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

I. *Preparations Treated to Reduce Lateral Interactions*

L. H. PINTO and W. L. PAK

From the Department of Biological Sciences, Purdue University, West Lafayette, Indiana 47907

ABSTRACT The time-course of the light-induced changes in membrane voltage and resistance were measured for single photoreceptors in the retina of *Gekko gekko*. In the surgically isolated retina, small stimuli directed toward the impaled receptor produced a membrane hyperpolarization the time-course of which was identical to that of the increase in membrane resistance. In the eyecup preparation nearly identical time-courses were evoked only after perfusion of the vitreous surface with solution having high (Mg^{++}). Disparate time-courses were obtained in (*a*) the isolated retina when large or displaced stimuli were used, and (*b*) the eyecup preparation when it was treated normally (see Pinto and Pak. 1974. *J. Gen. Physiol.* **64:49**) and when it was exposed to aspartate ions or hypoxia. These results are consistent with the hypothesis that the *receptor potential* (elicited in the impaled receptor as a result of quanta only it captures) is generated by a single ionic process that decreases membrane conductance. These measurements provide a means to distinguish the receptor potential from interactions. From direct measurements of membrane time constant and total resistance in darkness, total membrane capacitance was calculated. The mean capacitance was 7.1×10^{-5} μF. This high value is consistent with anatomical observations of membrane infoldings at the base of gecko photoreceptors.

INTRODUCTION

After a vertebrate photoreceptor captures quanta of light, its membrane potential becomes more negative than the resting (dark) value (Bortoff, 1964; Tomita, 1965; Werblin and Dowling, 1969) to produce a receptor potential. It has been demonstrated that certain pairs of receptors in the turtle retina are functionally connected and that horizontal cell feedback onto receptors exists

(Baylor et al., 1971). Therefore, the light-induced change in voltage, recorded intracellularly from a single receptor (the receptor response), may result not only from the receptor potential but also from interactions that involve the paled receptor.

Results of studies using the isolated retina support the hypothesis that the receptor potential results from decreased influx of Na^+ (Tomita, 1970; Hagins, 1972). It is a conclusion of the present study that interactions are minimized in the isolated retina. Therefore, the following results probably do not reflect the activity of receptor interactions. If the receptor membrane voltage was maintained above some positive value by extrinsic current, then a depolarizing receptor potential was obtained (Toyoda et al., 1969; Baylor and Fuortes, 1970). During the receptor potential, total resistance of the cell membrane increased (Toyoda et al., 1969; Baylor and Fuortes, 1970). Transretinal potentials evoked by light from the aspartate-treated retina, thought to be massed receptor potentials, decreased in magnitude with decreased external sodium concentration (Sillman et al., 1969 *b*). By measuring the extracellular voltage along rat rods, Penn and Hagins (1969) calculated the net membrane current. In the dark, there was a standing current that was decreased upon illumination and required the presence of external Na^+ (Hagins, 1972). Osmotic experiments on isolated rod outer segments have shown that light decreases the influx of Na^+ but not K^+ (Korenbrot and Cone, 1972). Exposure to a Na^+-free medium resulted in hyperpolarization of the receptor membrane and loss of response to light (Brown and Pinto, 1974).

Let us assume that the light-induced hyperpolarization of an individual photoreceptor is caused solely by the light-induced decrease in conductance for a single ion. If this is so, then at each instant during the response, the amplitude of the hyperpolarization should be proportional to the measured change in membrane resistance (see Eq. 7 and Appendix I). In this study we measured the time-courses of light-induced changes in the membrane potential and membrane resistance of single photoreceptors. This was done by using stimuli of various geometries in both the isolated retina and the eyecup preparation. The study had two purposes. First, we wished to establish the conditions under which we could record from an impaled receptor its receptor potential, uncontaminated by interactions. Secondly, we wished to identify the effects that the interactions have upon the receptor cell membrane. This paper presents results from retinas that were isolated or allowed to remain in the eyecup but treated chemically to minimize interactions, and the following paper (Pinto and Pak, 1974) studies the function of one type of interaction in an eyecup preparation, where interactions are preserved.

METHODS

DISSECTION Intracellular recordings were made from single photoreceptors of *Gekko gekko* which were cared for according to the suggestions of Pawley (1966). The

gecko was kept in darkness 18–24 h before each experiment and was then decapitated. The head was pithed and the eye was excised. A segment of the globe was sliced away (see Fig. 1). The remaining procedure differed for the isolated retina and eyecup preparation. The retina was isolated from the segment by permitting its vitreous side to adhere to a piece of moist filter paper and then removing the sclera and pigment epithelium. The retina was then surrounded by a wick which provided it with a con-

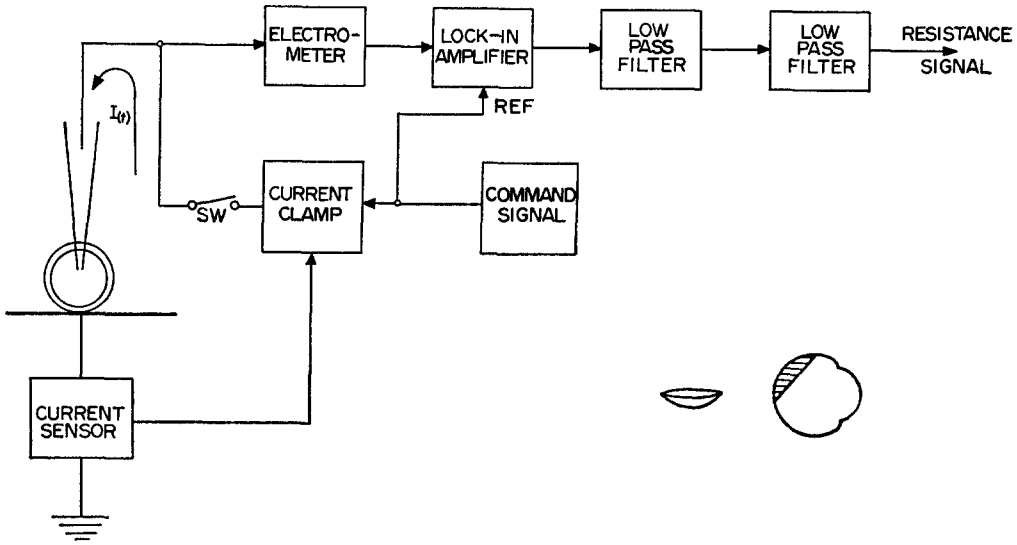

FIGURE 1. Block diagram of scheme for measuring changes in membrane resistance. The sinusoidal command signal caused the current clamp to force a sinusoidally modulated current across the cell membrane, via the electrode. Changes in the voltage induced by this current, which depended upon changes in membrane resistance were distinguished from the receptor potential by the lock-in amplifier, because it ignored signals which were not in phase with the command signal, its reference signal. Output from the lock-in amplifier was passed through two low pass filters to eliminate ripple caused by the command signal. This resistance signal was then averaged over several stimulus cycles to further improve signal to noise ratio. Receptor response was measured by opening switch SW and recording electrometer output. Not shown is a tape recorder used to record and replay signals. Inset: sketch of segment of globe removed to expose retina.

tinuous supply of Ringer's solution (see Table I). For the eyecup preparation the segment was mounted in a chamber (see next paper for details). All dissection was performed in dim red light ($\lambda > 650$ nm) except for a few experiments in which infrared illumination and image converters were used, with no noticeable improvement in results.

