REVERSAL OF PHOTORECEPTOR POLARITY RECORDED DURING THE GRADED RECEPTOR POTENTIAL RESPONSE TO LIGHT IN THE EYE OF *LIMULUS*

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ABSTRACT Intracellular electrodes were inserted into single photoreceptor units of the excised lateral eye of *Limulus*, and preparations were selected from which graded receptor potentials of relatively large amplitude could be recorded in response to light stimuli. The experimental data indicated that the graded receptor potential does not arise solely from a collapse of the resting membrane potential of the sensory cells of the eye, since a reversal of polarity of the photoreceptor unit could be demonstrated when the eye was stimulated by light. In the recovery period following stimulation, characteristic changes in the so-called resting potential were recorded. It is suggested that these changes in the so-called resting membrane potential are electrical signs of recovery processes occurring in the photoreceptor, because the potential changes were recorded when the eye was in darkness and because the magnitudes of the potential changes were a predictable function of the intensity and duration parameters of the preceding light stimulus.

INTRODUCTION

Retinal processes include mechanisms for the absorption of light energy and mechanisms for the production of an encoded output which is some function of the input light energy. The final encoded output of the retina occurs in the form of electrical nerve impulses which are transmitted to higher centers of integration *via* the optic nerve.

The intermediate photoreceptor processes which couple the absorption of light energy to the final encoded output have not been clearly defined. However, one important step in the intermediate processes has been demonstrated in the photoreceptor unit of *Limulus*. Hartline, Wagner, and MacNichol (1952) inserted micropipette electrodes into *Limulus* ommatidia and recorded graded receptor

potentials in response to light stimuli. The experimental evidence has been consistent with the hypothesis that this graded receptor potential is the immediate generator of impulses in the optic nerve of *Limulus* (MacNichol, 1956; Fuortes, 1958*a*; Fuortes, 1959; Rushton, 1959). The evidence indicates that it is reasonable to employ the term "generator potential" to describe the phenomenon that had previously been designated as the "slow potential response to light" or the "graded receptor potential response to light," and the more convenient term, generator potential, will be used throughout the remainder of this report.

A "reversal" of photoreceptor polarity, during the period in which the *Limulus* eye is responding to light stimuli, had been reported earlier (Benolken, 1959). The data which support this conclusion will be presented in this report. These data appear to be in general agreement with the independent work of M.G.F. Fuortes (1958*b*). The characteristic sequence of electrical events observed in recovery periods following stimulation of the eye will also be discussed.

A lateral eye of *Limulus* is a compound eye composed of about 600 photoreceptor units or ommatidia. A schematic representation of a single ommatidium is shown in Fig. 1. The clear conical area in the upper portion of the sagittal section represents the crystalline cone which provides the ommatidium with a primitive lens system. A group of 10 to 20 retinula cells are located proximal to the crystalline

