

Intracellular Recordings from Gecko Photoreceptors during Light and Dark Adaptation

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ABSTRACT Intracellular recordings were obtained from rods in the *Gekko gekko* retina and the adaptation characteristics of their responses studied during light and dark adaptation. Steady background illumination induced graded and sustained hyperpolarizing potentials and compressed the incremental voltage range of the receptor. Steady backgrounds also shifted the receptor's voltage-intensity curve along the intensity axis, and bright backgrounds lowered the saturation potential of the receptor. Increment thresholds of single receptors followed Weber's law over a range of about 3.5 log units and then saturated. Most of the receptor sensitivity change in light derived from the shift of the voltage-intensity curve, only little from the voltage compression. Treatment of the eyecup with sodium aspartate at concentrations sufficient to eliminate the *b*-wave of the electroretinogram (ERG) abolished initial transients in the receptor response, possibly indicating the removal of horizontal cell feedback. Aspartate treatment, however, did not significantly alter the adaptation characteristics of receptor responses, indicating that they derive from processes intrinsic to the receptors. Dark adaptation after a strongly adapting stimulus was similarly associated with temporary elevation of membrane potential, initial lowering of the saturation potential, and shift of the voltage-intensity curve. Under all conditions of adaptation studied, small amplitude responses were linear with light intensity. Further, there was no unique relation between sensitivity and membrane potential suggesting that receptor sensitivity is controlled at least in part by a step of visual transduction preceding the generation of membrane voltage change.

INTRODUCTION

There is now abundant evidence that many vertebrate photoreceptors undergo large changes of sensitivity during light and dark adaptation, and such receptor adaptation appears to be an important component of the adaptive process (Boynton and Whitten, 1970; Dowling and Ripps, 1971, 1972; Grabowski et al., 1972; Normann and Werblin, 1974). For example, a recent study on the skate showed that upon moderate to bright light adaptation and during slow (photochemical) dark-adaptation alterations in sensitivity of the

b-wave and ganglion cells closely parallel changes in receptor sensitivity (Green et al., 1975).

The mechanisms underlying receptor adaptation are largely unknown. Most of the findings so far reported are based on extracellular recordings and detailed information on the behavior of the membrane potential of photoreceptors during light and dark adaptation is lacking (but see Baylor and Hodgkin, 1974). Indeed, there remains some question as to what extent adaptational changes observed in receptor responses originate within the receptors. Baylor and his colleagues (1971) have shown that in the turtle retina individual cones receive signal input from horizontal cells as well as from other cones. This finding raises the possibility that adaptation phenomena observed in receptor recordings may not reflect intrinsic receptor events.

The present study was undertaken to learn more about the characteristics of receptor responses during light and dark adaptation and to determine if horizontal cell feedback is involved in receptor adaptation. We report here detailed measurements of *Gekko gekko* photoreceptor responses made during the course of light adaptation in normal eyecup preparations and in preparations treated with sodium aspartate. Sodium aspartate strongly depolarizes the horizontal cells and abolishes their response to light, thereby isolating the receptors from any light-induced horizontal cell influences which may normally be present (Dowling and Ripps, 1972; Cervetto and MacNichol, 1972). We also report a few observations of photoreceptor responses during the course of dark adaptation in the normal eyecup preparation.

The *Gekko gekko* eye was chosen for these experiments because its photoreceptors are rather large compared with other vertebrate photoreceptors, and we hoped that such large receptors would permit stable recordings for periods long enough to measure slow adaptation phenomena. A preliminary report of some of our results has appeared (Kleinschmidt, 1973).

METHODS

Adult *Gekko gekkos* (6–9 inches in length) were kept at room temperature in large terrarium tanks for periods of up to several weeks before use. Before each experiment the animal was dark adapted for at least an hour. The dissection and all subsequent preparatory procedures were carried out under dim red light. After enucleating and hemisecting the eye, its back part (i.e. the eyecup) was drained of most of the vitreous humor. The drained eyecup was transferred to a light-tight Faraday cage and placed in a fitted impression on a cotton pad saturated with reptilian physiological saline in contact with the reference electrode. The composition of the saline was: 130 mM NaCl, 4 mM KCl, 2 mM CaCl₂, 6 mM NaHCO₃. The electroretinogram (ERG) was continually monitored as an indicator of the physiological condition of the preparation. There was little deterioration of ERG sensitivity, amplitude, or waveform in most preparations during the 2–3 h of an experiment. All experiments were carried out at about 25°C.

For some experiments, the drained eyecup was filled with a saline of the following composition: 100 mM Na aspartate; 30 mM NaCl; 4 mM KCl; 2 mM CaCl_2 ; 6 mM NaHCO_3 . After 5–10 min, the solution was drained away from the eyecup. This treatment temporarily eliminated the *b*-wave of the ERG, thus isolating the receptors from influences of the more proximal cells (see Dowling and Ripps, 1972). After 1–2 h, the *b*-wave often reappeared. Aspartate concentrations of less than about 50 mM did not fully abolish the *b*-wave in the gecko. In the isolated perfused retina of *Bufo marinus*, on the other hand, 2 mM aspartate is sufficient to eliminate the *b*-wave (Dowling and Ripps, unpublished observations) which suggests the existence of a diffusion barrier to aspartate in the eyecup of the gecko.