STIMULATION A dual beam stimulator provided steady or modulated monochromatic lights of any desired spatial pattern. Beam A originated from a Xenon arc lamp and passed through a monochromator ($\lambda = 543$ nm). Beam B originated from a tungsten iodide lamp, and passed through heat filters and interference filters ($\lambda = 543$ nm). The beams were passed through separate field lenses, combined in a mixing

TABLE I
PERFUSATE COMPOSITION

	NaCl	Dextrose	KCl	$NaHCO_3$	Na_2SO_4	$MgSO_4$	$CaCl_2$	HEPES	Na^+ aspartate
				mM					
Normal Ringer	159	5.6	3.7	0.13	0.6	1.2	2.5	3.0	
Aspartate Ringer	109	5.6	3.7	0.13	0.6	1.2	2.5	3.0	50
High Mg^{++} Ringer	59	5.6	3.7	0.13	0.6	101.2	2.5	3.0	

HEPES: N-2 Hydroxyethylpiperazine N'-2 ethanesulfonic acid.
All solutions titrated to pH 7.8 with NaOH.

cube, and passed through a microscope objective (× 10, numerical aperture 0.22, 11 mm working distance). The objective cast an image upon the retina (reduced 40-fold) of any object located in a plane 45 cm from the lens. Positioned in this plane were circular or annular apertures; they were mounted upon micrometer drives so that their images could be moved across the retina. Electromagnetic shutters modulated, and neutral density wedges attentuated the stimulus beams. Unattenuated retinal illuminance from beam A was 1.3×10^{-3} $\mu W/cm^2$ and from beam B was 1.1×10^{-4} $\mu W/cm^2$, corresponding to 8×10^8 and 7×10^7 quanta/receptor·s, incident axially. Gecko receptors are 6.0 μm in diameter (Dunn, 1969). Calculations based on microdensitometric measurements have shown that gecko photoreceptors have 10^9 pigment molecules each (Murakami and Pak, 1970). The longest stimulus routinely used in this study (2 s) could not have bleached more than 1 % (10^7) of these molecules unless it came from beam A with attenuation less than 2.40 neutral density or from beam B with attenuation less than 1.40 neutral density.

IMAGE QUALITY Light scattered within the retina always caused the actual image size to be greater than the size calculated from the magnification factor. The effect of scatter was worse for the small stimuli. It was measured in the following way. An isolated retina was prepared as usual, except that it was mounted in a chamber having a microscope coverslip for its bottom. The chamber was placed on the stage of a microscope (Zeiss WL, Carl Zeiss, Inc., New York, N. Y.) and the condenser of the microscope (numerical aperture 0.93) was used to cast the image of each of several pinholes upon the receptor layer (which faced up). The images were observed visually using a water immersion objective (× 40, numerical aperture 0.75). No matter how small the pinhole was, its image never appeared smaller than 25 μm in diameter (an area containing about 12 receptors). Pinholes whose theoretical (ideal) images should have been smaller than 25 μm ideal diameter, used often in this study, had maximal illuminance of less than one-sixth their theoretical illuminance.

IMPALEMENTS The retina was jolted (Tomita, 1965) or electronic oscillations were produced at the tip of the electrode (Baylor et al., 1971) in order to impale the cells.

MEMBRANE RESISTANCE MEASUREMENT A sinusoidally modulated current, of constant frequency, was forced across the membrane via the electrode by using a current clamp. Changes in membrane resistance caused changes in the transmembrane voltage induced by the current. These voltage changes were measured with a lock-in amplifier (see Smith et al., 1967).

The electrical length constant of a gecko photoreceptor exceeds the length of the cell.

> The receptors have diameter of about 6 μm (except at the short connecting cilium and connecting fiber, see Pedler and Tilley, 1964) and total length of about 80 μm (dimensions taken from Pedler and Tilley, 1964 and Dunn, 1969). The electrical length constant for direct current is $\sqrt{\rho \cdot R_m/2 \cdot R_i}$, where R_m is specific resistance of the membrane, R_i specific resistance of the cytoplasm and ρ radius (Katz, 1966). The value of R_m can be calculated to be $2.6 \times 10^3\ \Omega \cdot cm^2$ from the area and dark resistance of the membrane of the gecko photoreceptor (see p. 41). Using $R_i = 200\ \Omega \cdot cm$ (Ruppel and Hagins, 1973) yields DC length constant of 440 μm. The length constant decreased to $1/\sqrt{2}$ of this value for alternating currents one-half of which flow through the membrane capacitance.

This allows the membrane to be approximated by a parallel combination of a lumped resistance, R, and capacitance, C. The transmembrane voltage induced by the applied current is proportional to the impedance of the parallel combination. Provided that measuring frequency is held constant and that membrane capacitance remains constant, changes in impedance depend only upon changes in membrane resistance. In general the impedance has both real and imaginary components. The constant sinusoidal current induces a voltage in phase with the current the magnitude of which is proportional to the real component of impedance. The magnitude of the voltage induced out of phase with the current is proportional to the imaginary component of impedance. It was these components of transmembrane voltage that were actually measured using the lock-in amplifier. How these components were used to calculate changes in membrane resistance is explained in Eqs. 1–6.

The measuring frequency, f, was chosen to give the highest signal to noise ratio at the output of the lock-in amplifier. For $2\pi f \ll 1/RC$, negligible current flows through the membrane capacitance. Under this condition a change in membrane resistance causes an equal change in the real component of membrane impedance, which in turn causes a change in only the component of the transmembrane voltage that is in phase with the current. Unfortunately, changes in electrode resistance cause changes in transelectrode voltage that are also in phase with the current. For this reason small fluctuations in electrode resistance often obscured the desired signal when measurements were made at low frequencies. For $2\pi f \gg 1/RC$ nearly all of the current flows through the membrane capacitance, regardless of changes in membrane resistance. High measuring frequencies were therefore avoided. For $2\pi f \approx 1/RC$, changes in membrane resistance produce changes in both the real and imaginary components of membrane impedance. For a given change in membrane resistance, the change in the real component of impedance decreases with increased measuring frequency, but the change in imaginary component reaches a maximal value for $2\pi f = 1/\sqrt{3} \cdot R \cdot C$. Most measurements were made using frequencies near

this value, and the component of voltage that was out of phase with the applied current was observed. This component was nearly always independent of changes in electrode resistance, but *did* depend upon changes in membrane resistance. It therefore yielded the highest signal to noise ratio. Using the imaginary component also made the measurement insensitive to other changes in resistance within the retina, unless those resistances were shunted by a capacitance that allowed the combination to have a time constant nearly equal to that of the photoreceptor membrane.

