the aftereffects of previous illumination (dark adaptation). This difference in time-course was seen throughout the course of dark adaptation as is shown in the different parts of Fig. 6. Fig. 6 C was included to show that this difference in time-course was not critically dependent on comparing responses at nearly identical thresholds, for even here the response of the dark-adapting cell was slower. Similar results were found in three other cells.

## DISCUSSION

### A. Photochemical Cycle and Adaptation

When we first began these experiments, we expected to find a component of dark adaptation that was correlated with the pigment concentration, as in the vertebrate eye (Dowling, 1963; Rushton, 1965; Dowling and Ripps, 1970), and possibly in the insect eye (Hamdorf et al., 1971). Cone and Cobbs (1969) had shown, in the rat, that the ERP could be used to assess pigment concentration in the retina, and that during the slow component of adaptation, the log sensitivity of the electroretinogram (ERG) was linearly proportional to the ERP amplitude, that is, to the pigment concentration. In psychophysical experiments, Goldstein and Berson (1969) have shown the same relationship between sensitivity and ERP amplitude.

However, while this work was in progress, Selden et al. (1972) found that dark adaptation in the larval mosquito ocellus does not depend upon pigment

regeneration. Our results indicate that dark adaptation in the ventral photoreceptors of *Limulus* is independent of the photochemical cycle, based on the observation that the threshold recovers more than 100 times slower than the ERP amplitude (Figs. 1 and 3). Thus in the ventral eye of *Limulus* (and in the larval mosquito ocellus), adaptation appears to take place at a stage of transduction subsequent to the photochemical cycle.

#### B. *Membrane Potential and Adaptation*

The results presented in Figs. 4 and 5 indicate that the instantaneous value of the membrane potential is not related to the sensitivity of the cell during ambient illumination and dark adaptation. However, some sort of dynamic model could be proposed where a signal composed of the membrane potential, its integral, and/or its derivatives was controlling the sensitivity during adaptation. Such a model would allow a time-dependent relationship to exist between the change of sensitivity and the change in membrane potential. The experiments using extrinsic currents are inconsistent with this model. When we did those experiments, we left the currents on for at least 1 min and found no indications that the sensitivity changed with time.

Several specific models have been proposed in which visual sensitivity depends upon the membrane potentials of photoreceptors (Fuortes and Hodgkin, 1964; Boynton and Whitten, 1970; Glantz, 1972). We have compared the results obtained in Fig. 4 to the results that would be predicted using these models. In Fig. 4 illumination caused a 2 log unit increase of threshold and a 10 mV response. 2 s after the light was turned off, the response had decreased to less than 1 mV (a factor of 10 decrease) and the threshold had decreased 0.4 log units. In the model calculations, we assumed an illumination that increased the threshold 2 log units and we assumed that this threshold elevation was caused by a response  $R$  of the receptor. Then we calculated the threshold if  $R$  were to decrease by a factor of 10. All these models predicted that the threshold 2 s after the light was turned off should be more than three-quarters of a log unit lower than was actually observed. The differences were smaller for the succeeding data points.

Finally, the demonstration by Hagins et al. (1962) that the photoreceptors of the squid could be adapted locally supports the conclusion that the membrane potential in this animal too is unrelated to the changes in threshold. When the tips of squid photoreceptors were exposed to an adapting flash, the response of the tips to subsequent test flashes was initially reduced more than 10-fold, whereas unilluminated parts of the same photoreceptors were not detectably affected by the adapting light. Since photocurrents in the distal parts of squid photoreceptors are propagated decrementally by the cable properties of these cells (Hagins, 1965) and elicit nerve impulses in more proximal regions (MacNichol and Love, 1960), the unilluminated regions of

the distal photoreceptor must also have been depolarized. The implication of this experiment is therefore that the membrane potential cannot be controlling the threshold in the squid either.