Stimulating and Recording

Light stimuli were delivered to the eyecup from a conventional dual-beam photostimulator. The light, derived from a 45 W tungsten iodine quartz lamp (General Electric Quartzline Lamp, General Electric Co., Cleveland, Ohio, type Q6.6A/T 2-1/2/CL), passed through two separate optical systems equipped with neutral density filters and wedges and mechanical shutters. The two beams were superimposed and focused onto one end of a flexible fiber optic of 10-mm diameter. The other end of the fiber optic was positioned about 15 mm above the bottom of the eyecup. White light and full field illuminations were used in all experiments. Retinal irradiance with light between 350 and 650 nm from the unattenuated background beam was measured with a calibrated thermopile placed at the position of the eyecup. It was found to be about 1.3 mW/cm². This intensity corresponds to $\log I = 0$ on all figures. Flash duration was always 0.1 s.

The ERG was recorded from the eyecup with a cotton wick electrode which gently touched the vitreous. ERG signals were amplified with a low-level differential DC amplifier and displayed on an oscilloscope. Intracellular potentials were recorded with glass micropipette electrodes, pulled on a Livingston-type puller from Pyrex tubing of 0.8-mm OD and 0.4-mm inside diameter (ID) (type 7740, Corning Glass Works, Corning, N.Y.). They were filled with 2 M potassium acetate either by boiling under reduced pressure or by the glass fiber method (Tasaki et al., 1968). The best microelectrodes had resistances between 200 and 250 M Ω measured in the vitreous.

Microelectrodes were driven into the retina by means of a remote-controlled hydraulic microdrive with stepping motor (David Kopf Instruments, Tujunga, Calif.). The long axis of the microelectrode formed an angle of about 30° with the optical axis of the eyecup, and its tip was usually placed halfway up the wall of the eyecup. Under this condition of placement, the microelectrode made a slanted penetration into the retina, its axis forming an angle of between 45 and 80° with the long axis of the photoreceptors. This angle of approach optimized the probability of penetrating a photoreceptor. Typically, several receptors were penetrated successively in one track because of the slanted course of the microelectrode. Penetration of cells was facilitated by gently tapping on the table on which the experimental cage was mounted.

Potentials recorded with microelectrodes were led into a wide-band DC isolation amplifier (model BAK ELSA-4, Electronics for Life Sciences, Rockville, Md.) and displayed on a Tektronix 502 A dual-beam oscilloscope (Tektronix Inc., Beaverton,

Ore.). The vertical amplifiers of the oscilloscope were tapped at the cathode follower stage, and their voltage signals were frequency modulated by a dual-channel wide-band FM recording adaptor (model 2D, A. R. Vetter Co., Rebersburg, Pa.). The audio signals from the FM adaptor were recorded and stored on magnetic tape on a four-track stereo tape recorder (Revox Mark III G 36, Elpa Marketing Industries, Inc., New Hyde Park, N.Y.).

The different types of light-evoked electrical potentials which can be recorded intracellularly from vertebrate retinas have been classified by several authors (e.g. Werblin and Dowling, 1969; Kaneko, 1970), and their cellular origin has been identified through staining of the cell after electrophysiological characterization. In the *Gekko gekko* retina intracellular responses characteristic of ganglion cells were encountered on many penetrations, those typical of amacrine cells rarely, and responses that had characteristics of bipolar or horizontal cell potentials, more rarely still. The most frequently obtained intracellular recordings were from distal cells of only one type as judged by the criteria of polarity, latency, details of waveform, maximum amplitude, and voltage-intensity relation of the responses. They conformed in all major respects to the more recent descriptions of photoreceptor responses given by other workers (e.g. Baylor and Fuortes, 1970; Fain and Dowling, 1973; Brown and Pinto, 1973). Although one type of horizontal cell (L type) and one type of bipolar cell generate responses with many characteristics similar to those of photoreceptors, these cells can be ruled out as the origin of potentials recorded in the distal *Gekko gekko* retina because their perikarya measure only about 5–7 μm in diameter compared with diameters of more than 10 μm for the large rods. Thus the probability of recording from horizontal or bipolar cells is rather low in this retina. Furthermore, in the *Gekko gekko* retina the receptor cell layer occupies the distal one-third of the entire thickness of the retina, and it was exclusively within that layer that the recordings were obtained. All observations reported in this paper were made on this one class of distal cell which we identify by the above criteria as photoreceptors (also see the following section).

Gecko Visual Cells

According to Underwood (1951), there are three classes of visual cells in the retinae of nocturnal geckos. The majority of the receptors are double rods with a large chief member and a smaller accessory member (called type B by Underwood). Less frequent are large single rods (type A) and least in frequency of occurrence are double rods with members of roughly equal size (type C). Walls (1934) considered the visual cells of nocturnal geckos to be rods "transmuted" from cones. He suggested that the extant nocturnal geckos derive from diurnal ancestors with pure cone retinas and that they adapted to the nocturnal habitat by evolving rod-like outer segments and abandoning the oil droplets while retaining other morphological characteristics of cones and the basic organization of an all-cone retina. Electron microscopy lends some support to this notion (Pedler and Tilly, 1964; Hedden, 1973). For example, the synaptic terminals of *Gekko gekko* photoreceptors closely resemble the complex pedicles of cones. However, the outer segments clearly show rod ultrastructure. Their shape is cylindrical and all saccules except for a few basal ones are detached from the plasma membrane.