The changes in membrane resistance were computed from the components of transmembrane voltage measured by the lock-in amplifier, as described below. Transmembrane voltage, $V'(j\omega)$, induced by the forcing current, expressed in vector form, has magnitude and phase given by the relation

$$V'(j\omega) = Z(j\omega) \cdot I(j\omega), \tag{1}$$

where $Z(j\omega)$ is membrane impedance; $I(j\omega)$ is forcing current; $\omega = 2\pi f$, and f is measuring frequency, which varied between 15–35 Hz. Membrane impedance for the parallel capacitance, C, which is assumed to be constant, and total membrane resistance, R, can be expressed by

$$Z(j\omega) = \frac{R(1 - j\omega RC)}{(\omega RC)^2 + 1}. \tag{2}$$

The lock-in amplifier actually measured changes in transmembrane voltage, $V'(j\omega)$, which were either in phase or out of phase with the current, $I(j\omega)$. Such changes were equal to changes in the real and imaginary components of membrane impedance, multiplied by current amplitude. These components of $Z(j\omega)$ can be expressed as follows:

$$Re\,[Z(j\omega)] = \frac{R}{(\omega RC)^2 + 1}; \qquad Im[Z(j\omega)] = \frac{-\omega R^2 C}{(\omega RC)^2 + 1}. \tag{3 a, b}$$

Both of these components underwent change when R changed:

$$\frac{\mathrm{d}\,[Re(Z(j\omega))]}{\mathrm{d}R} = \frac{1 - (\omega RC)^2}{[1 + (\omega RC)^2]^2} = C_R(j\omega), \tag{4 a}$$

and

$$\frac{\mathrm{d}\,[Im(Z(j\omega))]}{\mathrm{d}R} = \frac{-2\omega RC}{[1 + (\omega RC)^2]^2} = C_I(j\omega). \tag{4 b}$$

Every measurement was made using a fixed frequency of forcing current, and changes in R were always small. Thus, changes in the output of the lock-in amplifier actually were proportional to changes in total membrane resistance, ΔR:

$$\Delta Re[Z(j\omega)] = C_R(j\omega) \cdot \Delta R, \tag{5 a}$$

and

$$\Delta Im[Z(j\omega)] = C_I(j\omega) \cdot \Delta R. \qquad (5\,b)$$

In this equation, $\Delta Re[Z(j\omega)]$ and $\Delta Im[Z(j\omega)]$ represent changes in real and imaginary components of membrane impedance as a result of a change in total membrane resistance, ΔR. For every cell both $\Delta Re[Z(j\omega)]$ and $\Delta Im[Z(j\omega)]$ were measured using the lock-in amplifier. From Eqs. 4 *a*, it can be shown that the ratio of these changes, F, depends only upon membrane time constant, RC, and measuring frequency, ω:

$$F = \frac{\Delta Re[Z(j\omega)]}{\Delta Im[Z(j\omega)]} = \frac{C_R(j\omega)}{C_I(j\omega)} = -\frac{1}{2}\left[\frac{1}{\omega RC} - \omega RC\right]. \qquad (6)$$

From the value of F calculated from measurement of $\Delta Re[Z(j\omega)]$ and $\Delta Im[Z(j\omega)]$, the time constant RC was determined (see Results), and constants $C_R(j\omega)$ and $C_I(j\omega)$ were evaluated (Eqs. 4 *a*, *b*). The constants $C_R(j\omega)$ and $C_I(j\omega)$ were used to calibrate the lock-in amplifier.

Two checks were made to be certain that changes in membrane capacitance did not affect the measurements. First, measurements were made using measuring frequencies high enough to cause current to pass entirely through the membrane capacitance. No light-induced changes in $V'(i\omega)$ could be detected under these conditions. Secondly, the time-courses of the light-induced changes in $V'(j\omega)$ obtained with high and low measuring frequencies were obtained from the same cell. The time-courses did not differ.

Further improvement in signal to noise ratio was achieved by averaging the output of the lock-in amplifier after filtering by two, sequential, low pass filters. The time constants of the filters were short enough not to distort the signal waveform. An improved signal-to-noise ratio of the receptor response waveform was similarly achieved by averaging over several stimulus cycles.

This method was also used because application of a square wave of current (see Toyoda et al., 1969) resulted in an electrode potential which changed gradually for a few seconds, while sinusoidal currents gave an electrode potential which had a constant envelope. In addition, alternating half-cycles of inward and outward current prevented intracellular iontophoresis of the electrolyte.

Stable resistance measurements required electrodes with small tips, low resistance (60–300 MΩ) and free from rectification. The best electrodes for these purposes were drawn from tubing having 0.6-mm ID and 0.1-mm wall. They were boiled in distilled water to fill. Their stems were injected with 4 M potassium acetate, and they were allowed to sit 12–18 h in a moist container. Each electrode was microscopically examined and its currect-voltage characteristic was determined in Ringer's solution (see Table I). Only the few electrodes meeting the above specifications were used.

PERFUSION The vitreous surface of the eyecup was perfused in several experiments with the solutions listed in Table I. All solutions were oxygenated, maintained at room temperature, introduced by gravity feed at 10 ml/mm, and removed by suction.

PROTOCOL After impalement of the cell, its receptor response was elicited under the stimulus conditions desired. Resistance measurements were then made after engaging the necessary instruments. After their completion the control receptor response was measured. When several resistance measurements were to be made upon the same cell, the mean membrane resistance (about which stimulus-induced fluctuations occurred) was carefully monitored to check that the electrode was not coming out of the cell. Calibration was checked by switching resistor-capacitor combinations of known value into the circuit in series with the electrode.

RESULTS

Type of Receptor Studied

A tangential section through the receptors of *Gekko gekko* shows these cells to occur in a regular mosaic array (Dunn, 1969). Each location on the array is occupied by either a pair of receptors, a single receptor, or a triplet of receptors (Dunn, 1969). Members of a pair have either the same size (twins) or unequal size (doubles) (see Dunn, 1966 for classification). Triplets occur infrequently (see Dunn, 1969). The largest, frequently occurring cells are twins, the larger members of doubles, and singles (see Table I, Dunn, 1966). Hydroxylamine difference spectra of extracted pigments of *Gekko gekko* show the presence of a dominant pigment having λ_{max} of 521 nm and a minor pigment having λ_{max} of 478 nm (Crescitelli, 1963). The presence of two pigments has been confirmed electroretinographically (Crescitelli, 1966). Using microspectrophometry, Liebman (1972) has shown that the twin receptors, the larger member of the double receptors, and the single receptors contain a pigment that absorbs maximally at 518 nm, while the smaller member of the double receptors absorbs maximally at 467 nm. In the present study spectral sensitivity was measured at three wavelengths (430, 525, and 580 nm) for eight cells. For each cell the spectral sensitivity distribution was consistent with maximal absorption near 525 nm. None had spectral sensitivity consistent with maximal absorption at a shorter wavelength. We conclude from this that the majority of recordings were made from those cells bearing a pigment with absorption maximum near 518 nm.

Extracellular Measurements of Voltage and Resistance

No light-induced changes in potential or resistance were measured when the microelectrode was located outside the photoreceptor cell. This was the case for all stimuli in all preparations used. In addition, the resistance measuring technique was inherently insusceptible to extracellular resistance changes (see Methods).

Measurements in the Surgically Isolated Retina

RECEPTOR POTENTIAL MECHANISM STUDIED WITH RESTRICTED STIMULI The hypothesis that the receptor potential is caused by decreased sodium conduct-

ance is expressed formally in the model of Fig. 2 (Sillman et al., 1969 *a*, *b*). It can be seen that both membrane voltage, V_m, and membrane resistance, R_m, depend on the membrane conductance of sodium ions, G_{Na}, the reciprocal of which is R_{Na}. It follows that membrane voltage should be a function of membrane resistance. As shown in Appendix I, light-induced changes in membrane voltage, ΔV_m, are, according to the model, directly proportional to changes in membrane resistance ΔR_m;

$$\Delta V_m = \frac{-(E_{Na} + E_K)}{R_K} \cdot \Delta R_m, \qquad (7)$$

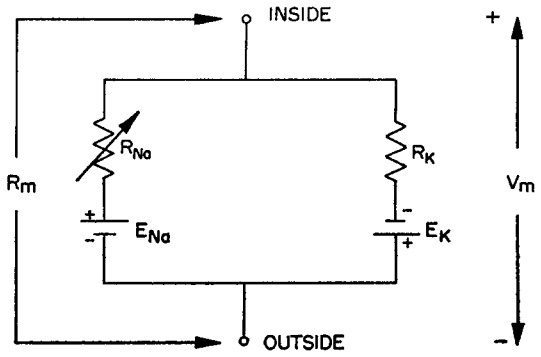

FIGURE 2. Formal model of photoreceptor membrane (from Sillman et al., 1969 *a*, *b*). E_{Na} and E_K are sodium and potassium equilibrium potentials. R_{Na} and R_K are resistances of membrane to sodium and potassium ions, respectively. ($R_{Na} = 1/G_{Na}$, where G_{Na} is sodium conductance of membrane). The receptor potential results from decreased G_{Na}.