#### C. *Membrane Resistance and Adaptation*

Voltage clamp experiments (Millechia and Mauro, 1969) on the ventral eye of *Limulus* have shown that the receptor potential is caused by a light-activated conductance increase mechanism. One can assume therefore, that the differences in membrane depolarization in Fig. 5, are caused by differences in the underlying light-activated conductance. On the other hand, it was possible that membrane resistance might change during dark adaptation with a time-course slower than that of the membrane potential (Stieve, 1965, Fig. 5). Therefore, we also determined that the membrane potential and membrane resistance had similar time-courses during dark adaptation (see Fig. 4 c). Hence, the conclusions (based on Figs. 4 and 5) about the role of the membrane potential in controlling the sensitivity during light and dark adaptation can be extended to the underlying light-activated conductance. It can then be said, as it was for the membrane potential, that the instantaneous value of the light-activated conductance does not control the sensitivity during adaptation. These results only indicate that the *instantaneous* value of the light-activated conductance does not control adaptation. It is possible that an after-effect of the light-activated conductance might be controlling adaptation, as has been proposed by Lisman and Brown (1972).

#### D. *Difference in Response Time-Courses between Light and Dark Adaptation*

The experimental findings of this paper indicate that adaptation is not a single unique process. This conclusion results from the findings in Fig. 6 that the time-course of the late receptor potential is not uniquely linked to the sensitivity of the photoreceptor. This conclusion does not depend upon the particular criterion used in light and dark adaptation to equate sensitivities (which here was equal amplitudes of response). For example, had equal latencies or equal peak times of response been chosen, the adapting background lights would have had to have been considerably diminished for the responses of the light-adapted cell to have been as slow as those of the dark-adapting cell. Obviously, the amplitudes of responses to the same flash in the two states of adaptation would then no longer have been the same.

Unlike the results reported here, Fuortes and Hodgkin (1964), working in the lateral eye of *Limulus*, found that the time-course of the responses from this eye were similar at a given sensitivity, whether this sensitivity was reached during dark adaptation or light adaptation.

Crawford (1947) has shown psychophysically that the threshold elevation during dark adaptation can be thought of as being caused by an equivalent

background illumination. The results of Fig. 6 show that in the ventral eye, dark adaptation can be thought of as being caused by an equivalent background illumination only if the threshold elevation alone is considered. If however the total response of the photoreceptor is considered, then dark adaptation cannot be thought of as being caused by an equivalent background illumination alone. Fig. 6 clearly shows that dark adaptation is not equivalent to light adaptation if the total response of the photoreceptor is considered.

Finally, the findings presented in this paper were not dependent on the absolute sensitivities or absolute membrane potentials of the individual cells studied. The membrane potentials for individual cells varied between 40 and 60 mV and the sensitivities in the dark varied by as much as two log units.

#### E. *Intermediate Processes*

By the elimination of membrane potential, membrane conductance and the photochemical cycle as the determinants of adaptation in the ventral photoreceptors, we are left with assigning adaptation to unknown process(es) intermediate between the photochemical cycle and the membrane electrical properties. Moreover, adaptation within these intermediate process(es) cannot be described in terms of a single, unique, if unknown parameter. Rather, it appears that incremental responses superimposed upon ongoing receptor activity in the presence of background illumination must reflect different states of the intermediate process(es) than do responses elicited during adaptation in the dark, even though the two types of responses are elicited by equal numbers of quanta and have nearly the same amplitudes.

Of a number of attempts to get at such intermediate processes, none appear satisfactory for explaining the results of this paper. On the one hand, formal kinetic models of the analog sort (Fuortes and Hodgkin, 1964; Pinter, 1966; DeVoe, 1967) may or may not describe the distinctions between responses of light- and dark-adapted receptors, but they rarely encompass such slow changes as occur in dark adaptation, and they have as yet no molecular basis. On the other hand, more molecular or mechanistic proposals for intermediate processes, such as the proenzyme-enzyme schema of Wald (1965) or the possibility that calcium may be involved in adaptation (Yoshikami and Hagins, 1971; Hagins, 1972; Lisman and Brown, 1972) have yet to be tested kinetically with such data as presented in this paper. If calcium were to mediate adaptation it would have to have a different effect during light adaptation than during dark adaptation. This possibility remains to be tested.

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The experiments reported here were described in a thesis presented by Alan Fein to the departments of Biomedical Engineering and Physiology, Johns Hopkins University, in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

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