Light-Induced Changes in Photoreceptor Membrane Resistance and Potential in Gecko Retinas

II. *Preparations with Active Lateral Interactions*

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ABSTRACT The time-course of light-induced changes in membrane voltage and resistance were measured in single photoreceptors in eyecup preparations of *Gekko gekko*. A small circular stimulus directed toward the impaled receptor produced membrane hyperpolarization. Application of a steady annular light to the receptor periphery resulted in diminution of the receptor's response to the stimulus. The effects of illumination of the surrounding receptors were isolated by directing a small, steady desensitizing light to the impaled receptor and then applying a peripheral stimulus. Brief stimuli produced a transient decrease in resistance with rapid onset and offset, a time-course similar to that of the response diminution. For some cells a depolarization that coincided with the resistance decrease was seen. During illumination with prolonged stimuli the resistance decrease was followed by a slow increase. After offset resistance rose transiently above the original value and then returned slowly to its original value. The slow resistance changes were not accompanied by changes in membrane voltage. The response diminution, resistance decrease, and depolarization were not observed in retinas treated with aspartate or hypoxia. It is therefore concluded that these effects are mediated by horizontal cells. The diminution is achieved by shunting the receptor potential and may play a role in field adaptation.

INTRODUCTION

In the eyecup preparation of the turtle retina, certain pairs of cones have been found to be functionally connected. In addition, feedback from horizontal cells to cones has been shown to be present (Baylor et al., 1971). This paper investigates the effects of interactions upon light-evoked changes in membrane resistance and membrane potential in single photoreceptors of the gecko eye-

cup preparation. Particular interest has been directed to interactions that are mediated by horizontal cells, since the identity of the mediating cell can be confirmed by using aspartate treatment (Cervetto and MacNichol, 1972). The interpretation of results in these experiments rests upon a conclusion from the previous paper (Pinto and Pak, 1974), that if changes in membrane voltage and membrane resistance in a single photoreceptor have parallel time-courses, then these changes must be evoked by a light stimulus that does not excite interactions from other receptors.

METHODS

Stimulation, recording, and resistance measurements were performed on *Gekko gekko* as described in the previous paper (Pinto and Pak, 1974). However, maintenance of interactions in the experimental preparation was aided by the following steps. After slicing away a segment of the globe (see Fig. 1, Pinto and Pak, 1974), the segment was mounted, vitreous side up, at an angle of 45° from vertical in a 2.0-ml chamber. Oxygen or a mixture of 95% O_2-5% CO_2, saturated with water, flowed into the chamber through a hole in the side at 200–500 ml/min and left through a 5-mm^2 opening in the top. Both stimulus light and electrode entered through the latter opening.

Aspartate treatments were performed as described by Kleinschmidt (1973). Hypoxia was induced by passing only nitrogen into the chamber.

Electrodes containing methanol were used in a few experiments and were prepared by boiling in methanol and displacing the contents of the stem with 4 M potassium acetate twice, once immediately after boiling and again 5–8 h later. Impalements were made 12–18 h after the second displacement.

RESULTS

The majority of the receptor cells studied were probably those having pigment that absorbs maximally at 518 μm. The results were not due to changes in resistance or potential that occurred extracellularly (Pinto and Pak, 1974).

Comparison between Responses from Eyecup and Preparations Treated to Reduce Interactions from Horizontal Cells

Responses from these two classes of preparations had greatly different waveforms. This is illustrated in Fig. 1 where responses from the normal eyecup preparation and isolated retina are compared. The larger responses obtained from the eyecup preparation all had a transient undershoot followed by a plateau, while all responses from the isolated retina and the smaller responses from the eyecup preparation had only a plateau. For five cells studied in the isolated retina, the response magnitude increased from 5% of its maximal value to 95% when stimulus illuminance was increased 1.75 log units or less. However, for four cells studied in the eyecup preparation, the increase in stimulus illuminance required was more than 2.75 log units. Thus, the receptors operate over a wider range of retinal illuminance in the eyecup preparation than in the isolated retina, as illustrated in Fig. 1 C. Dynamic range was not studied in

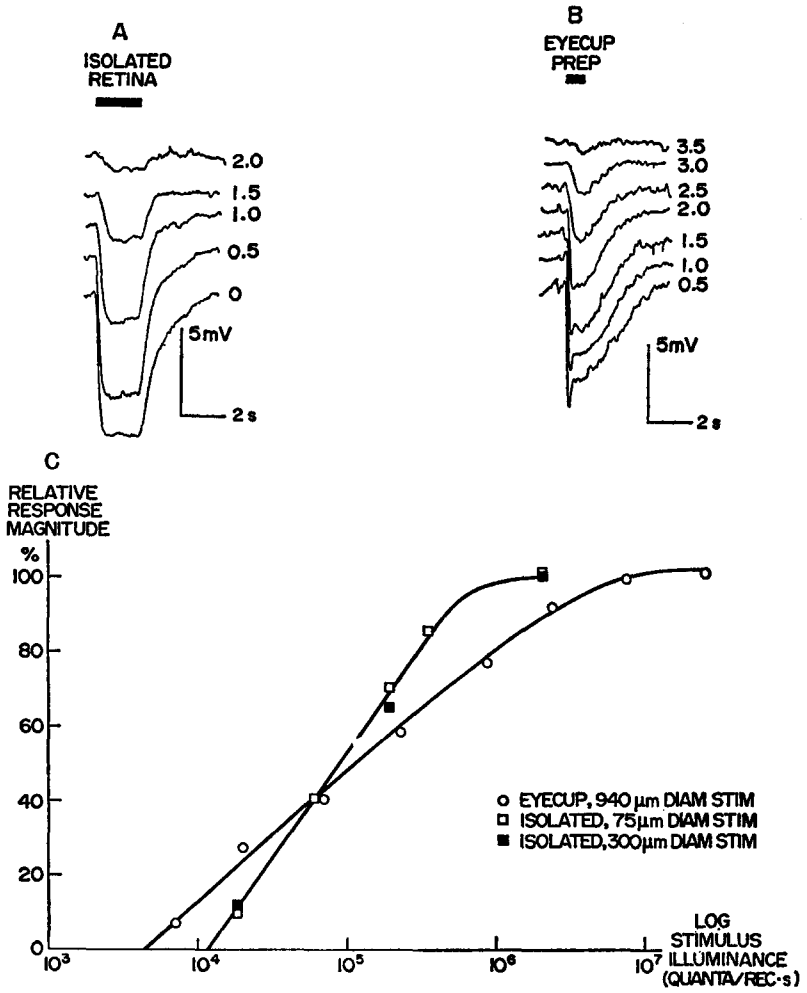

FIGURE 1. Comparison of shape and amplitude of receptor potentials elicited in isolated retina (A) and eyecup preparation (B). Relative response magnitude in plotted against log illuminance for these cells in C. Note the smaller operating range for receptors in the isolated retina. (Isolated retina, 10/22/71 Cell 1; eyecup preparation, 3/15/72 Cell 4.) In both cases the unattenuated stimulus illuminance was 8×10^8 quanta/receptor·s.

retinas treated with aspartate or hypoxia, but waveform in these preparations did not have transient undershoot, in confirmation of the results of Cervetto and MacNichol (1972) and Kleinschmidt (1973) on the aspartate-treated retina.