where E_{Na} = sodium equilibrium potential and E_K = potassium equilibrium potential. The essential element for a proportional relationship is that the receptor potential be the product of a single ionic process. For four cells, ΔV_m and ΔR_m were measured in the isolated retina using small (75-μm diam) stimulus lights the illuminance of which varied over a wide range (4×10^4–8×10^5 quanta/receptor·s). These stimulus conditions were chosen to avoid eliciting lateral interactions. The results from one of these cells is shown in Fig. 3. It can be seen that change in membrane voltage (ΔV_m) is directly proportional to change in membrane resistance (ΔR_m), as predicted from the model.

Further support for this simple model was obtained by passing *steady* extrinsic current into the cell (via the electrode) while recording the response to light. In the absence of light, inward current will depolarize the membrane (make V_m more positive) and outward current will hyperpolarize the membrane (make V_m more negative). For $V_m < E_{Na}$, Na^+ current will flow inward across the membrane, and for $V_m > E_{Na}$, Na^+ current will flow outward. A light-induced decrease in G_{Na} that occurs when $V_m < E_{Na}$ will cause

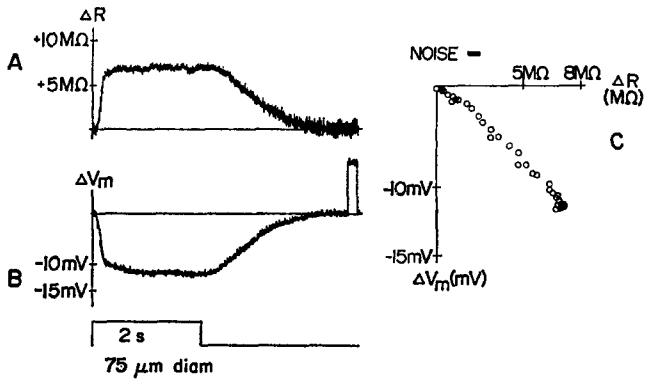

FIGURE 3. Comparison of light-induced change in total membrane resistance (A) with change in membrane potential (B). Stimulus was 75-μm diam. Change in membrane potential is plotted against change in membrane resistance in *C*, where dark rectangle indicates standard deviation of signals along each axis (noise) measured in darkness (Cell 10/6/71; stimulus illuminance 8×10^5 quanta/receptor·s.)

decreased inward Na^+ current and, therefore, a hyperpolarization. The magnitude of the hyperpolarization will be proportional to the difference ($E_{Na} - V_{md}$) where V_{md} is the membrane potential in the dark (see Appendix II). A light-induced decrease in G_{Na}, which occurs when $V_m > E_{Na}$, will cause decreased outward Na^+ current and therefore, depolarization. The magnitude of the depolarization will be proportional to the difference ($V_{md} - E_{Na}$). Thus, the peak magnitude of the response to a given stimulus should be a linear function of extrinsic current.

Five cells were studied in the following manner. The light stimulus centered upon the impaled receptor had a 75-μm diameter and lasted 2.0 s. The magnitude of the receptor's response to light is plotted against applied current in Fig. 4, for one cell. For each cell, the plot of peak response magnitude against extrinsic current was linear over the entire range of applied current. The responses of two cells (not shown) did not change sign, even when currents as large as 1.5 nA were applied. The slope of their plots was very small (< 1 MΩ), and the peak size of the receptor potential (measured with no current and intense stimulus light) was also small (2 or 3 mV), suggesting that these two cells were damaged during impalement. A linear relationship between response magnitude and extrinsic current was also found by Toyoda et al. (1969) and Baylor and Fuortes (1970).

RESISTANCE CHANGES STUDIED WITH DIFFUSE STIMULI The results we obtained with small spot stimulation of photoreceptors in the isolated retina are consistent with the hypothesis that the receptor potential is caused by a decrease in G_{Na} (Sillman et al., 1969 *a*, *b*; Toyoda et al., 1969; Tomita, 1970). However, the above conditions were deliberately chosen to minimize lateral

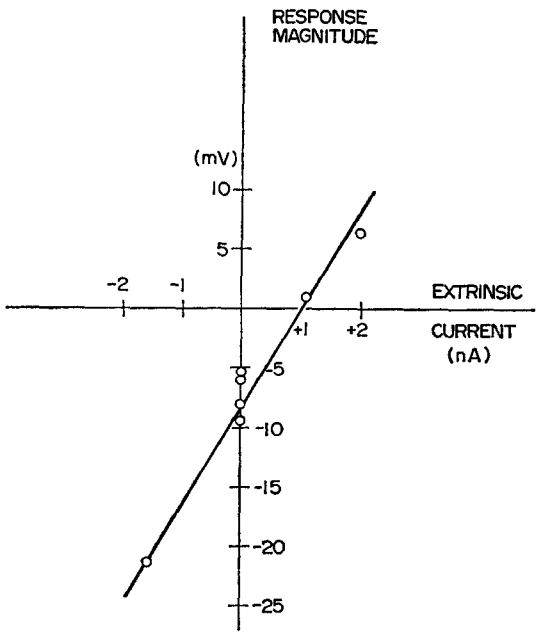

FIGURE 4. (A) Plot of response peak magnitude against steady extrinsic current. Line fitted by method of least squares. Note that response magnitude is linearly related to magnitude of extrinsic current. (Cell 10/6/71; stimulus illuminance, 4×10^5 quanta/receptor·s.)

interactions. We therefore repeated the resistance measurements for nine cells in the isolated retina using stimuli of large diameter, the illuminance of which varied over a wide range (10^3–10^6 quanta/receptor·s).

The results from one cell are shown in Fig. 5. It can be seen in A and B that after stimulus onset, the change in membrane resistance runs parallel with the change in membrane voltage. But after stimulus offset, membrane resistance takes longer to return to its original value. This discrepant time-course is also shown by the ellipticity of the graph plotting changes in membrane voltage against changes in membrane resistance (Fig. 5 C). Of the nine cells which gave results similar to that shown in Fig. 5, five were studied with stimuli that covered the entire retina, three with 300-μm diam stimuli, and one with a very intense stimulus (75-μm diam) that was deliberately centered 50 μm from the impaled receptor. The results obtained under these stimulus conditions are clearly not consistent with the simple model, suggesting that lateral interactions are involved in the receptor response of the isolated retina.