Crescitelli (1963) found by extraction two photosensitive pigments in the retina of

the *Gekko gekko*, a dominant pigment which absorbs maximally at 521 nm and a minor pigment with absorption maximum at 478 nm. Liebman (1972) observed by microspectrophotometry of isolated *Gekko gekko* outer segments that the majority of the receptors contain a 518-nm pigment. He also determined that the chief member of the class B double rod has the 518-nm pigment while its accessory member contains the short-wavelength pigment, placed by Liebman at 467 nm. His results suggest that there are probably only two basic types of photoreceptors in the *Gekko gekko* retina: a large rod with a 518-nm pigment (identical with Underwood's type A) and a more slender rod with a 467-nm pigment which occurs paired either with the large rod (forming the type B double rod) or with another member of its own kind (forming the type C double rod). Since our receptor recordings were so uniform in their characteristics (e.g. details of waveform, sensitivity, voltage-intensity function) as to suggest that they sampled only a single population of visual cells and since in the *Gekko gekko* the smaller rod is only about half the diameter of the larger rod (Dunn, 1969), it seems very likely that our recordings were always from the large rod.

RESULTS

Responses of Dark-Adapted Receptors to Brief Flashes

Penetration of a photoreceptor in the gecko retina was indicated by an abrupt potential drop of 30–40 mV magnitude and by the appearance of transient, graded, hyperpolarizing potential changes in response to flashes of light. Intracellular recordings for periods longer than a few minutes were obtained from about 250 receptors of which about 25% were stable long enough to yield data that could be used in this study. Results reported in this paper are based on data from 63 such cells (18 with aspartate).

Fig. 1 shows typical intracellular responses from a dark-adapted receptor to 0.1-s flashes of various intensities. For intensities up to about 2.5 log units above dark-adapted threshold (defined as the intensity of test flash which generates a 0.5-mV criterion response) responses were purely monophasic. At higher intensities responses displayed an initial transient wave which became very prominent for saturating and supersaturating intensities. The latency of the responses and their rise time decreased with increase of light intensity. Threshold responses had latencies of about 150 ms and a rise time of about 400 ms. With a saturating flash the latency decreased to about 30 ms and the rise time to about 65 ms. Time-course of recovery from peak back to base line also depended on light intensity. Threshold responses recovered within about 600 ms whereas responses elicited with bright flashes returned to base line only over the course of several seconds. Although the response amplitude saturated at an intensity about 4 log units above threshold, the latency and rise time of the receptor response continued to decrease with increase of stimulating intensity, and recovery became greatly prolonged.

As already noted the receptor response has a complex waveform when elicited with bright flashes. There is reason to suspect that this complexity may

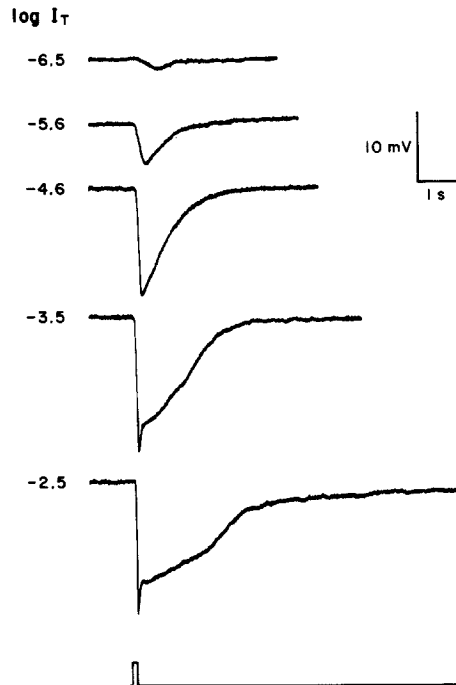


FIGURE 1. Intracellular responses from a dark-adapted receptor in the normal preparation to 0.1-s test flashes of different intensities. Sufficient time was allowed between flashes so that the receptor would fully dark adapt between flashes. Flash intensity is indicated to the left of each trace. Log I values refer to the attenuation by neutral density filters of the full intensity (i.e. log $I = 0$) available from the photostimulator. Log $I = 0$ corresponds to about 1.3 mW/cm². Note the fast initial transient in the receptor response at the brighter intensities.

not entirely derive from receptor events. For example, the fast initial transient wave observed in these responses resembles the initial transient observed in turtle cone responses elicited under comparable stimulus conditions. Such transients in the turtle were shown to be caused by horizontal cell feedback onto the receptors (Baylor et al., 1971). If the transients in gecko receptor responses are similarly shaped by feedback from the horizontal cells, treatment of the retina with Na aspartate should eliminate them (see Introduction). To test this hypothesis, receptor recordings were obtained from eyecups treated with 100 mM aspartate which in this preparation abolishes the *b*-wave of the ERG for at least 1–2 h (see Methods).