Waveform of Receptor Response in Eyecup Preparation Depends upon Stimulus Diameter

In their study of receptive fields in the eyecup preparation of the turtle, Baylor et al. (1971) found that the waveform of the receptor response depended upon

the size of the stimulus used. If stimuli of different size were used a constant waveform could not be attained by adjusting stimulus illuminance. The application of a tiny stimulus (10-μm diam or less, ideal size) elicited a response of a given waveform. Increasing the stimulus diameter to 140-μm always increased the size of this response because of the increased flux falling upon the impaled cone. However, when the illuminance of this larger stimulus was decreased until the response had the same peak size as that obtained with a tiny stimulus, the response had a slower onset. A further increase in stimulus diameter (to 1.2 mm) did not cause a greater flux to fall on the impaled receptor, but the trailing edge of the response rapidly diminished after the offset of light. Before studying changes in the waveform of gecko receptor responses, we confirmed that the observations made upon turtle cones could be repeated in our laboratory.

As with turtle cones, responses of gecko receptors displayed two types of waveform changes when studied with stimuli of different diameters. In Fig. 2 responses to tiny (10-μm ideal diam) and large (940-μm diam) stimuli are shown. Stimulus illuminances were adjusted to yield responses of nearly equal plateau size, but only the response to the large stimulus had a clear transient undershoot and a trailing edge that diminished rapidly after offset. In contrast with the turtle retina, stimuli of an intermediate diameter yielded responses of intermediate waveform. Similar changes in waveform due to stimulus size were observed in a total of nine cells. No noticeably different waveforms were seen with circular stimuli of different diameters in retinas that were surgically isolated or treated with aspartate. However, a small waveform difference was observed in one cell in the aspartate-treated retina when its responses to small spots and annular stimuli were compared. This difference was unlike that found in the eyecup preparation (Pinto and Pak, 1974).

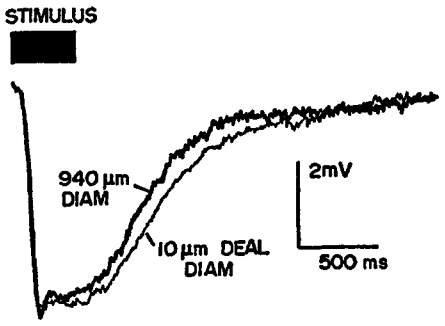

FIGURE 2. Comparison of waveshape of receptor responses elicited by stimuli having 10-μm ideal diam (thin tracing) and 940-μm diam (thick tracing). (Cell 3/23/72 #4; theoretical illuminance for tiny stimulus, 6×10^7 quanta/receptor·s; illuminance for large stimulus, 5×10^5 quanta/receptor·s.)

Diminution of Receptor Response by Steady Peripheral Illumination

In the eyecup preparation the response of a receptor to a stimulus directed toward it was reduced by steady illumination of its receptive field periphery. An example of the results obtained is given in Fig. 3 A. In the absence of the peripheral stimulus, the small (10-μm ideal diam) test stimulus produced a hyperpolarization of nearly 6 mV. When the annular light was turned on, a small hyperpolarization resulted. Scattered light from the annulus was probably responsible for the major portion of this hyperpolarization. In the presence of the continuous annular light, the test stimulus produced an additional

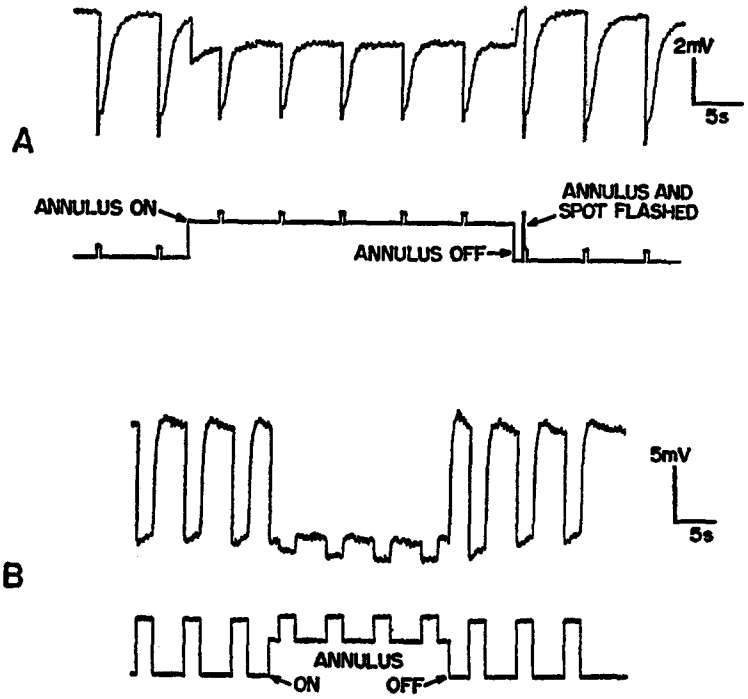

FIGURE 3. (A) Eyecup preparation. Steady peripheral illumination diminished the response of receptor cell to a test stimulus directed toward it. Membrane potential is shown in upper tracing, and stimulus and steady lights are displayed in lower tracing. Small vertical deflection in lower tracing indicates stimulus, and large deflection indicates steady annular light. (B) Aspartate-treated retina. Steady peripheral illumination fails to diminish response to test stimulus. Upper and lower tracings same as in A. Stimulus conditions: (A) 5/2/72 Cell 1. Test stimulus had 10-μm ideal diam and illuminance of 1.1×10^7 quanta/receptor·s. Steady annular light (160-μm ID, 940-μm OD) had illuminance of 2×10^7 quanta/receptor·s. (B) 10/10/73 Cell 2. Test stimulus had 25-μm ideal diameter and illuminance of 1.1×10^8 quanta/receptor·s. Steady annular light (150-μm ID, 1.0-mm OD) had illuminance of 2.8×10^7 quanta/receptor·s.

hyperpolarization. However, the sum of the hyperpolarizations due to the test light and scattered light from the annulus was smaller than that due to the test stimulus alone.

The diminution was tested five times while the annular light was held steadily on; the earliest test was 3 s after onset and the latest was 4 s before offset of the annular light. Diminution was constant over this interval, which spanned 24 s. If the annular light was presented briefly, the diminution of the test response did not occur. Similar results were obtained with four other cells. The least illuminous annular light producing noticeable (20%) diminution cast 2×10^5 quanta/receptor·s upon an area of 7.5×10^5 μm^2. This area contains about 2×10^4 receptors (see Dunn, 1969). Thus, the annulus cast 4×10^9 quanta/s upon the receptors.