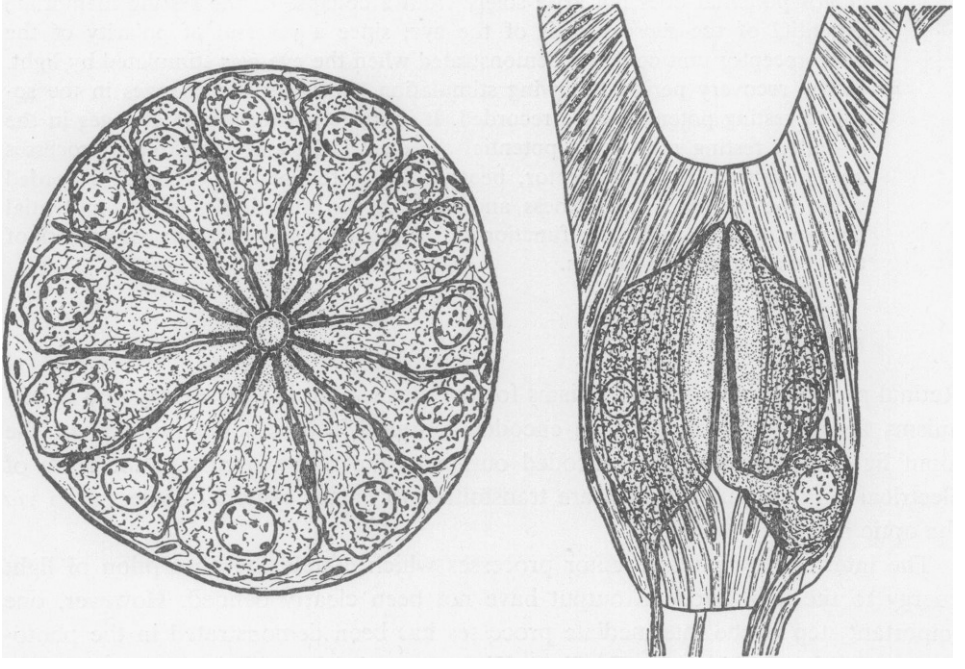

FIGURE 1 Schematic representation of transverse and sagittal sections of a *Limulus* ommatidium from Demoll (1917).

cone, and these cells are arranged around a central canal in a radial fashion as indicated in the transverse section. A single eccentric cell is shown to one side of the central canal in the sagittal plane. A distal process of the eccentric cell is enclosed in a portion of the central canal, and the axon of the eccentric cell and axons of smaller diameter from the retinula cells extend into a complicated nerve plexus behind the ommatidia. The axons from the 600 or so ommatidia ultimately converge in the nerve plexus to form the optic nerve of the eye. The reader is referred to Miller (1957) for the details of the histology and ultrastructure of the *Limulus* ommatidium.

When a micropipette is inserted into a cell of an ommatidium, a resting potential is recorded such that the micropipette becomes polarized about 55 mv negative with reference to an extracellular electrode. As the micropipette is probed through an ommatidium, the electrode may or may not record an electrical response to light. The success or failure of recording a response to light presumably depends upon the location of the micropipette in the photoreceptor unit. If the micropipette has been positioned in a region where an electrical response to light can be recorded, the response takes the form of (*a*) a graded receptor potential (generator potential) and (*b*) nerve impulses propagated from the optic nerve. Impulses propagated in the optic nerve appear to be generated near the eccentric cell (MacNichol, 1956). Presumably the electrical activity associated with the propagated impulses is recorded *via* passive conduction through the various structures of the ommatidium when the micropipette is placed in a location which is remote from the eccentric cell.

The relative amplitudes of the generator potential and the amplitudes of impulse activity which were recorded from the eye were markedly dependent upon electrode placement. In general, whenever the micropipette was positioned so that generator potentials of relatively large amplitude (60 to 90 mv) could be recorded in response to intense illumination, nerve impulse activity of relatively small amplitude (less than 1 mv) was recorded. Conversely, whenever large-amplitude (40 to 50 mv) nerve impulses were recorded, the generator potential amplitude (50 mv or less) was reduced in response to intense illumination. For the experiments to be discussed in this report, the micropipette was always positioned to permit recording generator potentials of maximum amplitude. Under these conditions, it is likely that the micropipette was located in a region of the ommatidium which was distal to the body of the eccentric cell. However, the precise location of the micropipette was difficult to establish since the ommatidia of *Limulus* are densely pigmented.

EXPERIMENTAL PROCEDURE

Recording Methods. Intracellular micropipette electrodes, filled with 2 M KCl, formed a salt bridge between an impaled photoreceptor unit and a small glass tube which contained an Ag–AgCl electrode immersed in sea water. The Ag–AgCl electrode made contact with the high impedance side of a negative capacitance preamplifier de-

signed by MacNichol and Wagner (1954). An indifferent Ag–AgCl electrode completed the circuit through sea water surrounding the eye. The amplified output of the preamplifier was monitored by an oscilloscope and a recording potentiometer. A Grass camera provided permanent records of the oscilloscope trace.

Stimulator. A single channel optical stimulator provided a maximum illumination of roughly 1 lumen/cm^2. The intensity of the stimulus was varied by attenuating a constant source intensity with a neutral density wedge having an attenuation range of 5.0 log units in reproducible 0.01 log unit steps (MacNichol, 1952). The intensity range of the stimulator was extended to 12 log units with the addition of neutral density filters. All relative stimulus intensities were defined in log units of attenuation of the constant source intensity. The stimulus intensity, I, is related to the constant source intensity, I_0, by the relative stimulus intensity expressed in log units $= -\log_{10}(I/I_0) = \log_{10}(I_0/I)$. For example, a relative stimulus intensity of 1.0 log unit indicates that $I = (0.1)I_0$.