Fig. 2 shows receptor responses recorded under these conditions. Responses to dim flashes (log $I_T = -6.5$, -5.6 , and -4.6) were virtually identical to responses elicited in the normal eyecup with dim flashes. However, for bright flashes (log $I_T = -3.5$ and -2.5), waveforms were dramatically different from those in the normal preparation. The responses were simpler in shape

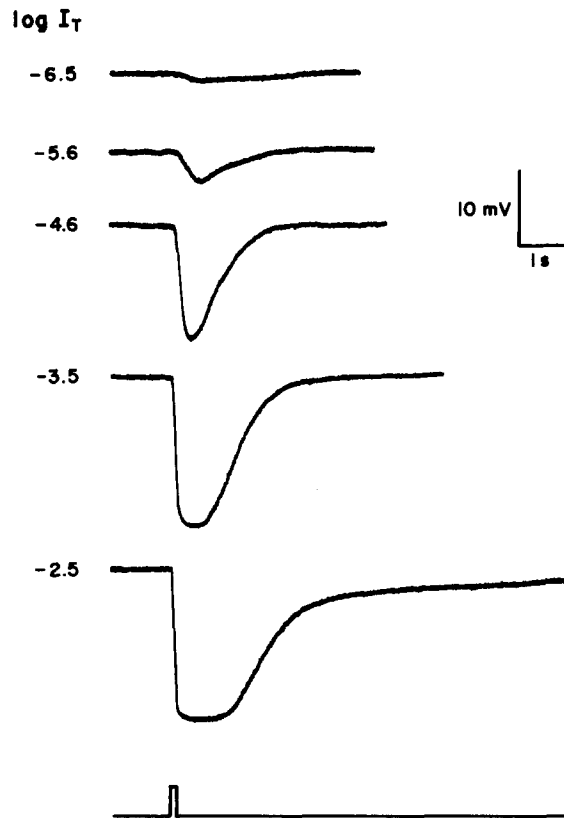


FIGURE 2. Intracellular responses to 0.1-s test flashes from a dark-adapted receptor in the aspartate-treated eyecup. Waveforms are simple and monophasic at all intensities; no initial transients are present.

with no trace of an initial transient. Also, peak amplitudes often were somewhat larger. With aspartate, the maximum peak amplitude sometimes exceeded 30 mV, compared with values of at most 20 mV in normal receptors. Resting potentials in the presence of aspartate were between -15 and -25 mV, which contrast with resting potential values of between -30 and -40 mV for receptors in the normal eyecup. Responses in the aspartate-treated eyecup, therefore, rise from a more depolarized level of membrane potential than do normal responses, but they appear to saturate at roughly the same absolute potential. This potential (about -55 mV) appears to be a fixed ceiling potential which cannot be exceeded in the direction of further hyperpolarization. This held for all states of light and dark adaptation tested in both treated and untreated preparations, although under bright background illumination and at the beginning of dark adaptation after strong (bleaching) light adaptation receptor responses were found to saturate at a *less* hyperpolarized potential level (see below).

The abolition of receptor transients by aspartate is consistent with the hypothesis that in the gecko, as in the turtle (Baylor et al., 1971), these transients are caused by horizontal cell feedback onto the receptors. However, Brown and Pinto (1973) have shown that similar transients in responses from the rods of *Bufo marinus* are unaffected by concentrations of aspartate (2 mM) which when applied to the isolated retina produce depolarization and inactivation of the horizontal cells. Thus, the change in waveform which we observed in gecko rods could also be due to a direct effect of aspartate on the receptors. It is interesting to note, though, that a concentration of aspartate (10 mM) five times that required to abolish the *b*-wave in the perfused isolated retina of *B. marinus* still does not affect receptor waveforms in this animal (Lipton and Dowling, unpublished observations). In the gecko eyecup a concentration of aspartate (50 mM) which is just sufficient to eliminate the *b*-wave, fully abolishes receptor transients.

Fig. 3 shows a plot of the peak amplitude of receptor responses to 0.1-s test flashes as a function of the logarithm of light intensity for dark-adapted receptors in the normal and in the aspartate-treated preparation. The data points have been fitted with the hyperbolic function $V/V_{\max} = I_T/(I_T + \sigma)$, first used by Naka and Rushton (1966) to fit voltage-intensity data for S-potentials in the tench. Fig. 3 plots data from one cell each under the two experimental conditions. Similar voltage-intensity curves were determined for 13 other dark-adapted cells in the normal retina from which very stable recordings were obtained, and all could be closely fitted with the same function. However, the value of σ varied over a range of about 0.5 log units resulting in a parallel shift of the curves along the abscissa over that range. Data points obtained from seven stable dark-adapted cells under aspartate could also be closely fitted with this same function, and Fig. 3 shows voltage-intensity data from one such cell. However, receptors under aspartate were more variable and in five other cells steeper voltage-intensity curves were found in which V rose from 5 to 95% of V_{\max} over an intensity range of only about 2.1 log units (in contrast with 2.6 log units for the cells that could be fitted with the template function).

For dim test flash intensities (i.e. when I_T is small compared with σ) the above function simplifies to the linear relation $V = V_{\max} \cdot I_T/\sigma$. Thus if this function correctly describes visual transduction in vertebrate photoreceptors in general, the receptor response should be directly proportional to light intensity over the initial portion of its instantaneous range. That this is so in the gecko is seen clearly in the inset of Fig. 3 in which the data from the normal receptor were replotted in a double logarithmic plot. The initial straight line segment of the curve has a slope of one, indicating a linear relation between voltage and intensity. The maximum amplitude of this receptor was 19.4 mV; the voltage-intensity curve was linear up to response amplitudes of about 3 mV.