To confirm that light scattered onto the impaled receptor did not cause the response diminution in Fig. 3 A two control experiments were performed. The experiment was repeated using seven cells in the aspartate-treated retina and three cells in hypoxic retinas. The results from one cell in the aspartate-treated retina are illustrated in Fig. 3 B. It can be seen that the hyperpolarization during illumination by the test spot alone was less than that which occurred when the test spot was shone in the presence of the annulus. That is, the annulus was ineffective in producing a diminution of the receptor response. No diminution was found in these preparations using any combination of stimulus and annulus of any size or illuminance. The second control experiment was performed in two cells in the eyecup preparation. A stimulus was directed toward the receptor and the response was obtained. Next, a small steady light was also directed toward the receptor. This light elicited a hyperpolarization of about the same size as that due to the annular stimulus in Fig. 3 A. The response to the test stimulus was recorded in the presence of the steady light. If scattered light were the explanation for the effect in Fig. 3 A, it should also have been seen in this situation. However, in contrast to the case of a steady annular light, the magnitude of the response elicited by the test stimulus was not measurably smaller in the presence of the small steady light. When this experiment was repeated with small but very illuminous steady lights, producing near maximal hyperpolarization of the receptor, the response to the test stimulus was diminished. Even in this case, the sum of the responses due to the test and steady lights was often larger than that due to the test stimulus alone. Lights of this great illuminance were used to selectively desensitize receptors in other experiments.

Isolation of the Resistance Changes Due to Light Falling Remote from the Impaled Receptor

When an impaled receptor was selectively desensitized, it became possible to measure changes in its membrane resistance due to illumination of receptors

in its periphery. To do this, a tiny (10-μm ideal diam) steady light was aimed at the receptor. It had to be made extremely bright in order to desensitize the receptor adequately. (Theoretical illuminance was at least 5×10^7 quanta/receptor·s, but actual illuminance was less because of image spread.) After this treatment peripheral illumination was applied.

In all 17 cells studied, selective desensitization abolished the initial resistance increase which usually occurs upon stimulation. The resistance changes that did occur were strongly dependent upon the duration of peripheral illumination. This is illustrated in Fig. 4 A and B for one of eight cells studied with more than one duration of peripheral illumination. Fig. 4 A shows resistance changes due to a brief stimulus (1.7 s). Before this record was begun a desensitizing light (10-μm ideal diam) was applied to the impaled cell and remained on throughout the experiments to be described. This light caused a steady, increased membrane resistance. The fluctuations shown in Fig. 4 A occurred about this new steady value and were induced by a brief large diameter stimulus. At onset, resistance decreased rapidly to a minimal value, after a short delay. This decrease went to completion only for stimuli longer than about 2.0 s. Resistance then returned to nearly the prestimulus value, sometimes with a small overshoot. When the stimulus was prolonged, as in Fig. 4 B (12 s), after the initial decrease, resistance slowly rose to a new steady value during illumination. This slow rise reached the steady state only during very long stimuli. At offset there was a delay of almost ½ s, after which resistance rose rapidly to a value higher than the original value and then slowly fell to its original level. The transient overshoot in resistance at the offset was larger for stimuli of long duration than for stimuli of short duration.

The changes in membrane potential that accompanied the changes in membrane resistance are illustrated in Fig. 4 C and D for two cells. In Fig. 4 C are shown the changes that were often elicited with brief peripheral stimuli. Before the records in Fig. 4 C were begun, the small desensitizing light was applied to the cell. This light caused a slight steady hyperpolarization and increase in membrane resistance. The changes in Fig. 4 C were measured about these steadily altered values. The large diameter stimulus fell upon both the impaled receptor and peripheral receptors and was applied while the steady light was held on. The stimulus produced a small hyperpolarization (Fig. 4 C). On the other hand, membrane resistance decreased at onset after a short delay, reached its minimal value soon after stimulus offset, and then returned to its original value in less than 2 s. No resistance increase was observed, but this does not necessarily mean that the stimulus light falling upon the impaled receptor had no effect upon its membrane resistance. For example, an increase in resistance may have reduced the magnitude of the resistance decrease. Five cells that were studied with selective desensitization yielded resistance records with only a single decrease. For three of these cells,

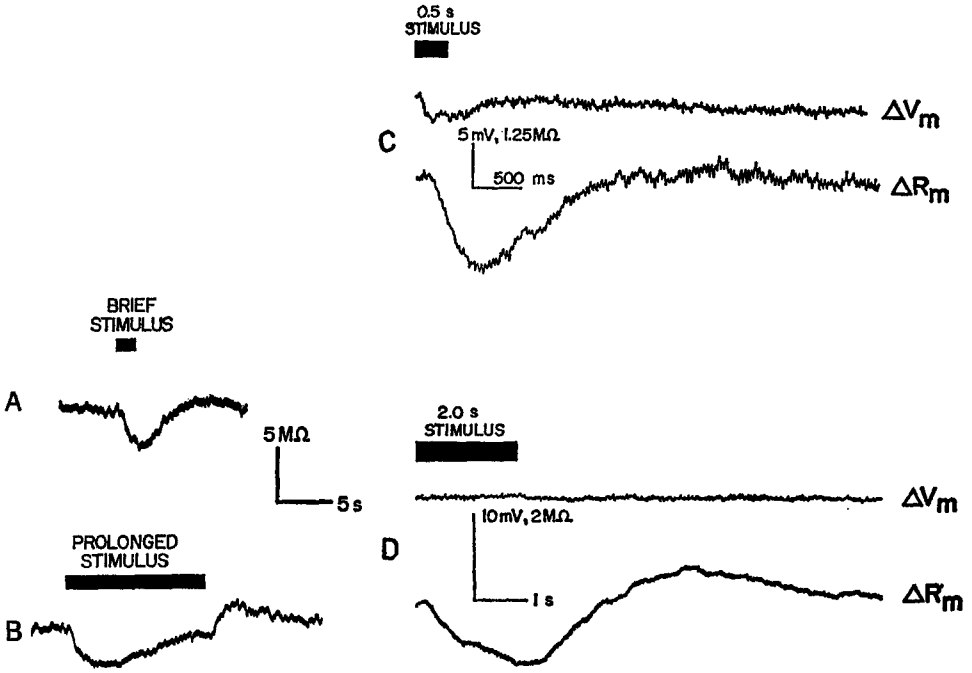

FIGURE 4. (A and B) Change in membrane resistance of receptor cells which were selectively desensitized by tiny (10-μm diam) steady lights directed toward them (not shown). The gradual resistance increase during stimulus and the transient overshoot of resistance after stimulus offset were more pronounced for stimuli of long duration. Stimulus conditions: (A and B) 5/11/72 Cell 1. Desensitizing light had ideal size of 10-μm diam, and theoretical illuminance was 3×10^7 quanta/receptor·s. Circular stimulus (940-μm diam) had illuminance of 5×10^5 quanta/receptor·s. (C and D) Changes in membrane resistance and potential in receptor cells which were desensitized by tiny steady lights. (C) Large circular (940-μm diam) stimulus of short duration caused a decrease in membrane resistance. The small hyperpolarization shown in the upper trace was probably due to stimulus light falling upon this incompletely desensitized receptor. (D) Annular (50-μm ID, 940-μm OD) stimulus of long duration caused resistance decrease followed by transient resistance increase. Stimulus conditions: (C) 3/23/72 Cell 2. Desensitizing light had ideal size of 10-μm diam, and theoretical illuminance was 9×10^7 quanta/receptor·s. The stimulus light had 940-μm diam and illuminance of 6×10^5 quanta/receptor·s. (D) 5/11/72 Cell 3. Desensitizing light had ideal size of 10-μm and theoretical illuminance was 3×10^7 quanta/receptor·s. Annular stimulus (50-μm ID, 940-μm OD) had illuminance of 5×10^6 quanta/receptor·s.

stimuli of 400-ms duration were used; an annular stimulus was used for one of these cells and circular stimuli for the other two cells. For the remaining two cells, stimuli of 2.0-s duration were used; the stimulus was annular for one cell and circular for the other cell.