CONSTANCY OF EQUILIBRIUM POTENTIALS DURING RESPONSE TO LIGHT

There are two principal ways in which the model of Fig. 2 could fail. The first is that illumination causes conductance changes in addition to G_{Na}. A less likely possibility is that the equilibrium potential for some ion (or ions)

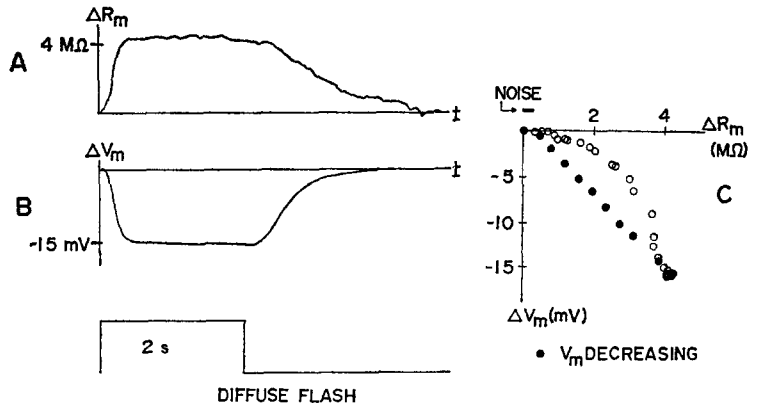

FIGURE 5. Comparison of light-induced change in total membrane resistance (A) with change in membrane potential (B). Stimulus was a diffuse flash which flooded the entire retina. Change in membrane potential is plotted against change in membrane resistance in *C*, where dark rectangle indicates noise. (Cell 1/15/71 # 1; stimulus illuminance 9 × 10^5 quanta/receptor·s.)

changes during illumination. The latter possibility was shown to be unlikely by the following experiment. Changes in equilibrium potential can be brought about by changes in the ratio of ionic concentrations across the membrane. If changes in concentration result from altered ionic fluxes elicited by illumination, then the changes should become greater the longer the light is held on. In two cells, the changes in membrane voltage and membrane resistance were studied with diffuse stimuli of short (0.2 s) and long (2.0 s) durations. The results from one of these cells are shown in Fig. 6, in which the change in membrane voltage is plotted against the change in membrane resistance for the two stimulus conditions. It can be seen that the plot is elliptical, even for a very short stimulus. The fact that the model fails in the same way for the long and the short stimulus suggests that changes in equilibrium potential are not responsible for its failure.

An attempt was made to discover the nature of the deviation from the model by studying the waveform of the responses of single photoreceptors as a function of stimulus size. However, the recordings were not stable for a long enough time to permit completion of the experiments.

Chemical Isolation of Receptor Potential

Membrane resistance and membrane potential run parallel time-courses only when small stimuli are used (see Fig. 3). This suggests that interactions might be responsible for the deviations seen with large stimuli (Figs. 5 and 6). Therefore, we attempted to eliminate these interactions using chemical treatments. If the "interaction hypothesis" is correct, successful abolishment of interactions, accomplished without direct effects upon the receptor cell, should allow

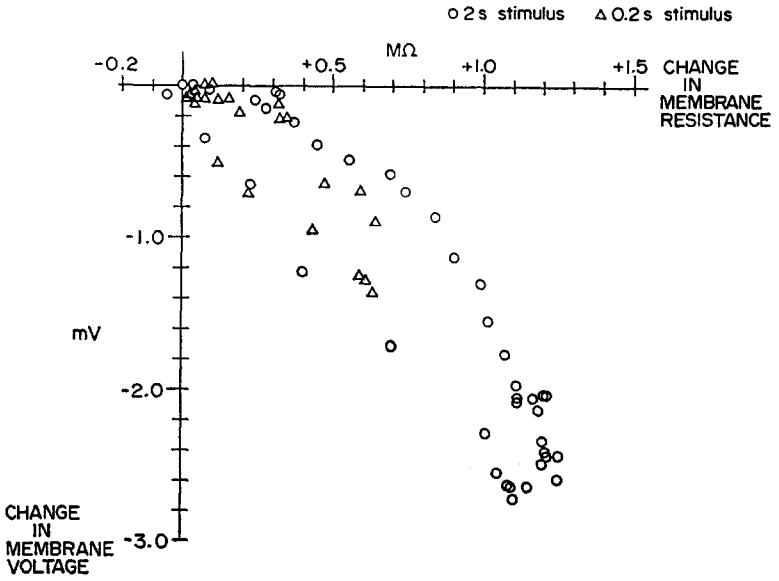

FIGURE 6. Change in membrane voltage plotted against change in membrane resistance for a cell studied with a diffuse stimulus which lasted either 0.2 s (Δ) or 2.0 s. The ellipticity seen for *both* plots implies that significant changes in equilibrium potential do not occur during illumination. (Cell 2/14/71 #1; stimulus illuminance 2×10^5 quanta/receptor·s.)

light-induced changes in receptor membrane resistance and membrane potential to run parallel time-courses. Because it was difficult to obtain stable recordings in the isolated retina these experiments were done in the eyecup preparation.

Responses elicited from receptors in the normal eyecup preparation by saturating stimuli of large area have a transient undershoot at onset. This waveform is due to delayed negative feedback from horizontal cells (Cervetto and MacNichol, 1972; Kleinschmidt, 1973) that causes decreased resistance of the photoreceptor membrane (Baylor et al., 1971; Pinto and Pak, 1974)[4]. Because of this feedback the time-course of the change in receptor membrane resistance in the untreated retina bears no resemblance to that of the change in membrane potential, even when small stimuli are used (Pinto and Pak, 1974). The onset of response to a saturating stimulus is rapid (< 100 ms risetime), but after stimulus offset membrane potential takes longer (at least 1 s) to return to its resting value.

ELEVATED (Mg^{++}) Perfusion of the vitreous surface of the retina with high Mg^{++} Ringer (see Table I) gave the most successful isolation of the receptor potential by chemical means. This treatment has also been used to isolate the *a*-wave of the electroretinogram (Winkler, 1972). Diffuse stimuli were used in these experiments on 10 cells. The test solution was applied only

when desired during impalement. Gecko Ringer's was used at all other times. Upon application of high Mg^{++} Ringer the receptor membrane hyperpolarized in the dark, responses became smaller (50–80% of original size) and lost their transient undershoot. The responses did not become slower, but one response developed an after-depolarization after 3-min exposure to the test solution. The membrane hyperpolarization was greatest (5–10 mV) for the first exposure to test solution. Hyperpolarization was not completely reversed after return to gecko Ringer. After each exposure to high Mg^{++} Ringer the responses lost a successively greater fraction of the transient undershoot. Responses obtained after a total of 10-min exposure, even though recorded during perfusion with gecko Ringer, completely lacked transient undershoot. This is consistent with the results of Dowling and Ripps (1973) who recorded membrane potential of horizontal cells in the skate retina during application of high Mg^{++} Ringer to the vitreous surface. They noted prolonged hyperpolarization and loss of response to light in these cells.

Resistance measurements were made in five cells either during perfusion with high Mg^{++} Ringer or during perfusion with gecko Ringer after a total of 10 min exposure to high Mg^{++} Ringer previously. For each cell, resistance change lagged behind voltage change by 100–200 ms at both onset and offset. This small lag was the least noticeable discrepancy between time-courses that were obtained by chemical means. It should be mentioned that long-lasting changes in response did not occur if a stagnant pool of high Mg^{++} Ringer was applied to the vitreous surface.