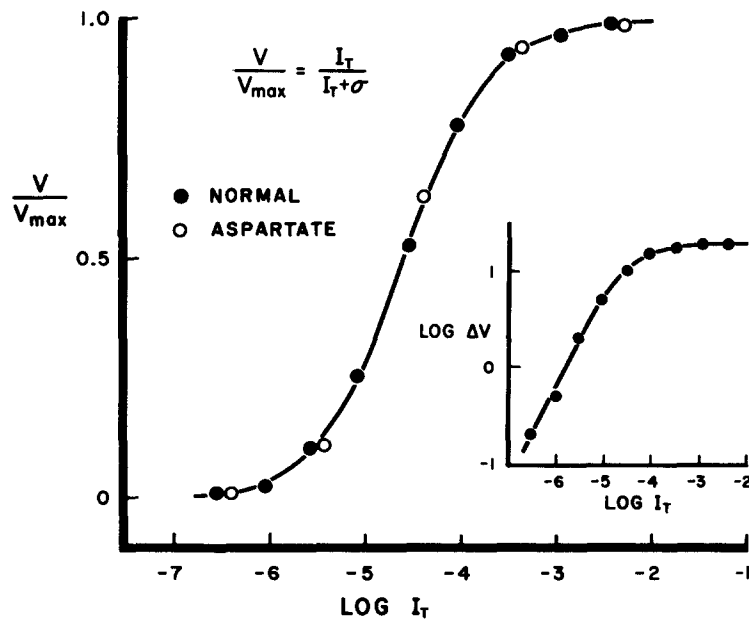


FIGURE 3. Voltage-intensity data for intracellular responses from two dark-adapted receptors to 0.1-s test flashes in the normal preparation (filled circles) and in the aspartate-treated preparation (open circles). On the ordinate are plotted amplitudes of the peak of response normalized with respect to the maximum peak amplitude V_{\max} which was 19.4 mV for the normal receptor and 23.4 mV for the receptor under aspartate. The solid line plots the function $V/V_{\max} = I_T/(I_T + \sigma)$. Data points from the receptor in the aspartate-treated eyecup have been shifted to the right along the abscissa by 0.15 log units so that all points could be fitted with one curve. Inset shows the data from the normal receptor replotted in double logarithmic coordinates. The slope of one of the initial segments of the curve in this plot indicates a linear relation between voltage and intensity. Similar voltage-intensity curves were obtained from 14 normal cells and 7 cells under aspartate.

For higher intensities, the response deviates from linearity and with bright flashes it saturates. The same behavior has been observed in rat (Penn and Hagins, 1972), skate (Dowling and Ripps, 1972), and toad rods (Fain, 1975), and in the rods and cones of the mudpuppy (Fain and Dowling, 1973), and turtle (Baylor and Hodgkin, 1974).

Light Adaptation

The decrease of sensitivity that occurs when the visual system is exposed to a steady background light is termed light adaptation. The following section first describes the characteristics of receptor responses to the onset and termination of steady background illumination ("background responses") and then describes the characteristics of responses to test flashes which were superimposed upon the backgrounds ("increment responses").

BACKGROUND RESPONSES Typical intracellular responses to illumination of 10-s duration from a dark-adapted receptor in the normal preparation are shown in Fig. 4. Upon the onset of the background light the receptor rapidly hyperpolarized, and the potential reached a maximum within a second. However, with all backgrounds except the dimmest ($\log I_B = -6.5$) the

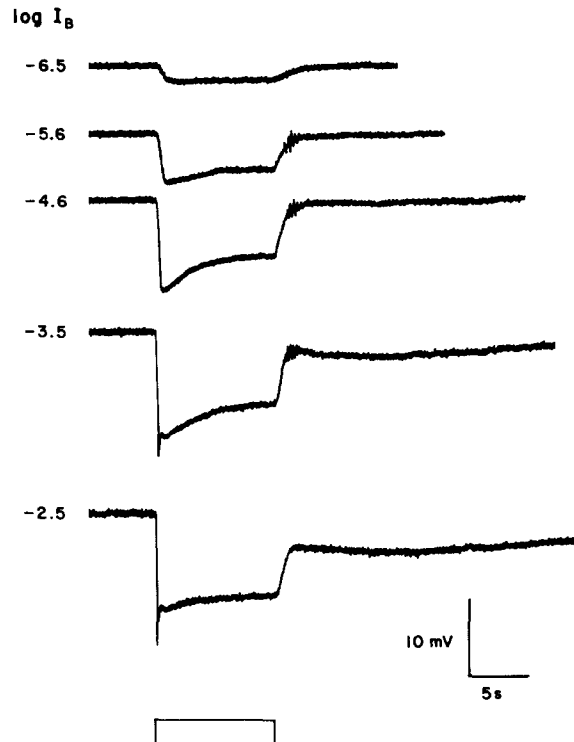


FIGURE 4. Intracellular responses from a dark-adapted receptor in the normal preparation to illumination of various intensities and of 10-s duration. Such background responses have two components: an initial peak and a later sustained plateau. Note the prominent initial transients for the brightest two backgrounds. The oscillations during the decay phase of the responses were not usually observed.

voltage maximum was not maintained. Rather the potential decayed and eventually reached a stable plateau of hyperpolarization. Thus two components of the receptor response to steady background illumination can be distinguished, a peak and a plateau.