The changes in membrane potential and resistance that were often elicited

by prolonged peripheral stimuli are illustrated in Fig. 4 D. In this case an annular stimulus was used and it produced no detectable change in membrane potential. However, membrane resistance decreased at stimulus onset after a short delay. At offset there followed a transient increase to a value higher than the original resistance (Fig. 4 D). The maximal resistance occurred 2–3 s after stimulus offset, and resistance returned to its original value 4–5 s after stimulus offset. For seven cells giving resistance changes similar to that shown in Fig. 4 D, stimuli of 2.0-s duration were used. The stimulus was annular for two of these cells and circular for the other five cells. For the remaining two cells, stimuli of 400-ms duration were used; again, the stimulus was annular for one cell and circular for the other cell.

When the same experiments were performed with five cells in aspartate-treated retinas and six cells in hypoxic retinas, the only effects that could be detected upon peripheral illumination were those expected from light scattered onto the impaled receptor: small hyperpolarization and slightly increased membrane resistance during illumination.

The receptor potential was suppressed for four cells by impaling them with electrodes that contained methanol (see Methods). No desensitizing light was used in these experiments, and the stimulus was a large (1.0-mm diam) spot of 2.0-s duration. The changes in membrane potential resembled that in Fig. 4 C. However, the single resistance decrease that occurred was more prolonged than that in 4 C. During illumination a small hyperpolarization, but no resistance increase, was seen. Starting about ½ s after onset there began a resistance decrease that reached its peak value (about -0.5 MΩ) about 2.5 s after onset and decayed to zero in 8–10 s.

Resistance Decrease Depends upon Peripheral Stimulus Illuminance and Distribution

The magnitude of the decrease in resistance due to peripheral stimulation was studied as a function of illuminance of the stimulus. This was done by applying a steady light to the cell under study and then applying brief peripheral stimuli which evoked only decreases in resistance. The magnitude of the resistance decrease is plotted against log illuminance in Fig. 5 A. The lowest stimulus illuminance which produced a detectable (500 kΩ) resistance decrease cast 2×10^3 quanta/receptor·s upon a retinal area of 7×10^5 μm^2, or about 4×10^7 quanta/s incident upon a total of 4×10^4 receptors. Fig. 5 B gives the plot of magnitude of resistance decrease (similarly isolated) against stimulus diameter. It can be seen that for a stimulus of constant illuminance, the magnitude of the resistance decrease was greatest when using large stimuli. Results from two cells showed that stimuli falling as far as 500 μm from the impaled receptor elicited resistance decreases from the receptor.

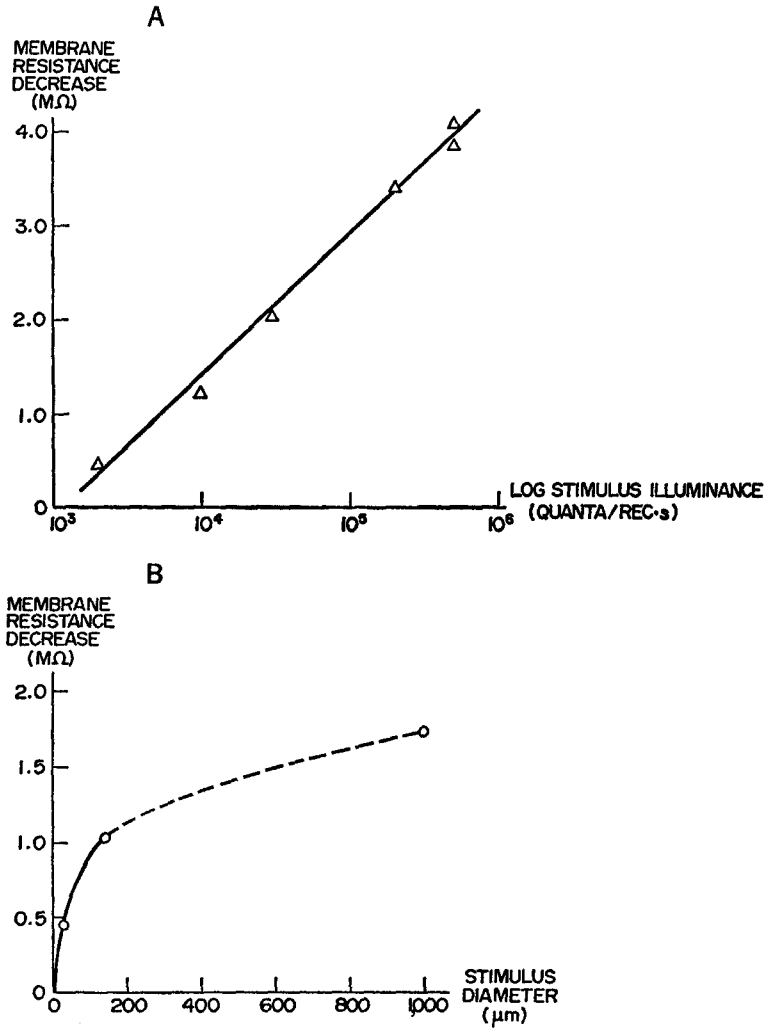

FIGURE 5. (A) Magnitude of decrease in membrane resistance elicited by peripheral stimulus plotted against stimulus illuminance. (B) Magnitude of decrease in membrane resistance elicited by brief peripheral stimulus plotted against diameter of stimulus. Stimulus conditions: (A) 5/11/72 Cell 2. Desensitizing light had ideal size of 10-μm diam, theoretical illuminance of 3×10^7 quanta/receptor·s, and was held on steadily. Circular stimulus was 940-μm in diameter and 0.4 s in duration. (B) 3/24/72 Cell 3. Desensitizing light had ideal size of 10-μm diam, and theoretical illuminance of 3×10^7 quanta/receptor·s. Circular stimulus had illuminance of 6×10^5 quanta/receptor·s and duration 0.4 s.

Isolation of Peripheral Illumination Effect on Membrane Potential of Impaled Receptor

The effect of peripheral illumination on membrane potential was studied in 15 cells. In six of these cells, the waveform of the potential was different from that

expected if the potential were due only to scattering of light onto the impaled receptor. An example of this is given in Fig. 6. An annular stimulus, centered upon the impaled receptor, produced the response shown in A. This response consists of a hyperpolarization and is what one would expect from light scattered from the annulus onto the receptor. When the effect of scattered light was minimized (Fig. 6 B) by application of a steady desensitizing light to the receptor, the annulus elicited a short-lived hyperpolarization followed by a small depolarization. The hyperpolarization can be explained as the result of light scattered from the annulus onto the receptor, but the depolarization cannot be similarly explained. The depolarization produced under these conditions always occured at approximately the time membrane resistance, measured under similar conditions, was minimal (see Fig. 4 C, D).