Preparation of the Photoreceptor. A lateral eye of *Limulus* was excised and mounted after the manner described elsewhere (Benolken, 1959). A micropipette was placed into artificial sea water which surrounded the mounted preparation, and the impedance of the micropipette was measured. If the impedance was less than 30 megohms or the tip potential exceeded 5 mv with respect to the Ag–AgCl reference electrode, the micropipette was rejected. Typical values of micropipette impedances ranged from 30 to 100 megohms, and tip potentials were rarely as large as 1 mv. An acceptable micropipette was probed very slowly through an ommatidium until a generator potential, 60 to 90 mv in amplitude, could be recorded in response to an intense stimulus. The photoreceptor unit was isolated optically so that the stimulator illuminated only the facet of the ommatidium impaled by the micropipette. Then the preparation was enclosed in a light-tight box and remained in total darkness until the resting potential achieved a constant level.

EXPERIMENTAL RESULTS

Reversal of Photoreceptor Polarity

Fig. 2 shows a generator potential which was recorded in response to a relatively intense light stimulus of 1 second duration. The two characteristic components of

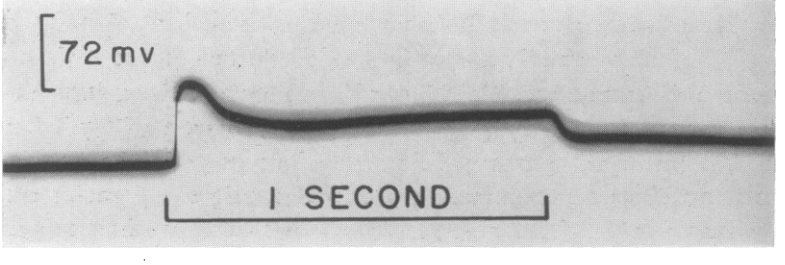

FIGURE 2 A generator potential recorded in response to a light stimulus of 1 second duration and 0.00 log unit of relative intensity. The peak amplitudes of the generator potential were measured with respect to the base line established by the dark potential at the time that the stimulus was delivered. The broadening of the response record was due to a diffuse flare surrounding the scope trace which has been accentuated in reproduction.

the generator potential response are clearly defined on the record: an initial transient component and a second (steady-state) component which was maintained for the duration of the stimulus.

The amplitude of the initial transient component of this response was +83 mv, and the steady-state amplitude of the second component was +45 mv. All generator potential amplitudes were measured from the baseline established by the so called resting potential which was recorded at the time that the stimulus was turned on.

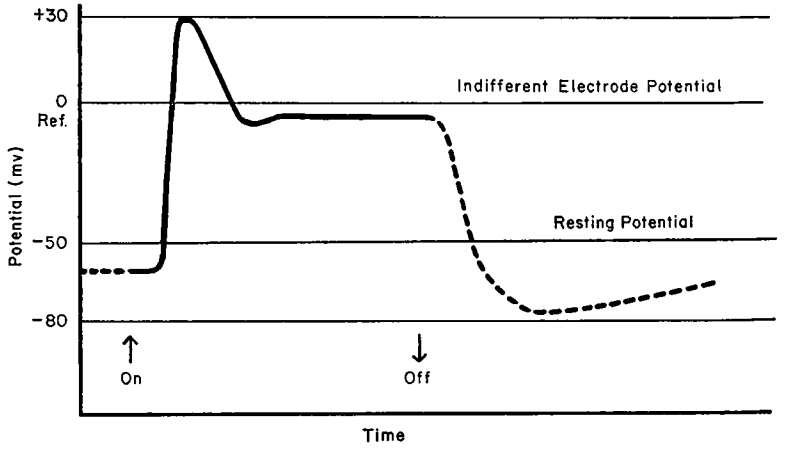

FIGURE 3 Schematic representation of potentials recorded during experimental runs similar to those of Fig. 2. The potentials recorded during the stimulus period are indicated by solid lines, and the dotted lines indicate potentials recorded when the eye was in the dark. The time scale of the dotted portions of the potential record has been collapsed relative to the time scale for the solid portions of the record (see text).

The baseline for the experiments under discussion was 60 mv negative with respect to an extracellular reference electrode.