As shown in Fig. 4, the decay from peak to plateau occurred in a single slow phase for dim backgrounds ($\log I_B = -5.6$ and -4.6). For bright backgrounds ($\log I_B = -3.5$ and -2.5) this transition consisted of two separate phases, an initial very rapid transient of less than 0.1-s duration followed by a much slower recovery of potential lasting many seconds. Fig. 5 shows a series of background responses from a receptor in the aspartate-

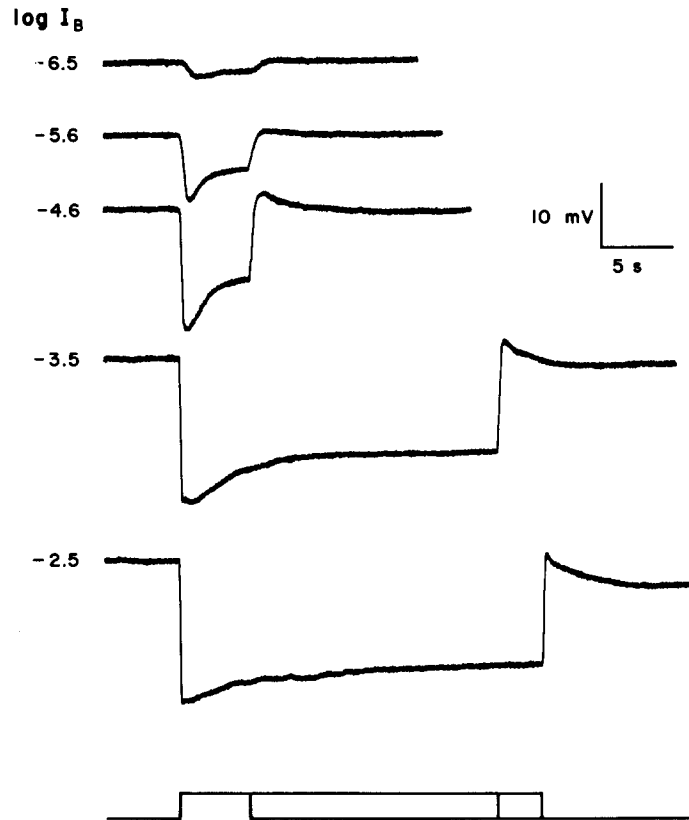


FIGURE 5. Intracellular responses from a dark-adapted receptor in the aspartate-treated preparation to steps of illumination of various intensities (indicated to the left of each trace) and durations (indicated at the bottom of the figure). The decay of membrane potential in the light occurs in a single smooth transition for all backgrounds. At the offset of light, the membrane potential often rebounded sharply toward the dark-adapted resting potential as shown here, especially when the light had been on for a long duration. In other cases, however, recovery was on a similar time scale as shown in Fig. 4 for a normal receptor.

treated eyecup. Under these conditions only a slow decay from peak to plateau is observed with all backgrounds. This result indicates that the slow decay is of receptor origin, and it further suggests that the initial transient in the normal response may be due to receptor interaction with the horizontal cells.

The duration of the slow decay of the background response is dependent on the background intensity. For a dim background ($\log I_B = -5.6$) it lasted about 5 s; for a bright background ($\log I_B = -2.5$) it increased to more than 15 s (note that in Fig. 4 the sustained plateau potential had not yet been reached when the brighter background lights were turned off). When the background light was extinguished, the membrane potential returned to the dark-adapted level with a time-course that also was dependent upon the

intensity of the previous illumination. For the dimmest backgrounds, the membrane potential returned to base line within a few seconds whereas for the brighter backgrounds the return had two phases and took up to several minutes (only the beginning of the later slow return phase is shown in Figs. 4 and 5). *Off* transients, as shown in Fig. 5, were frequently observed in aspartate-treated receptors but were never seen in untreated receptors. Also, in a few cells under aspartate (e.g. Fig. 5) the fast return phase occurring immediately after extinction of a bright background was somewhat more rapid than in cells in the normal preparation.

Fig. 6 shows a plot of the peak and the plateau potential from three receptors in the normal dark-adapted preparation as a function of the logarithm of background intensity. Amplitudes are normalized with respect to the maximum peak amplitude (V_{max}) obtained from the dark-adapted receptor with a saturating flash. The voltage-intensity relation for the initial peak of responses to *steps* of light (i.e. to steady backgrounds) is shifted to the left on the intensity axis, but it is significantly flatter than the voltage-intensity relation for the peak of responses to 0.1-s *flashes* of light (broken line, replotted