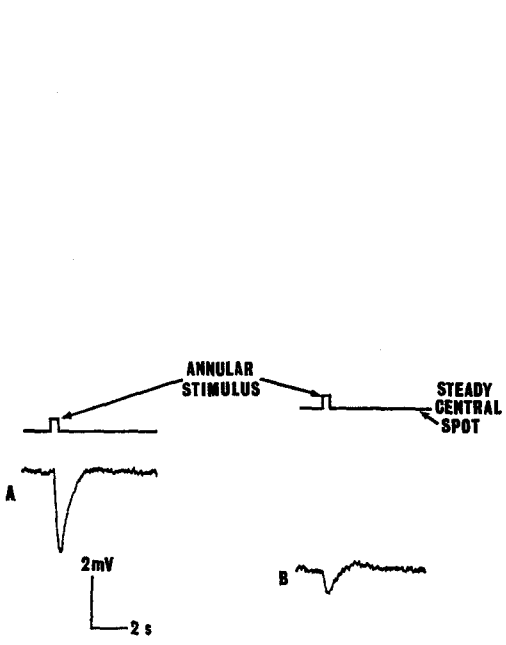

FIGURE 6

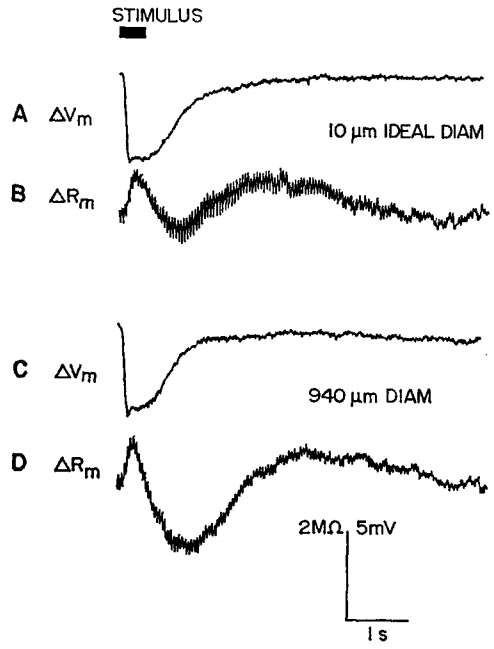

FIGURE 7

FIGURE 6. Effect of peripheral illumination upon membrane potential of receptor cell. (A) Response elicited by annulus centered upon impaled receptor. (B) Response to same annulus in presence of steady desensitizing light. Note that application of desensitizing light changed the character of the response elicited by the annulus. Stimulus conditions: 5/4/72 Cell 3. (A) Annular stimulus (50-μm ID, 940-μm OD) had illuminance of 3×10^5 quanta/receptor·s. (B) Steady desensitizing light of 10-μm ideal diam had theoretical illuminance of 3×10^7 quanta/receptor·s. Annular stimulus same as in A.

FIGURE 7. (A and B) Changes in membrane voltage (A) and membrane resistance (B) elicited by stimulus of 10-μm ideal diam. (C and D) Changes in membrane voltage (C) and membrane resistance (D) elicited by stimulus of 940-μm diam. Responses from this cell have been compared in Fig. 2.

Resistance Changes Elicited by Circular Stimuli in the Eyecup Preparation

Changes in membrane resistance were compared with changes in membrane voltage in a total of 11 cells by studying with circular stimuli. Four of these cells were studied using stimuli of both small and large diameters (10-μm ideal diam and 940-μm diam), and the remaining seven cells were studied using only the small stimulus. These 11 cells can be grouped into two classes according to the kind of resistance change obtained.

The first class consisted of two of the four cells studied with both small and large stimuli. They displayed both a transient increase and a decrease in resistance. The second class included the remaining nine cells. These displayed only a transient increase in resistance.

Light-evoked changes in membrane voltage and resistance obtained from the first class of cells are illustrated in Fig. 7. The illuminances of the small and large stimuli were adjusted to yield receptor responses with nearly the same plateau size (Fig. 7 A, C). These responses had slightly different waveforms (see comparison in Fig. 2). The time-course of the resistance change accompanying each response was more complicated than would be expected if only one conductance change were causing the response (see Eq 7 of Pinto and Pak, 1974). For the cell type shown in Fig. 7, both small and large stimuli evoked a transient increase in resistance, diminishing before stimulus offset (Fig. 7 B and D). After stimulus offset, resistance temporarily decreased to a value lower than that found in darkness. The magnitude of this resistance decrease was greater when the large stimulus was used (Fig. 7 D). A few seconds after stimulus offset, a second transient increase in resistance occurred. This had slower onset and offset than the first transient increase.

The remaining nine cells, including the two other cells studied with both small and large diameter stimuli, belong to the second class of cells. In the two cells, the receptor responses had waveforms similar to those shown in Fig. 7 A and C, but the resistance changes were very different from those shown in Fig. 7 B and D. The small stimulus evoked a single, transient increase in resistance. This increase began 20–40 ms after the response onset and lasted 1.5–2.0 s when evoked by stimuli of 500-ms duration. The resistance waveform was not a "mirror image" of its partner receptor response. The large stimulus (940-μm diam) also evoked only one transient increase in resistance. The magnitude of this increase was smaller than that elicited by the tiny stimulus. It began 0.5–1.0 s after stimulus onset, was maximal 1.0–1.5 s later, and lasted 2–3 s. The seven cells studied using only the small stimulus all responded in a manner similar to the cells just described.

DISCUSSION

Response Waveform in Isolated Retina and Eyecup Preparation

The responses of gecko photoreceptors, recorded in the eyecup preparation (see Fig. 2), have waveforms similar to those of turtle cones recorded in the eyecup preparation (Baylor et al., 1971). Application of aspartate or glutamate to the vitreous side of the turtle retina (Cervetto and MacNichol, 1972) causes sustained depolarization of horizontal cells, and the cone responses lose their transient undershoot. These results have been interpreted to mean that the waveform of the cone response, under normal conditions, is in part due to delayed feedback from horizontal cells, and that the feedback is interrupted by aspartate or glutamate (Cervetto and MacNichol, 1972). Similar results for the responses of gecko photoreceptors have been reported by Kleinschmidt (1972). After isolation of the retina, the responses of gecko photoreceptors also lose their transient undershoot. Resistance measurements indicate that interactions mediated by horizontal cells are not active in the isolated gecko retina (Pinto and Pak, 1974). These results suggest that the change in the waveform seen after isolation of the gecko retina is also due to interruption of signals necessary for delayed feedback from horizontal cells.