The maximum intensity which was available from the optical stimulator was used to obtain the record of Fig. 2. The response mechanisms had not saturated at this intensity level, since the amplitude of the transient component of the generator potential increased to +90 mv when the eye was stimulated with a more intense microscope illuminator. Further tests were not made to determine whether or not the response mechanisms had saturated for this +90 mv response.

The relative magnitudes of the generator potential, the resting potential, and the potential of the extracellular reference electrode are shown in Fig. 3. The potential of the extracellular (indifferent) electrode was chosen to define the zero potential reference of the system. Before stimulation, the photoreceptor unit was polarized in a negative sense to the level of the resting potential, but the polarity reversed in a positive sense at the peak of the generator potential response to light. The mag-

nitude of the reversal was about 30 mv positive of the extracellular zero reference. Even if an unlikely accumulative error of 10 mv were assumed for electrode potentials and errors in amplitude measurements, the photoreceptor must have been polarized at least 20 mv positive with respect to the reference electrode at the peak of the response. Data such as these are inconsistent with the hypothesis that the generator potential arises solely from a partial or complete collapse of the resting potential of the sensory cells, since the photoreceptor response could never exceed the zero reference on this hypothesis.

The reversal of photoreceptor polarity which was observed during the peak of the generator potential response is qualitatively similar to the reversal of polarity observed in nerve cells during the peak of the nerve impulse, and perhaps the initial transient component of the generator potential is derived from ionic mechanisms similar to those demonstrated in nerve (Hodgkin, 1958). Unfortunately, studies on the relation between external ion concentrations and the generator potential response are incomplete. The generator potential has been studied when the eye of *Limulus* was subjected to various external concentrations of potassium and calcium (Yeandle, 1957), but the effects of varying external sodium concentrations have not been reported.

When micropipettes were probed through the eye in a stochastic fashion, generator potentials of maximum amplitude exceeding 60 mv were recorded much less frequently than maximum amplitudes ranging from 20 to 40 mv. This observation may indicate that the volume of the photoreceptor unit which reverses polarity upon intense stimulation is quite small, and it may be that these small volumes of high current density are very close to the morphological site of origin of the generator potential. Obviously, it would be desirable to locate the anatomical regions of the photoreceptor unit of *Limulus* which are associated with the production of the generator potential (see Hartline, 1959). Thus far, the morphological sites from which large-amplitude generator potentials can be recorded have not been identified experimentally.

It was possible to demonstrate a reversal of photoreceptor polarity during the initial transient component of the generator potential response in at least 30 different preparations. It was not possible to demonstrate a reversal of photoreceptor polarity during the second (steady-state) component of the generator potential response for any of the preparations tested. Frequently the potential difference between the micropipette electrode and the indifferent electrode approached zero during the second component of the generator potential. However, this potential difference was consistently several millivolts negative of zero. If the generator potential response to light arises from changes in specific ionic permeabilities in the photoreceptor unit, it is likely that there is a pronounced quantitative difference in the permeability of at least one ionic species during the transient and steady-state components of the response.

Changes in the Dark Potential During Recovery Periods Following Stimulation

Under certain conditions, the photoreceptor unit of *Limulus* may not be polarized to a steady resting level even when the eye is in complete darkness. As had been noted by Yeandle (1957), the resting potential may show significant changes after the eye has been stimulated by light.

The changes in the so-called resting potential were especially pronounced whenever generator potentials of large amplitude (60 to 90 mv) could be recorded from a preparation. The characteristic time course of this type of "rebound" phenomenon is shown schematically in the dotted trace of Fig. 3. When the stimulus was turned off, the photoreceptor became hyperpolarized about 20 to 30 mv more negative than the resting level. Thereafter, the polarity of the photoreceptor increased again in a positive sense to recover to the resting level. The peak of this hyperpolarization phenomenon usually occurred about 30 seconds after the stimulating light had been turned off. The time scale of the dotted portions of the trace in Fig. 3 has been collapsed about 50 times relative to the time scale for the solid portions of the trace for convenience of presentation.