ASPARTATE It was not possible to confirm the isolation of the receptor potential in retinas presoaked in aspartate Ringer for 5 min (see Table I). Perfusion of the vitreous side of the turtle retina with aspartate causes sustained depolarization and loss of light response of horizontal cells and receptor responses lose their transient undershoot, presumably through loss of delayed negative feedback from horizontal cells (Cervetto and MacNichol, 1972). In gecko eyecup preparations that were presoaked for 5 min in aspartate Ringer and maintained in room air, Kleinschmidt (1973) reported absence of transient undershoot of receptor responses. Response onset was also slowed. After confirming these observations we compared the time-courses of the light-induced changes in membrane resistance and membrane potential for nine cells. Five of these cells were in retinas maintained in room air; the rest were maintained with oxygen. For the cells studied in room air, the membrane hyperpolarized monotonically at onset and returned monotonically to its resting value after offset. This happened with both small (25-μm diam) and large (1-mm diam) stimuli. Membrane resistance monotonically increased after stimulus onset and monotonically returned to its original value after offset. However, the resistance changes at both onset and offset lagged behind the voltage changes by 0.4–2.0 s. Evidence for lateral interactions was found in

one of these cells. Responses were elicited from this cell by a series of small spots (25-μm diam) of each of several illuminances and a similar series of annuli (50-μm ID, 1.0-mm OD). Response waveforms differed in that a short-lived after depolarization of 1–2 mV was elicited after offset of the annuli, but not of the spots.

In the normal eyecup preparation studied in the accompanying paper (Pinto and Pak, 1974), oxygen (rather than room air) was allowed to flow into the side of the chamber to preserve lateral interactions. Resistance measurements were therefore made in cells from retinas that were presoaked in aspartate and maintained with oxygen. For each of four cells so studied the changes in membrane resistance and membrane potential were qualitatively similar to the results obtained from cells maintained in room air. However, resistance changes lagged behind voltage changes by at least 1 s at both onset and offset. In addition, response onset was very slow in these cells (200–400-ms risetime). These observations were made with all stimuli used in this study: small (25-μm diam) and large (1-mm diam) spots and annuli (50- or 150-μm ID, 1.0-mm OD).

HYPOXIA An attempt was made to eliminate interactions by making the retina hypoxic. This was done for nine cells by flowing nitrogen (saturated with water) into the chamber instead of oxygen. The response of each cell was elicited by a saturating stimulus of large area and normally had a transient undershoot. This undershoot disappeared within 1–2 min after replacement of oxygen. While response waveform was changing response size became smaller (30–80% of original size), onset became slower (about 200-ms risetime) and resting membrane potential slowly drifted. For four cells the membrane became depolarized in the dark by 1–5 mV. The other cell membranes hyperpolarized by 1–5 mV. The effects of the first hypoxic exposure only were reversible, and then only if the exposure lasted less than about 1 min. Membrane resistance was measured in two cells. For both cells, resistance increased monotonically after stimulus onset and returned monotonically to its original value after offset. The resistance change lagged behind the change in membrane potential by 0.1–0.4 s at both onset and offset.

In summary, the chemical treatment that most nearly gave parallel time-courses of membrane potential and resistance was perfusion with high Mg^{++} Ringer. All other treatments yielded discrepant time-courses that very likely resulted from direct effects upon receptor cells.

Membrane Capacitance

Membrane capacitance was computed from the measurements of membrane time constant and membrane resistance. The time constant was measured in 19 cells (see Methods). In 13 cells studied in isolated retinas, the mean time constant was 2.75 ms (range 1.37–5.21 ms). However, for six cells in the eyecup

preparation (see Pinto and Pak, 1974), the mean time constant was 3.05 ms (range 2.53–3.59 ms). The total membrane resistance in darkness was measured in four cells. While current was being passed through the electrode, electrode voltages were compared when the tip of the electrode was inside and outside the cell. However, the electrode resistance was slightly higher with the electrode tip in 0.15 M NaCl than in 0.15 M KCl. Measurements were corrected for this. The mean total membrane resistance was 40 MΩ (range 30–48 MΩ). This is higher than the range of resistances reported for either turtle cones (10–20 MΩ) (Baylor and Fuortes, 1970) or gecko photoreceptors (10 MΩ) (Toyoda et al., 1969). From the pooled mean time constant (2.84 ms) and our measurement of membrane resistance, the total membrane capacitance was calculated to be 7.1×10^{-5} μF.

For a cylinder having the length (38 μm) and radius (3 μm) of a gecko photoreceptor (Dunn, 1969), the surface area is 7.2×10^{-6} cm². Extracellular dye patterns (Laties and Liebman, 1974, in preparation) indicate that in *Gekko gekko* between 30–100 disks located at the base of the outer segment are connected to the plasma membrane. Electron microscopic studies (A. Cohen, personal communication) also indicate that there are persistent connections of the plasma membrane to the disks which are located at the base of the photoreceptors of *Gekko gekko*.

If 100 disks are functionally connected with the plasma membrane, then the membrane surface area, calculated from the geometry of the cell, would be 6.4×10^{-5} cm². With this value of membrane surface area, a membrane capacitance of 1.11 μF/cm² is obtained. This value is in close agreement with the generally accepted value of 1 μF/cm² (Cole, 1968) for cell membranes.

DISCUSSION

IDENTIFICATION OF THE RECEPTOR POTENTIAL If it is accepted that the receptor potential results from one ionic process involving primary alteration in membrane conductance, then it follows that when a normal receptor potential is recorded, changes in membrane potential and membrane resistance will run parallel time-courses. However, the converse is not necessarily true because it is possible that the decreased conductance of a given receptor is caused in part by interactions from neighboring cells that result in changes in membrane properties identical to those resulting from light captured by the receptor. Occurrences of such interactions seem highly improbable. Even if they do exist, they are probably small, since receptive field size for receptors in the isolated retina is as small as 25 μm. In light of these considerations, we will consider a receptor potential to be pure, i.e. uncontaminated by interactions, whenever its time-course parallels the time-course of increase in membrane resistance.

IONIC BASIS FOR RECEPTOR POTENTIAL The following results support the hypothesis (see Fig. 1, Sillman et al., 1969 *a*, *b*) that decreased sodium influx, caused by decreased sodium conductance, results in the receptor potential. We obtained these results from receptors in isolated retinas which were studied with stimuli of small diameter, so that no evidence for lateral interactions was found. The change in membrane resistance was directly proportional to the change in membrane voltage (Fig. 3). The relationship between peak magnitude of the receptor response and the magnitude of extrinsic current was linear (Fig. 4). These results do not exclude the possibility that the receptor potential is caused by a simultaneous decrease in the membrane conductance for two or more ions having a compound equilibrium potential more positive than resting potential, e.g. a process opposite to that causing the muscle endplate potential (Takeuchi and Takeuchi, 1960). Additional support comes from the cells in the Mg^{++}-treated retina that gave nearly identical time-courses of change in membrane resistance and membrane potential.

REDUCTION OF INTERACTIONS BY SURGICAL ISOLATION Lateral interactions that are mediated by horizontal cells were not readily observable in the isolated retina. These interactions are readily observed in the normal eyecup preparation and caused decreased membrane resistance of the cell under study (Pinto and Pak, 1974). This is in contrast to the isolated retina, in which receptor membrane resistance always increased. The reduction of interactions upon removal of the pigment epithelium does not necessarily imply that the pigment epithelium normally mediates the interactions. During the process of isolation many receptor outer segments were torn off or damaged (even when surgery was performed under infrared illumination to prevent migration of pigment epithelial cell granules). Recordings in this preparation were made from remaining undamaged cells, which we believe were few in number because we had only 1 successful impalement per 20 penetrations vs. 1 impalement per 2 penetrations in the eyecup preparation. Also, it is conceivable that damage to other retinal cells occurred.