Effects of Peripheral Illumination

In the previous paper (Pinto and Pak, 1974) we gave evidence that the changes in membrane resistance and voltage had parallel time-courses only under conditions in which the receptor potential was uncontaminated by the feedback signals. Using this criterion, "pure" receptor potentials were never elicited in the eyecup preparation. The changes in membrane resistance that result from peripheral illumination were isolated by selectively desensitizing the impaled receptor (Fig. 4). For peripheral stimuli of short duration, the resistance decreased at onset and returned to the original value at offset. However, the resistance changes resulting from prolonged stimuli were more complicated. After the initial resistance decrease at onset there followed a slow rise to a new steady value during illumination. After offset, resistance rose transiently to a value higher than original. As a working hypothesis, we propose that the resistance change consists of two processes, as illustrated in Fig. 8. The first is a resistance-decreasing process (Fig. 8 B), responsible for the rapid decrease in resistance at onset and rapid increase in resistance at offset (RD and RI in Fig. 8 A). The second, or resistance-increasing, process (Fig. 8 C) reaches a steady state only during exposure to stimuli of prolonged duration. It is responsible for the gradual increase (GI) during illumination and gradual decrease (GD) in resistance after offset seen in Fig. 8 A.

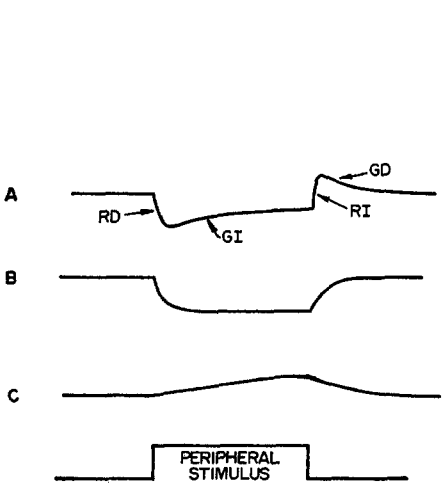

FIGURE 8

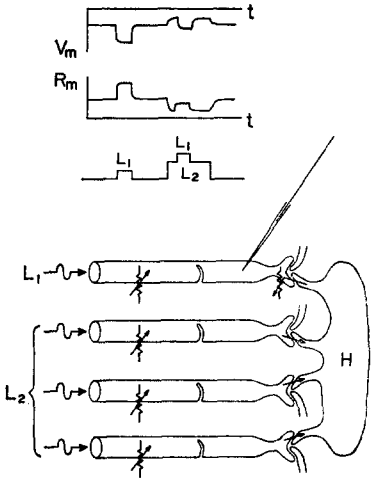

FIGURE 9

FIGURE 8. Working hypothesis for the change in receptor membrane resistance that occurs as a result of peripheral illumination. (A) Resistance undergoes rapid decrease (RD) at onset, rapid increase (RI) at offset, gradual increase (GI) during, and gradual decrease (GD) after offset of peripheral illumination. (B) Resistance change which results from the resistance-decreasing process thought to cause the rapid decrease (RD) and rapid increase (RI) in A. (C) Resistance change which results from the slower resistance increasing process thought to cause the gradual increase (GI) and gradual decrease (GD) in A.

FIGURE 9. Summary of findings in the present study. Receptors generate the receptor potential by decreased conductance involving a single ionic process. Thus, changes in V_m and R_m of the top receptor are proportional when elicited by a light that stimulates only this receptor (L_1). The top receptor is shown to receive signals from only the horizontal cell illustrated. These signals are evoked by light that stimulates only the lower three receptors (L_2) and cause the conductance of the membrane of the top receptor to increase. The conductance increase is for an ion(s) having equilibrium potential more positive than the dark potential. Thus L_2 elicits both a resistance decrease and depolarization from the top receptor. The conductance increase shunts the membrane of this receptor and results in a diminution of its response to L_1. The resistance-increasing process (Fig. 8) is ignored in this diagram.

It is important to note that the response diminution due to steady peripheral illumination (Fig. 3) had a time-course similar to the resistance-decreasing process (Fig. 8 B). The diminution was not effective for peripheral lights of short duration, but once effective, it was constant as long as the peripheral light was held on. Moreover, the diminution disappeared shortly after offset of peripheral illumination. In light of the similarity of the time-courses, the process causing the resistance decrease (Fig. 8 B) may be responsible for the response diminution. It has also been shown that the depolarization caused by peripheral illumination (Fig. 6) occurs at approximately the time when membrane resistance, measured under similar conditions (Fig. 4), is minimal. Thus

the resistance-decreasing process (Fig. 8 B) may also be responsible for the depolarization. Perhaps the resistance decreasing process is due to an increase in membrane conductance for an ion(s) having equilibrium potential more positive than the potential in complete darkness.

O'Bryan (1973) also found that onset of illumination of the far periphery of turtle cones brought about a decrease and an increase in cone membrane conductance. He interpreted these conductance changes to be mediated by horizontal cells. However, for turtle cones the conductance decrease occurred first.

Resistance-Increasing Process

In the experiments involving response diminution due to illumination of the periphery (Fig. 3 A), the peripheral light was always held on long enough to cause the gradual increase in resistance during illumination and the transient resistance overshoot after offset. However, the time-course of these resistance changes (Fig. 4 B) did not run parallel with changes in the response diminution (Fig. 3). Thus, the resistance-increasing process (Fig. 8 C) seemed to have little effect upon response diminution. In addition the depolarization caused by peripheral illumination (Fig. 6) preceded the resistance-increasing process (Fig. 8 C), indicating that the resistance-increasing process could not be responsible for the depolarization. What then is the origin of the resistance-increasing process? No resistance changes could be detected when the electrode was located outside the cell. It is therefore unlikely that extracellular factors, such as migration of granules in pigment epithelial cells (see Ali, 1971), could explain the resistance increase recorded intracellularly. The increased resistance could be detected by measuring the component of transmembrane potential that was out of phase with the measuring current. This implies that the element that increased resistance was shunted by a capacitance (Pinto and Pak, 1974). The value of the capacitance had to be such that the time constant of the element was nearly the same as the time constant of the plasma membrane. It is unlikely that this element was the disk membrane, since its conductance increases upon illumination (Falk and Fatt, 1973). It also seems unlikely that the resistance increase was caused by photomechanical movements of the receptor cell, since the cell was steadily illuminated by the desensitizing light and peripheral stimuli were not likely to add greatly to the flux falling upon the cell. We are not certain of the origin of the resistance-increasing process. We do not understand how it can affect membrane resistance but not membrane potential or responsiveness, and we cannot attribute the process to a structure other than the plasma membrane.

Mechanism for Interactions

As a result of illumination of the periphery of a gecko photoreceptor, the following effects, all thought to be caused by the resistance-decreasing process

(Fig. 8 B), were observed: (*a*) membrane resistance decreased, (*b*) receptor response to light was diminished, and (*c*) membrane became depolarized. These effects were not seen in retinas treated with aspartate or hypoxia. This suggests that the effects were mediated by horizontal cells. An experiment of Baylor et al. (1971) supports this suggestion. Using two different electrodes, they simultaneously impaled a horizontal cell and a cone in the turtle retina. Next, the cone's response to a stimulus directed toward it was obtained. Hyperpolarizing current was then applied to the horizontal cell, causing a depolarization of the cone in the dark. While the current was being applied, the response of the cone to the same light stimulus was again obtained. It was smaller than the original response. It was suggested that this response diminution might have been due to increased conductance of the cone membrane as a result of feedback from the horizontal cell.