To avoid confusion, all potentials recorded when the eye was in darkness will be referred to as dark potentials, and the term resting potential will be restricted

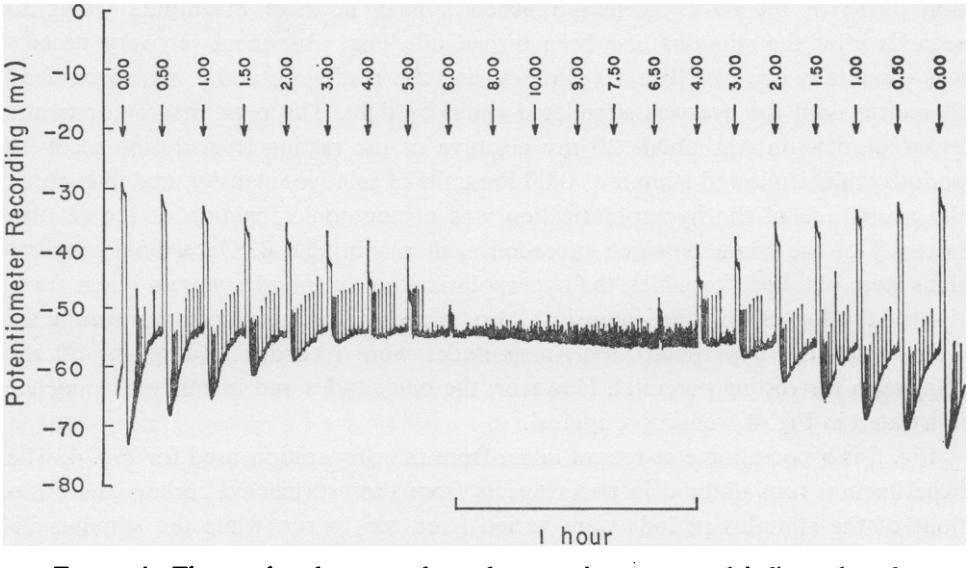

FIGURE 4 The numbered arrows above the potentiometer record indicate the relative intensity in log units of 60 second stimuli which initiated each experimental run. The broadening of the trace between arrows numbered 6.00 and 6.50 was due to an increase in the spontaneous activity which is observed as the eye approaches complete dark adaptation. The sharp positive spikes recorded during the runs were responses to short test flashes of low intensity (see text).

to the steady potentials recorded after the preparation had remained in darkness for 10 minutes or longer. Fig. 4 shows a recording potentiometer trace which was used to monitor the relatively slow changes of the dark potential. These records were obtained by stimulating the eye for 60 seconds; then the preparation remained in darkness for 9 minutes so that the changes in the dark potential could be followed after periods of stimulation. The numbered arrows at the beginning of each experimental run indicate the relative intensity of each 60 second stimulus in log units of attenuation of a constant source intensity (see methods section). The more positive potentials recorded during each period of stimulation were primarily a recording of the second component of the generator potential response, since the potentiometer was too slow to record the initial transient component of the generator potential faithfully. The sharp spikes which were recorded in the 9 minute dark period between stimuli were elicited by short test flashes of low intensity. These low intensity flashes were used to follow sensitivity changes which occurred in the eye during the course of dark adaptation. In previous tests it was demonstrated that the test flashes did not measurably affect the time course of the dark potential for experimental runs initiated by stimuli of relative intensity greater than or equal to 5.00 log units, and no test flashes were delivered in the dark period which followed stimuli of relative intensity less than 5.00 log units.

The resting potential of this preparation was about −55 mv. The hyperpolarization phase of the dark potential reached a peak negative magnitude about 30 seconds after the stimulus had been turned off. The subsequent recovery process was essentially completed in 10 minutes, and the resting potential was maintained thereafter until the eye was stimulated again by light. The peak magnitude of the hyperpolarization was about 20 mv negative of the resting level during recovery periods which followed stimuli of 0.00 log units of relative intensity, and in general, the magnitude of the hyperpolarization was a monotonic function of the relative intensity of the stimulus which preceded each run in Fig. 4. Occasional preparations were obtained for which the hyperpolarization magnitudes were a linear function of the log of stimulus intensity. Also, occasional preparations were obtained from which the hyperpolarization magnitudes were recorded as large as 30 mv relative to the resting potential. However, the magnitudes and intensity dependence illustrated in Fig. 4 were more typical.

Fig. 5 is a potentiometer record taken from the preparation used for Fig. 4. The experimental runs differed in two respects from those discussed earlier: the durations of the stimulus periods were varied from run to run while the stimulus intensity was maintained constant at 0.00 log units and the experimental runs were repeated at 5 minute intervals. The numbered arrows in this figure indicate the duration, in seconds, of the stimulus which preceded each run. The records of Fig. 5 differ in one important respect from those of Fig. 4 in that the dark potential did not recover to the resting level in the shorter 5 minute intervals between runs.