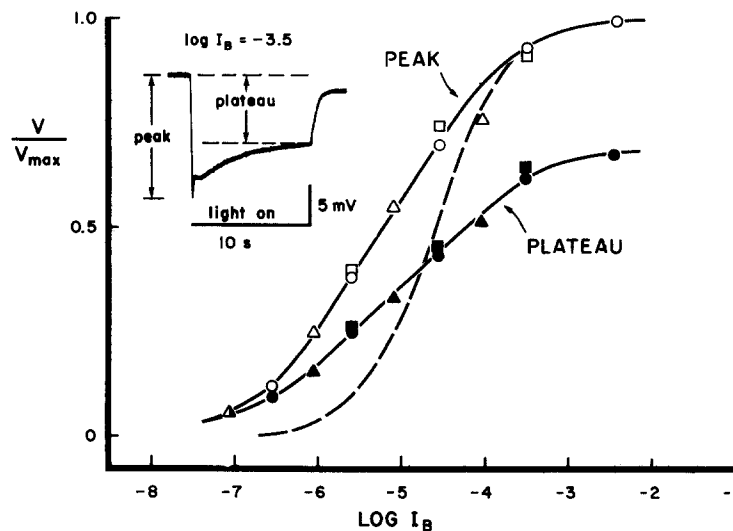


FIGURE 6. Voltage-intensity data for the peak (open symbols) and the plateau potential (closed symbols) of the background response from three dark-adapted receptors in the normal preparation. The inset illustrates how the peak and the plateau potential were measured. On the ordinate are plotted the peak and plateau potential normalized with respect to the maximum peak potential obtained with a saturating 0.1-s flash. Curves were drawn by eye through the data points. The dashed line is taken from Fig. 3 and represents the voltage-intensity function for the peak of responses to 0.1-s flashes. The shape and relative positions of the three curves along the abscissa were fairly constant from cell to cell, although the whole set of curves could be shifted along the abscissa by up to 0.7 log units.

from Fig. 2). Thus both functions saturate at about the same intensity. The shift of the curve to the left with a step of light is to be expected from temporal summation beyond the 0.1-s duration of a flash, whereas the flattening of the curve most likely reflects the early onset of the adaptation process at the brighter backgrounds. The plateau potential increases even less with background intensity than does the peak potential and its voltage-intensity curve is approximately logarithmic over a range of about 3 log units. Similar sets of curves were obtained from four other receptors. The shape and relative positions of the three curves were quite constant from cell to cell (i.e. as in Fig. 6) but the position of the whole set of curves varied along the abscissa over a range of about 0.7 log units.

Voltage-intensity curves for the peak and the plateau of the background response in aspartate-treated receptors were generally similar. Occasionally, however, the voltage-intensity curve for the plateau was much steeper in the aspartate-treated preparation and did not level off at about $0.7 V/V_{\max}$. Rather, it continued to grow and reached the receptor's saturation potential at about $\log I_B = -2.5$. In those cases, there was no slow relaxation of membrane potential in bright light but rather the membrane potential stayed at its maximally hyperpolarized level.

INCREMENT RESPONSES The absolute saturation potential of the dark-adapted receptor appears to be a fixed ceiling potential (see above). Thus, the initial peak of the receptor response to backgrounds causes a substantial reduction of the voltage range available for increment responses. The decay of membrane potential after the peak brings about an increase of this voltage range. Thus the recovery of membrane potential with time in the light serves as a mechanism to restore the receptor's responsiveness.

This phenomenon is clearly evident in Fig. 7 which shows increment responses evoked by 0.1-s test flashes of fixed intensity presented immediately after the onset of steady background lights. In all cases, the amplitude of increment responses grew in proportion to the expansion of the incremental voltage range brought about by recovery of the membrane potential. For the dimmer background (top records), recovery of membrane potential and concurrent growth of increment responses were identical for both normal and aspartate-treated receptors. With the brighter background and test flashes (bottom records), the initial transients were absent in the receptor treated with aspartate. However, the slow decay of membrane potential and increase in amplitude of increment responses with time in the light were similar in both cases. Thus aspartate treatment which in other retinas has been shown to eliminate horizontal cell activity alters receptor response waveforms in the gecko but does not significantly affect the receptors' adaptation behavior.

To study the effect of backgrounds on increment responses during steady-

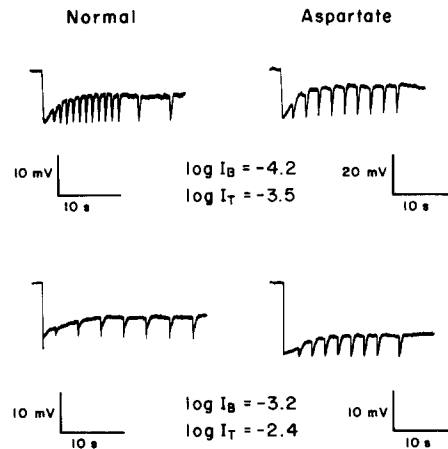


FIGURE 7. Background responses and increment responses from receptors in the normal and in the aspartate-treated eyecup at two different sets of background intensity (I_B) and test flash intensity (I_T). The background light was turned on immediately before the rapid hyperpolarization. 0.1-s test flashes of constant intensity were then superimposed upon the background at regular intervals. The fast initial transient observed in the normal receptor response at the brighter adapting intensity is absent in the aspartate-treated preparation (bottom records). Slow recovery of membrane potential and growth of increment responses, however, are unaffected by aspartate.

state light adaptation, test flashes of different intensities were superimposed upon the background at a time when the membrane potential had stabilized. Fig. 8 shows a series of increment responses obtained in the presence of a background of moderate intensity from a normal receptor and from a receptor in the aspartate-treated retina. The decay of increment responses was considerably faster than the decay of responses from dark-adapted receptors whether the two were compared for the same test flash intensity or for the same response amplitude (note that the time scale in Fig. 8 is the same as in Figs. 1 and 2). Increment responses after aspartate treatment differed from normal increment responses in that they lacked the fast initial transient and had larger amplitudes. These are the same differences that were found between responses of dark-adapted receptors under the two experimental conditions.