Our results may be explained as follows (see Fig. 9). The impaled receptor transmits signals to horizontal cells and receives signals from horizontal cells (probably not the same horizontal cells, see Kaneko, 1971). With no horizontal cell signal, the receptor hyperpolarization results exclusively from decreased sodium conductance due to light captured by the impaled receptor. However, with horizontal cell signals there is an increase in conductance for some ion or ions having an equilibrium potential more positive than the resting potential. This increase in conductance attenuates the receptor potential for two reasons. First, the increased conductance (decreased resistance) will tend to shunt any light-induced current resulting from decreased sodium conductance. Second, the equilibrium potential associated with the conductance increase is more positive than resting potential.

In light of the above events, the following scheme may be proposed for the depolarization (Fig. 6) and response diminution (Fig. 3 A) due to peripheral illumination. When a stimulus is applied in the periphery of an impaled receptor (selectively desensitized to minimize the effects of scattered light), the peripheral receptors will cause hyperpolarization of horizontal cells. Some of the horizontal cells are presumably able to send signals to the impaled receptor. Therefore, after a transmission delay, the impaled receptor receives signals from the mediating horizontal cell(s). These signals cause an increase in conductance (decreased resistance) of the receptor membrane. The conductance increase then causes a depolarization of the receptor membrane, if the effects of scattered light have been sufficiently reduced by selective desensitization. The same scheme may be used to explain diminution of the receptor response due to steady peripheral illumination. A peripheral light, held on steadily, will diminish the response of the receptor to a light directed toward it. The diminution arises from the maintained conductance increase (decreased resistance) of the receptor membrane, caused by horizontal cell signals.

Function of the Observed Interactions

The most pronounced effect observed in the present study was a decrease in the amplitude of the receptor's response as a result of steady illumination of the region surrounding the receptor. We now consider the role of this effect in light adaptation and in the information processing that generates the responses of retinal ganglion cells.

Adaptation is a light-induced change in the sensitivity of some retinal element(s). Three types of light adaptation have been distinguished electrophysiologically. These are bleaching adaptation, "neural" or fast adaptation, and field adaptation. Under many conditions these three types of adaptation occur simultaneously, but each has distinguishing photochemical, temporal, and spatial features.

Bleaching adaptation is caused by exposure to an adapting light bright enough to bleach a substantial number of visual pigment molecules, and it is characterized by a slow recovery (Rushton, 1965 *a*; Dowling, 1967). Bleaching shifts the operating range of photoreceptors (Grabowski et al., 1972). But the interactions we observed could be evoked by lights far too dim to bleach a significant amount of pigment, and they were rapid in onset and offset. It is therefore unlikely that they have mechanism(s) in common with bleaching adaptation.

Neural or fast adaptation has a more rapid time-course than bleaching adaptation, taking at most a few minutes to reach completion (Dowling, 1967), and is unrelated to the time-course of pigment regeneration (Dowling, 1967). In addition, neural adaptation can be observed with adapting lights too dim to bleach a significant amount of visual pigment (Weinstein et al., 1967). Although it was originally thought that the rapid recovery of sensitivity was due to alteration of synaptic input to proximal retinal neurons (Dowling, 1967), recent work has shown that this adaptation can also be observed in single photoreceptor cells (Grabowski et al., 1972) and with receptor potentials in the aspartate-treated retina (Dowling and Ripps, 1971, 1972; Ernst and Kemp, 1972; Hood and Mansfield, 1972). These observations have led to the conclusion that such adaptation occurs in individual receptors. Since the diminution we observed was not seen in functionally isolated photoreceptors, it probably is unrelated to neural or fast adaptation.

Field adaptation is a decrease in sensitivity that occurs as a result of the presence of a shower of quanta from an adapting field (Rushton, 1965 *a*, *b*; Easter, 1968; Cleland and Enroth-Cugell, 1968). The adapting field can be effective even if it does not fall directly upon the retinal element under study, and it need not be illuminous enough to bleach a significant amount of visual pigment. The onset of field adaptation is very rapid (less than 1 s), and recovery after removal of the adapting field is also very rapid. The decrease in

sensitivity in electrophysiological studies is unrelated to quantal fluctuations in light (Barlow and Levick, 1969; Enroth-Cugell and Shapley, 1973), although quantal fluctuations may affect detectability in psychophysical studies that use adapting fields (Rose, 1948). Sensitivity measured on one part of the retina may be decreased by presenting an adapting light in a remote region of the retina (Rushton, 1965 *a* and *b*; Cleland and Enroth-Cugell, 1968).

The receptor interactions noted in this study do involve the rapid diminution of the receptor response to a test stimulus in one part of the retina as a result of application of a steady adapting field in another part. Thus, they may play a role in field adaptation. In fact, receptors in the gecko eyecup preparation, in which lateral interactions were active, operated over a 2.75-log unit range of illuminance. Receptors in the isolated retina, in which lateral interactions were minimal, operated over a range of only 1.75 log units. Such an increase in operating range in the eyecup preparation would be expected from field adaptation and could, therefore, be due to the observed interactions.

The least intense adapting field which could reduce the amplitude of the receptor response by 20% cast 4×10^9 quanta/s upon a retinal area of 0.79 mm^2. Granted that the adapting flux may have been ineffectively distributed, this value is still high compared with the 10^4 quanta/s flux that must fall upon the central response mechanism of cat retinal ganglion cells (covering retinal areas as large as 1.25 mm^2) in order to reduce ganglion cell sensitivity to half the dark-adapted (highest possible) value (Enroth-Cugell and Shapley, 1973). The receptor interactions we have described probably play an important role in field adaptation. However, the interactions may be much stronger in an intact animal and they may not be the sole determining factor for field adaptation.

Lateral interactions have not been studied extensively in the reptilian retina. Therefore, we do not know whether the interactions we observed can partly account for complex information processing such as that inherent in the responses of retinal ganglion cells in the retina of the frog (Hartline, 1938; Barlow, 1953) and goldfish (Wagner et al., 1960). However, we do not believe that the interactions we observed can serve as the direct basis for center-surround interaction of the mammalian retinal ganglion cells (Kuffler, 1952). It has been shown that a light applied to the receptive field periphery does not decrease the sensitivity of the center mechanism (Cleland and Enroth-Cugell, 1968), and that signals from the center mechanism do not substantially alter the properties of the surround mechanism (Enroth-Cugell and Pinto, 1972). This is in contrast to the interactions we observed, in which the response of a receptor was diminished by a steady light falling on the region surrounding it.