The experimental runs numbered 0.00 in Fig. 4 and the runs numbered 60 in Fig. 5 were initiated by identical stimuli of 60 second duration and 0.00 log units of relative intensity. The magnitudes of the hyperpolarization phase of the dark potential were the same for these 4 particular runs although the level of the dark potential recorded before the start of the runs ranged in value from −55 mv to greater than −65 mv. Under the experimental conditions of Figs. 4 and 5, the

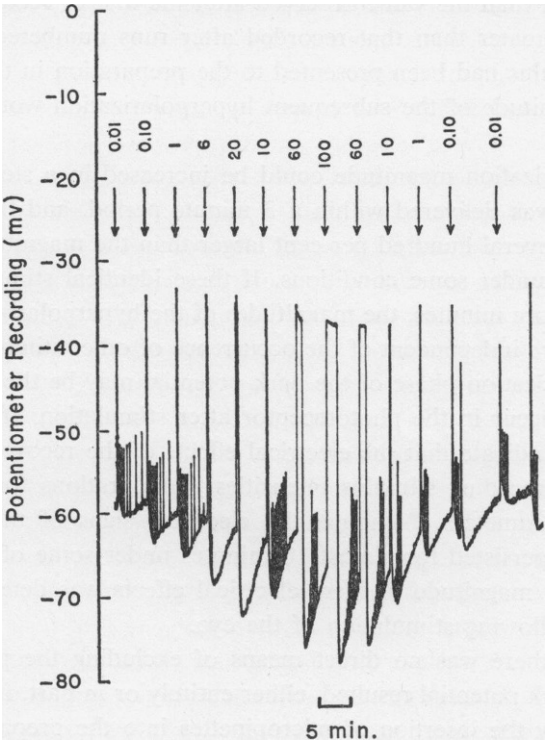

FIGURE 5 The numbered arrows above the potentiometer record indicate the duration, in seconds, of stimuli of 0.00 log units of intensity which initiated each experimental run. The other general features of the record are similar to those described for Fig. 4.

hyperpolarization magnitudes were independent of (*a*) the previous stimulus history of the preparation and (*b*) the level of the dark potential prior to the start of an experimental run. This is contrary to the effect of the dark potential upon the amplitude of the generator potential. Evidence (unpublished) indicates that the amplitude of the initial transient component of the generator potential may be markedly affected by the level of the dark potential at the time of stimulation.

Under some conditions the magnitude of the hyperpolarization may be modified by the history of stimulation of the preparation. The second run numbered 0.10 in fig. 5 shows an example of the way in which stimulus history may affect the

hyperpolarization phase of the dark potential. This particular 5 minute run was initiated by a stimulus of 0.10 second duration and 0.00 log units of relative intensity. The preparation recovered from the stimulus and exhibited a hyperpolarization magnitude which was comparable to that of an earlier run numbered 0.10. However, 60 seconds later a 0.01 second stimulus (not numbered) was presented to the preparation, and the magnitude of the subsequent hyperpolarization was significantly larger than the value observed after the first or second runs numbered 0.10 and much greater than that recorded after runs numbered 0.01. If another 0.01 second stimulus had been presented to the preparation in the next 60 second interval, the magnitude of the subsequent hyperpolarization would have increased further.

The hyperpolarization magnitude could be increased in a stepwise fashion if a series of stimuli was delivered within a 3 minute period, and the final magnitude was as much as several hundred per cent larger than the magnitude observed after a single stimulus under some conditions. If these identical stimuli were separated in time by 3 or more minutes, the magnitudes of the hyperpolarization phase of the dark potential were independent of the occurrence of other stimuli in the sequence.

The hyperpolarization phase of the dark potential may be the result of recovery processes which occur in the photoreceptor after stimulation. If this hypothesis is correct, the data indicate that the electrical effects of the recovery processes are a function of the preceding stimulus intensities and durations for a wide range of these stimulus parameters. Although the electrical signs of the hypothetical recovery processes persisted for almost 10 minutes under some of the stimulus conditions tested, the magnitude of these electrical effects was determined within the first 3 minutes following stimulation of the eye.