RESIDUAL INTERACTIONS IN THE ISOLATED RETINA In the isolated retina, change in membrane resistance was directly proportional to change in membrane voltage when small diameter stimuli were used. If, on the other hand stimuli of larger diameter were used, resistance took longer than voltage to return to its original value after offset. This implies that lateral interactions involving receptors are functional, at least to some degree, in the isolated retina. It was not possible to study changes in voltage and resistance for stimuli of several diameters in a single cell in the isolated retina. Hence, we do not know with certainty whether the discrepancy in their time-courses was due to an ionic mechanism that (*a*) elevated membrane resistance after offset of a large stimulus, but had little effect on membrane voltage, or an ionic mechanism

that (*b*) tended to depolarize the membrane after offset of a large stimulus but had little effect upon membrane resistance. However, indirect evidence supports the first alternative. In the isolated retina the responses of cells studied with small stimuli were similar to the responses of cells studied with large stimuli, when illuminances were nearly equal. The magnitude of responses elicited by diffuse stimuli have been studied as a function of extrinsic current (Toyoda et al., 1969). The plot of magnitude vs. current is linear. We have confirmed this result for small stimuli, and have also studied response magnitude as a function of stimulus illuminance in the isolated retina. The plot of magnitude vs. illuminance is the same for both small and large stimuli (Fig. 1 C, Pinto and Pak, 1974). Since potential was not affected in any of these cases we suspect that the residual interactions impart only a delayed increase in membrane resistance to the receptor cell. This residual interaction probably is not mediated by horizontal cells, since the horizontal cell-mediated interactions produce decreased membrane resistance shortly after stimulus onset (Pinto and Pak, 1974). Perhaps the residual interactions are mediated directly from receptor to receptor. Delayed resistance increases were recorded in aspartate-treated and hypoxic retinas, but these increases may have been due either to direct effects of the treatment upon the receptor or to residual interactions.

The discrepancy between time-courses of change in membrane resistance and potential had a similar character when studied with large diameter stimuli of long and short durations (Fig. 6). This implies that equilibrium potentials did not change as a result of ionic fluxes associated with the receptor response. Also, it seems unlikely that the discrepancy was due to altered resistance of photoreceptor disks (Yoshikami and Hagins, 1973). If this were the case, then only the resistance should be affected. However, the longitudinal resistance along isolated rod outer segments decreases with a delay after illumination (Falk and Fatt, 1973). This could not explain the delayed resistance increase seen in Fig. 5.

CHEMICAL ISOLATION OF THE RECEPTOR POTENTIAL Successful isolation of the receptor potential by chemical means requires that the chemical agent eliminate interactions mediated by horizontal cells and direct receptor-to-receptor interactions and that it does not affect receptor membrane properties directly.

The mechanism of receptor isolation by high Mg^{++} Ringer is of interest, since this was the most successful chemical treatment. Application of a similar solution causes hyperpolarization of horizontal cells in the skate retina. It is possible that hyperpolarization results from interruption of release of depolarizing transmitter substance from receptor terminals by Mg^{++} ions (Dowling and Ripps, 1973). Nearly two-thirds of Na^+ was removed from the high Mg^{++} Ringer used in these experiments. The lower Na^+ concentration would be ex-

pected to cause some receptor hyperpolarization (Cervetto, 1973; Brown and Pinto, 1974). Such hyperpolarization was observed and may have somehow interfered with transmitter release. Both mechanisms may play a role in interfering with transmission of signals by receptors to postsynaptic cells and give complete isolation. Mg^{++} appears to have no direct effect on the receptor membrane. When applied in seven times normal concentration to the receptor side of the isolated perfused retina, Mg^{++} has no effect upon waveform or magnitude of rod responses (Brown and Pinto, 1974).

Our inability to obtain parallel time-courses for change in membrane resistance and membrane potential in the aspartate-treated retina may be attributed to two factors. The more important is probably the direct effect that aspartate ions seem to have upon receptor responses when recorded intracellularly (Brown and Pinto, 1974) or when using the *a*-wave of the electroretinogram (Dowling and Ripps, 1973). In addition some residual lateral interactions appear to be present in the aspartate-treated retina. These interactions probably are not mediated by horizontal cells and their effects on the time-courses of membrane potential and resistance are relatively minor.

Horizontal cells are rendered unresponsive by hypoxia (Fatehchand et al., 1966). A possible explanation is the interruption of release of synaptic transmitter substance (see Hubbard and Løyning, 1966) from receptor terminals. Inactivity of horizontal cells was probably the reason why only resistance increases were recorded from photoreceptors in hypoxic retinas, even when studied with large area stimuli. Such stimuli usually evoke resistance decreases in addition to resistance increases (Pinto and Pak, 1974). Hypoxia may also affect the receptors directly. It is known that receptors have a high metabolic requirement to maintain dark current (Hagins et al., 1970). It would therefore be expected that hypoxia has a direct effect upon receptor cells and causes the response size to decrease. Perhaps this direct effect led to an alteration of the receptor membrane and caused the change in membrane resistance to lag behind change in membrane voltage.

APPENDIX I

The relationship between light-induced change in membrane voltage, ΔV_m, and light-induced change in membrane resistance, ΔR_m, is desired. Using the symbols of Fig. 2, membrane voltage is given by

$$V_m = E_{Na} - \frac{R_{Na}}{R_K + R_{Na}} (E_{Na} + E_K). \qquad (I A)$$

Total membrane resistance is the parallel combination of the individual resistors:

$$R_m = \frac{R_K \cdot R_{Na}}{R_K + R_{Na}}. \qquad (I B)$$

Substitution of I B into I A yields the desired proportionality between V_m and R_m, assuming constant R_K :

$$V_m = E_{Na} - \frac{R_m}{R_K}(E_K + E_{Na}), \qquad (I\,C)$$

and

$$\Delta V_m = \frac{-(E_{Na} + E_K)}{R_K} \cdot \Delta R_m. \qquad (I\,D)$$

APPENDIX II

The relationship between magnitude of the receptor potential as a function of time, $\Delta V_m(t)$, and extrinsic current, I is desired. The receptor potential, according to the model of Fig. 2, is caused by increased R_{Na} (decreased G_{Na}). Let R_{Na} be the sum of a fixed and light-induced (and hence time-varying) resistance: $R_{Na} = R_o + \Delta R_{Na}(t)$. Taking account of the extrinsic current I, V_m can be expressed by:

$$V_m = \frac{E_{Na} \cdot R_K + (I \cdot R_K - E_K)(R_o + \Delta R_{Na}(t))}{R_K + R_o + \Delta R_{Na}(t)}. \qquad (II\,A)$$

This can be rewritten:

$$V_m = \frac{E_{Na} \cdot R_K + (I \cdot R_K - E_K)(R_o + \Delta R_{Na}(t))}{R_K + R_o} \left[\frac{1}{1 + \frac{\Delta R_{Na}(t)}{R_K + R_o}}\right]. \qquad (II\,B)$$

The contents of the brackets can be expanded:

$$\frac{1}{1 + \frac{\Delta R_{Na}(t)}{R_K + R_o}} = 1 - \frac{\Delta R_{Na}(t)}{R_K + R_o} + \left[\frac{\Delta R_{Na}(t)}{R_K + R_o}\right]^2 + \cdots \qquad (II\,C)$$

Ignoring higher terms from this expansion, and substituting into (II B) yields

$$V_m \approxeq \frac{E_{Na} \cdot R_K + (I \cdot R_K - E_K)(R_o + \Delta R_{Na}(t))}{R_K + R_o} \left[1 - \frac{\Delta R_{Na}(t)}{R_K + R_o}\right]. \qquad (II\,D)$$

Changes in membrane potential induced by light (i.e. the receptor potential) can be measured. Neglecting higher terms of $\Delta R_{Na}(t)$,

$$\Delta V_m(t) \approxeq \left[I \cdot R_K - E_K + \frac{-E_{Na} \cdot R_K + R_o(E_K - I \cdot R_K)}{R_K + R_o}\right] \frac{\Delta R_{Na}(t)}{R_K + R_o}. \qquad (II\,E)$$

This can be simplified to

$$\Delta V_m(t) = \frac{R_K(I \cdot R_K - E_K - E_{Na})}{(R_K + R_o)^2} \Delta R_{Na}(t). \qquad (II\,F)$$

The slope, m, of the plot of the peak size of receptor potential, $\Delta V_m(t)$, against current I is

$$m = \frac{R_K^2 \cdot \Delta R_P}{(R_K + R_o)^2}, \qquad (II\ G)$$

where ΔR_P is the peak magnitude of change in resistance $\Delta R_{Na}(t)$.