Dependence of Waveform upon Stimulus Diameter

We observed differences between the time-courses of the receptor responses elicited by tiny (10-μm diam) and large (940-μm diam) stimuli in the eyecup

preparation. For the larger stimulus, the transient hyperpolarization (undershoot) was more pronounced, and the trailing edge of the response diminished more rapidly. The same differences in waveform were also noted with stimuli of intermediate size, but they were not as large. However, this type of waveform difference was not seen in the aspartate-treated retina, suggesting that horizontal cells mediate the interaction that causes the difference. This is a simpler situation than that observed in the turtle retina, where changes of response waveform occur in definite stages in three ranges of stimulus size (Baylor et al., 1971). The difference in waveform observed for the responses of gecko receptors studied with tiny (10-μm diam) vs. large (940-μm diam) stimuli was similar to the difference in waveform for the responses of turtle cones studied with intermediate sized (140-μm diam) vs. large (1.2-mm diam) stimuli (Baylor et al., 1971). These differences were explained on the basis of delayed feedback from horizontal cells in the turtle retina (Baylor et al., 1971). Nonlinear feedback from horizontal cells to receptor cells has also been advanced to explain several aspects of the response of cat retinal ganglion cells (Enroth-Cugell and Shapley, 1973). The same explanation also probably applies to the waveform difference in gecko receptors (see Fig. 2). Evidence for direct receptor-to-receptor interactions has been found in the turtle retina (Baylor et al., 1971). The abundance of double and triple receptors in the retina of *Gekko gekko* (Dunn, 1969) makes it likely that such interactions exist. In fact the small waveform differences (of a different type than those discussed above) that were found in the aspartate-treated retina may have been due to such interactions (Pinto and Pak, 1974).

Dissimilar Time-Courses with Circular Stimuli

Parallel time-courses between changes in membrane resistance and potential were never obtained in the eyecup preparation. Using circular stimuli two classes of cells were distinguished: those for which a transient increase and decrease in membrane resistance were elicited, and those for which only a transient increase in resistance was obtained. We suggest that these classes of cells differ only in that peripheral interactions were stronger in the first class. Because of the complexity of the interactions and their many unknown features, it was not possible to predict the time-course of the response from the time-course of the change in membrane resistance. At the onset of all responses, change in membrane resistance lagged behind change in membrane voltage. This was observed before the resistance-decreasing process (thought to be mediated by horizontal cells) became active. A similar lag was observed in retinas that were treated chemically to reduce interactions mediated by horizontal cells (Pinto and Pak, 1974), but this lag may have been due, in part, to direct effects of the chemicals upon the receptor cell. We might speculate that the lag seen in the eyecup preparation is due to direct receptor-to-receptor interactions that have very short latency.

We thank Dr. P. M. O'Bryan for teaching us the proper execution of the eyecup technique and Drs. Christina Enroth-Cugell, W. K. Ernst, P. Lennie, and R. M. Shapley for their critical reading of the manuscript.

Supported by N.I.H. grant EY 00033.

Received for publication 13 July 1973.

REFERENCES

ALI, M. A. 1971. Les responses retinomotrices: caracteres et mecanismes. *Vis. Res.* **11**:1225.

BARLOW, H. B. 1953. Summation and inhibition in the frog's retina. *J. Physiol. (Lond.)*. **119**:69.

BARLOW, H. B., and W. LEVICK. 1969. Three factors limiting the reliable detection of light by retinal ganglion cells of the cat. *J. Physiol. (Lond.)*. **200**:1.

BAYLOR, D. A., M. G. F. FUORTES, and P. M. O'BRYAN. 1971. Receptive fields of cones in the retina of the turtle. *J. Physiol. (Lond.)*. **214**:265.

CLELAND, B. G., and C. ENROTH-CUGELL. 1968. Quantitative aspects of sensitivity and summation in the cat retina. *J. Physiol. (Lond.)*. **198**:17.

CERVETTO, L., and E. F. MACNICHOL JR. 1972. Inactivation of horizontai cells in turtle retina by glutamate and aspartate. *Science (Wash. D. C.)*. **178**:767.

DOWLING, J. E. 1967. The site of visual adaptation. *Science (Wash. D. C.)*. **155**:273.

DOWLING, J. E., and H. RIPPS. 1971. Aspartate isolation of receptor potentials in the skate retina. *Biol. Bull. (Woods Hole)*. **141**:384.

DOWLING, J. E., and H. RIPPS. 1972. Adaptation in skate photoreceptors. *J. Gen. Physiol.* **60**: 698.

DUNN, R. F. 1969. The dimensions of rod outer segments related to light absorption in the gecko retina. *Vis. Res.* **9**:603.

EASTER, JR. S. S. 1968. Adaptation in the goldfish retina. *J. Physiol. (Lond.)*. **195**:273–281.

ENROTH-CUGELL, C., and L. H. PINTO. 1972. Properties of surround response mechanism of cat retinal ganglion cells and centre-surround interaction. *J. Physiol. (Lond.)*. **220**:403.

ENROTH-CUGELL, C., and R. M. SHAPLEY. 1973. Adaptation and dynamics of cat retinal ganglion cells. *J. Physiol. (Lond.)*. **233**:271.

ERNST, W., and E. M. KEMP. 1972. The effects of rhodopsin decomposition on PIII responses of isolated rat retinae. *Vis. Res.* **12**:1937.

FALK, G. and P. FATT. 1973. An analysis of light-induced admittance changes in rod outer segments. *J. Physiol. (Lond.)*. **229**:185.

GRABOWSKI, S. R., L. H. PINTO, and W. L. PAK. 1972. Adaptation in retinal rods of axolotl: intracellular recordings. *Science (Wash. D. C.)*. **176**:1240.

HARTLINE, H. K. 1938. The response of single optic nerve fibres of the vertebrate eye to illumination of the retina. *Am. J. Physiol.* **121**:400.

HOOD, D. C., and A. F. MANSFIELD. 1972. The isolated receptor potential of the frog isolated retina: action spectra before and after extensive bleaching. *Vis. Res.* **12**:2109.

KANEKO, A. 1971. Electrical connexions between horizontal cells in the dogfish retina. *J. Physiol. (Lond.)*. **213**:95.

KLEINSCHMIDT, J. 1973. Adaptating properties of intracellularly recorded Gekko photoreceptor potentials. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag KG, Berlin.

Kuffler, S. E. 1952. Neurons in the retina: organization, inhibition and excitation problems. *Cold Spring Harbor Symp. Quant. Biol.* **17**:281.

O'BRYAN, P. M. 1973. Properties of the depolarizing synaptic potential evoked by peripheral illumination in cones of the turtle retina. *J. Physiol. (Lond.)*. **235**:207.

PINTO, L. H., and W. L. PAK. 1974. Light-induced changes in photoreceptor membrane resistance and potential in gecko retinas: I. Preparations treated to reduced lateral interactions. **64**:000.

ROSE, A. 1948. The sensitivity performance of the human eye on an absolute scale. *J. Opt. Soc. Am.* **38**:196.

RUSHTON, W. A. H. 1965 *a*. The Ferrier Lecture, 1962. Visual adaptation. *Proc. Roy. Soc. Lond. B Biol. Sci.* **162**:20.

RUSHTON, W. A. H. 1965 *b*. The sensitivity of rods under illumination. *J. Physiol.* (*Lond.*). **178**: 141.

WAGNER, H. G., E. F. MACNICHOL, ad M. T. WOLBARSHT. 1960. The response properties of single ganglion cells in the goldfish retina. *J. Gen. Physiol.* **43**:45.

WEINSTEIN, G. W., R. R. HOBSON, and J. E. DOWLING. 1967. Light and dark adaptation in the isolated rat retina. *Nature* (*Lond.*). **215**:134.