Unfortunately there was no direct means of excluding the possibility that the changes in the dark potential resulted, either entirely or in part, from photoreceptor damage caused by the insertion of micropipettes into the preparation. Obviously, unless intracellular recordings can be checked independently by some method which does not require penetration of cell membranes, all intracellular recordings are subject to similar reservations. However, the extreme stability of the preparations indicated that if photoreceptor damage was significant, it was not extensive. The experimental results were consistently reproducible, and no signs of preparation deterioration could be detected in many preparations over a period of 8 to 10 hours. If the penetration of a photoreceptor unit damaged the membranes in such a way as to allow significant ionic leakage, the photoreceptor must have been able to adjust exactly to the leakage (presumably by increasing all ionic pumping rates) in a reproducible manner and to maintain the adjustment over a period of many hours. It is unlikely that the hyperpolarization phase of the dark potential originated from a leakage of potassium ions through a damaged membrane, since Yeandle's data (1957) indicate that this type of potassium leakage should be expected to de-

polarize rather than hyperpolarize the photoreceptor unit. Moreover, it seems unlikely that the hyperpolarizations were generated by leakage of potassium from the micropipette, because the hyperpolarization phase of the dark potential was only recorded after the photoreceptor had been depolarized by light, and the rate of potassium leakage should tend to be reduced rather than increased by a depolarization of the photoreceptor unit.

DISCUSSION

There are important distinctions between the type of electrical activity which occurs in a propagated nerve impulse and the type of electrical activity which gives rise to a graded receptor potential. The nerve impulse is an "all-or-none" response such that the amplitude of the potential difference of the nerve impulse is relatively independent of stimulus intensities and durations which equal or exceed a threshold level. The amplitude of a graded receptor potential, on the other hand, is typically a function of stimulus intensity.

MacNichol (1956) and Fuortes (1958*a*) have shown that the amplitude of the second component of the generator potential response to light in the *Limulus* eye is a linear function of the log of stimulus intensity for a given state of adaptation of the eye. Thus, although the data presented earlier demonstrate that the photoreceptor unit does reverse polarity in response to intense stimuli, it is always possible to reduce the stimulus intensity so that the polarity of the receptor membrane does not reverse during the light response. It is possible to argue that two mechanisms are involved in the receptor processes such that one mechanism operates when a stimulus elicits an electrical response which reverses the polarity of the receptor membrane and another mechanism operates at lesser stimulus intensities. Present data cannot exclude this type of argument. However, for simplicity it will be assumed that the generator potential arises from processes of a single kind and that the magnitude of the effects of these processes changes in a quantitative way as a function of stimulus intensity to produce a graded type of electrical response.

An equivalent electrical circuit of the receptor membrane has been proposed in Fig. 6 on the hypothesis that the generator potential arises from light reactions which decrease the value of resistance R_2. This model is the simplest parallel configuration which is consistent with the data. In order to account for the graded nature of the generator potential response to stimuli of various intensities, the extent to which R_2 is reduced by the light reactions must be a function of stimulus intensity.

Yeandle's data (1957) suggest that the voltage generator E_1 is derived primarily from a concentration gradient of potassium ions across the membrane with the potassium gradient increasing in an inward sense across the receptor membrane to establish the polarity of E_1 as shown. The voltage generator E_2 must be of the polarity indicated in Fig. 6 if this model is to be consistent with the observation that

the polarity of the receptor membrane can reverse during stimulation. Presumably the change in the value of the membrane resistance R_2 would result from specific permeability changes to ionic species 2 such that the membrane would become more permeable to ion 2 when the eye is stimulated by light. Unfortunately, the chemical nature of ion 2 has not been established.

If the leakage resistance across the capacitor C (not shown) is very much greater than R_1R_2/R_1+R_2, the steady-state voltage across the membrane model is defined by the relations:

$$V = (E_1 + E_2)(R_1/R_1 + R_2) - E_1 = E_2 - (E_1 + E_2)(R_2/R_1 + R_2) \quad (1)$$

and the condition for a reversal of receptor polarity during stimulation is:

$$R_2 < (E_2/E_1)R_1 \quad (2)$$

The model predicts that the steady-state membrane voltage should be a linear function of the DC resistance, R_m, of the equivalent circuit for constant values of E_1, E_2, and R_1 since

$$R_m = R_1R_2/R_1 + R_2 \quad (3)$$

and from equations 1 and 3

$$V = E_2 - (E_1 + E_2/R_1)R_m \quad (4)$$

Consequently, a plot of the steady-state membrane potential, V, *versus* R_m should be a straight line with a slope E_1+E_2/R_1 and with an ordinate intercept of E_2. In order to test the validity of both equation 4 and the assumptions under which it was derived, V and R_m should be measured during the second (steady-state) component of the generator potential response. Fuortes (1959) has made these measurements and has shown indirectly that the steady-state amplitude of the second component of the generator potential is a linear function of membrane impedance.