It is instructive to note that the magnitude of the receptor potential is proportional to the difference in voltage $(V_m - E_{Na})$ which is imposed by the current I in darkness. From Eq. (II A), the membrane voltage in darkness, V_{md} can be found

$$V_{md} = \frac{E_{Na} \cdot R_K + (I \cdot R_K - E_K)\, R_o}{R_K + R_o}. \qquad (II\ H)$$

This equation can be solved for current I:

$$I = \frac{V_{md}\,(R_K + R_o) - E_{Na} \cdot R_K + E_K \cdot R_o}{R_o \cdot R_K}. \qquad (II\ I)$$

This expression for I can be substituted into equation (II F):

$$\Delta V_{md} = (V_{md} - E_{Na}) \left[\frac{R_K}{R_o} + 1\right] \frac{R_K \cdot \Delta R_{Na}(t)}{(R_K + R_o)^2}. \qquad (II\ J)$$

Thus, the peak value of receptor potential elicited by a constant intensity light is proportional to the difference between membrane potential in darkness and E_{Na}, i.e. $(V_{md} - E_{Na})$.

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REFERENCES

BAYLOR, D. A., and M. G. F. FUORTES. 1970. Electrical responses of single cones in the retina of the turtle. *J. Physiol. (Lond.)*. **207**:77.

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.

BORTOFF, A. 1964. Localization of slow potential responses in the *Necturus* retina. *Vis. Res.* **4**:627.

BROWN, J. E., and L. H. PINTO. 1974. Ionic mechanism for the photoreceptor potential of the isolated retina of *Bufo marinus*. *J. Physiol. (Lond.)*. **236**:575.

CERVETTO, L. 1973. Influence of sodium, potassium and chloride ions on the intracellular responses of turtle photoreceptors. *Nature (Lond.)*. **241**:401.

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

COLE, K. S. 1968. Membranes Ions and Impulses, University of California Press, Berkeley, Calif. 51.

CRESTICELLI, F. 1963. The photosensitive retinal pigment system of *Gekko gekko*. *J. Gen. Physiol.* **47**:33.

CRESTICELLI, F. 1966. The spectral sensitivity of the gecko eye in relation to the state of adaptation. *Vis. Res.* **6**:129.

DOWLING, J. E., and H. RIPPS. 1973. Effect of magnesium on horizontal cell activity in skate retina. *Nature (Lond.)*. **242**:101.

DUNN, R. F. 1966. Studies on the retina of the *Gecko coleonyx variegatus*. I. Visual cell classification of the gecko. *J. Ultrastruct. Res.* **16**:651.

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

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

FATEHCHAND, R., G. SVAETICHIN, K. NEGISHI and B. DRUJAN. 1966. Effects of anoxia and metabolic inhibitors on the S-potential of isolated fish retinae. *Vis. Res.* **6**:271.

HAGINS, W. A. 1972. The visual process: excitatory mechanisms in the primary receptor cells. *Annu. Rev. Biophys. Bioeng.* **1**:131.

HAGINS, W. A., R. D. PENN, and S. YOSHIKAMI. 1970. Dark current and photocurrent in retinal rods. *Biophys. J.* **10**:380.

HUBBARD, J. I., and Y. LØYNING. 1966. The effects of hypoxia on neuromuscular transmission in a mammalian preparation. *J. Physiol. (Lond.)*. **185**:205.

KATZ, B. 1966. Nerve, Muscle and Synapse. McGraw-Hill Book Company, New York. p. 75.

KLEINSCHMIDT, J. 1973. Adaptation properties of intracellularly recorded *Gekko* photoreceptor potentials. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag New York, Inc., New York. 219.

KORENBROT, J. I., and R. A. CONE. 1972. Dark ionic flux and the effects of light in isolated rod outer segments. *J. Gen. Physiol.* **60**:20.

LIEBMAN, P. A. 1972. Microspectrophotometry of Photoreceptors. *In* Photochemistry VII/I. J. A. Dartnall, editor. Springer-Verlag KG, Berlin. 590.

MURAKAMI, M., and W. L. PAK. 1970. Intracellularly recorded early receptor potential of vertebrate photoreceptors. *Vis. Res.* **10**:965.

PAWLEY, R. 1966. Geckos as Pets. T.F.H. Publications, Inc., Neptune City, New Jersey.

PEDLER, C., and R. TILLEY. 1964. The nature of the gecko visual cell: a light and electron microscopic study. *Vis. Res.* **4**:499.

PENN, R. D., and W. A. HAGINS. 1969. Signal transmission along retinal rods and the origin of the electroretinographic *a*-wave. *Nature (Lond.)*. **223**:201.

PINTO, L. H., and W. L. PAK. 1974. Light-induced changes in photoreceptor membrane resistance and potential in gecko retinas. II. Preparations with active lateral interactions. **64**: 49.

RUPPEL, H., and W. A. HAGINS. 1973. Spatial origin of the fast photovoltage in retinal rods. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag New York, Inc., New York. 257.

SILLMAN, A. J., H. ITO, and T. TOMITA. 1969 *a*. Studies on the mass receptor potential of the isolated frog retina. I. General properties of the response. *Vis. Res.* **9**:1435.

SILLMAN, A. J., H. ITO, and T. TOMITA. 1969 *b*. Studies on the mass receptor potential of the isolated frog retina. II. On the basis of the ionic mechanism. *Vis. Res.* **9**:1443.

SMITH, T. G., R. B. WUERKER, and K. FRANK. 1967. Membrane impedance changes during synaptic transmission in cat spinal motenurones. *J. Neurophysiol.* **30**:1072.

TAKEUCHI, A., and N. TAKEUCHI. 1960. On the permeability of end-plate membrane during the action of transmitter. *J. Physiol. (Lond.)*. **154**:52.

TOMITA, T. 1965. Electrophysiological study of the mechanisms subserving color coding in the fish retina. *Cold Spring Harbor Symp. Quant. Biol.* **30**:1072.

TOMITA, T. 1970. Electrical activity of vertebrate photoreceptors. *Q. Rev. Biophys.* **3**:179.

TOYODA, J. I., H. NOSAKI, and T. TOMITA. 1969. Light-induced resistance changes in single photoreceptors of *Necturus* and *Gekko*. *Vis. Res.* **9**:453.

WERBLIN, F. S., and J. E. DOWLING. 1969. Organization of the retina of the mudpuppy, *Necturus maculosus*. II. Intracellular recording. *J. Neurophysiol.* 32:339.

WINKLER, B. 1972. The electroretinogram of the isolated rat retina. *Vis. Res.* 12:1183.

YOSHIKAMI, S., and W. A. HAGINS. 1973. Control of the dark current in vertebrate rods and Cones. *In* Biochemistry and Physiology of Visual Pigments. H. Langer, editor. Springer-Verlag New York, Inc. New York. 245.