The model of Fig. 6 is consistent with these impedance data as is an alternate

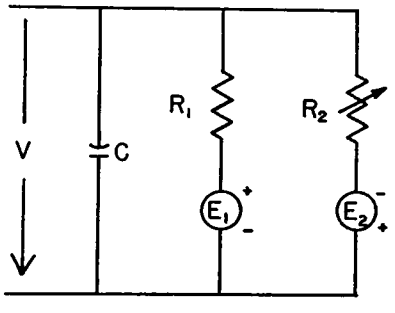

FIGURE 6 Equivalent circuit of photoreceptor membrane. Regions inside the receptor membrane correspond to areas below the figure, and regions outside the membrane correspond to areas above the figure.

model which has been proposed by Fuortes (1959). In the "equivalent circuit of a cell producing a generator potential" proposed by Fuortes, the polarity of E_2 is the same as that of E_1. A reversal of membrane polarity is impossible for the latter model. However, so far as the steady-state component of the generator potential is concerned, either model is adequate since it was not possible to demonstrate a reversal of photoreceptor polarity during the steady-state component of the generator potential. It is obvious that the differences in the models could be reconciled by again postulating two mechanisms for production of the generator potential—one which would operate when the polarity of the membrane reverses and one which would operate when the polarity of the membrane does not reverse during the response period. However, as discussed earlier, the simpler hypothesis which invokes quantitative differences in mechanisms of a single kind is adequate and will be assumed until such time as the experimental data indicate the necessity of further complication. One further remark in this regard involves a consideration of the recording situations under which the two models were proposed. Fuortes (1959) apparently positioned his electrodes in the eye so that impulse activity of reasonably large amplitude could be recorded. As indicated in the Introduction, the present experiments were performed on preparations from which generator potentials of large amplitude could be recorded; impulse activity was of such reduced amplitude in these cases that it could not be detected at the level of amplifier sensitivity used to record the generator potential response (for example, see Fig. 2).

Implicit in the model of Fig. 6 is an increase in ionic pumping rates to restore the concentration gradient of ion 2 during and/or after periods of stimulation. If R_2 is reduced by the light reactions, the component of membrane current contributed by ion 2 would be increased during the response period. Presumably the increase in membrane current would be the result of an increase in the rate of passive transport of ion 2 down its electrochemical potential gradient. In the absence of recovery mechanisms which would pump ion 2 against its electrochemical gradient, the net charge transport associated with the light response would change the concentration gradient of ion 2 across the receptor membrane and hence the value of E_2 would not be maintained.

It is possible to explain the electrical signs of presumed recovery processes that occur in the eye on the basis of ionic pumping mechanisms and the permeability model of Fig. 6. Unfortunately this can be accomplished in a number of equally satisfactory ways, and it is not possible to test the various possibilities with present experimental data. However, the observation that the magnitude of the hyperpolarization phase of the dark potential is a function of the intensity and duration of the preceding stimulus suggests that the recovery processes may be controlled by the net charge transport of ion 2 which results from the light reactions. As the reduction in R_2 (and hence the increase in membrane current) appears to be a function of stimulus intensity, the net charge transport which results from stimulation

should be a function of the product of stimulus intensity and duration on the hypothesis of Fig. 6.

If the magnitude of the electrical effects of the recovery processes were a function of the net charge transport resulting from the light reactions, this magnitude should remain constant for constant products of stimulus intensity and duration under the simplest experimental conditions. These experimental conditions would be fulfilled if (*a*) the stimulus periods were separated in time so that summation of recovery events was negligible and (*b*) the stimulus durations were sufficiently long to permit neglecting the transport which is assumed to be associated with the transient component of the generator potential. Experimental tests of the reciprocity relation for the electrical effects of presumed recovery processes which follow stimuli of constant $I \times t$ product will be discussed at a future date.

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