Representative voltage-intensity data for increment responses to 0.1-s flashes from two receptors in the untreated retina are plotted in Fig. 9 for six different backgrounds, along with the dark-adapted voltage-intensity function for one of the two receptors. On the ordinate are plotted peak potentials of dark-adapted responses and of increment responses measured from the level of the dark-adapted resting potential, expressed as a fraction of the maximum response amplitude obtained from the dark-adapted cell with a saturating flash. The abscissa plots the logarithm of intensity of the test flashes. Incre-

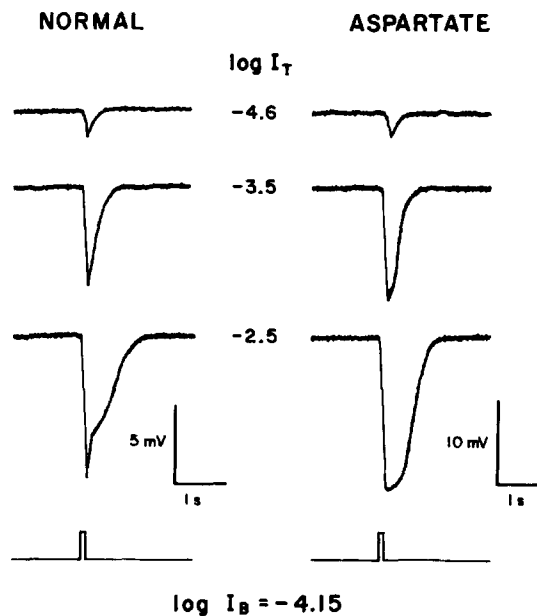


FIGURE 8. Increment responses to 0.1-s test flashes of different intensities (I_T) superimposed upon a steady background of moderate intensity ($\log I_B = -4.15$) during the steady state of light adaptation (i.e. at a time when the membrane potential had stabilized at the plateau level). To the left, responses from a normal receptor, to the right, responses from a receptor in the aspartate-treated preparation.

ment responses are plotted from the sustained levels of hyperpolarization associated with the different backgrounds (circles with crosses, dashed lines). Voltage-intensity curves were usually measured about 2 min after the membrane potential had stabilized. In many cells, we measured the incremental voltage-intensity curve for a given background repeatedly at various times (between 0 and 5 min) after the membrane potential had stabilized. No differences were found between these curves.

For the dimmer backgrounds ($\log I_B = -5.1$ and -4.15) increment responses saturated at a potential level close to the dark-adapted saturation potential. In the presence of backgrounds brighter than $\log I_B = -4.15$, however, the saturation potential for increment responses was found to be at a less hyperpolarized level than that of the dark-adapted cell. This lowering of the saturation potential was more pronounced the brighter the background. A similar lowering of the saturation potential in the presence of bright backgrounds has also been observed by Brown and Pinto (1973) and by Fain (1974) in the rods of *B. marinus*. The very bright background of $\log I_B = -1.15$ saturated the receptor in the sense that no increment responses could be generated with the brightest light available, even after several minutes of continuous background illumination. This was observed even though the

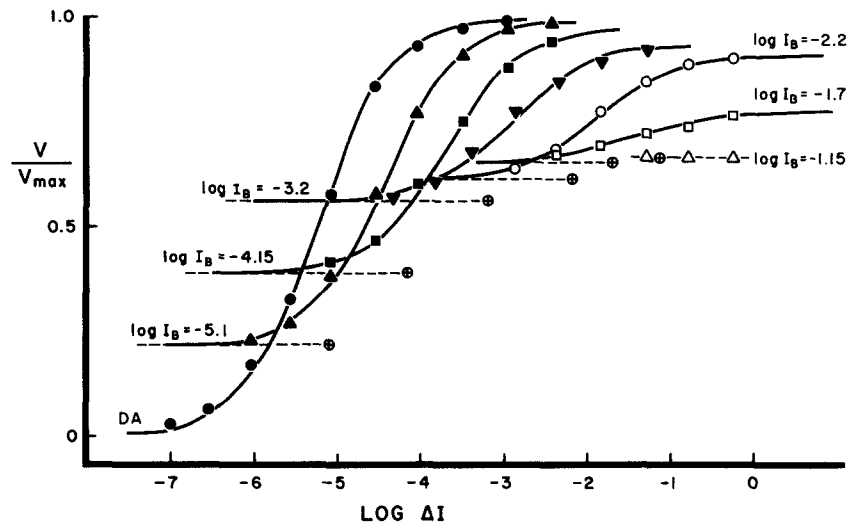


FIGURE 9. Voltage-intensity data from two receptors in the normal preparation for responses to 0.1-s test flashes in the dark-adapted state (DA) and to 0.1-s test flashes superimposed upon six different backgrounds. On the ordinate are plotted peak amplitudes of the responses measured from the dark-adapted resting potential and normalized with respect to the maximum peak amplitude obtained from the dark-adapted cell with a saturating flash (V_{\max} was 16.3 mV in the cell plotted with closed symbols and 18.0 mV in the cell plotted with open symbols). On the abscissa is plotted the intensity ΔI of the test flashes. Dashed lines and circles with crosses indicate the normalized plateau potentials prevailing for the various backgrounds. Solid lines are plots of the function $\Delta V/\Delta V_{\max} = \Delta I/(\Delta I + \sigma)$ for six sets of values of the parameters ΔV_{\max} and σ . See text for details.

receptor's membrane potential was poised at a level about 5 mV less hyperpolarized than the dark-adapted saturation potential. We observed such saturation of receptors with $\log I_B = -1.15$ in each of the four cells which we tested with that background.

Fig. 9 shows that steady backgrounds have two major effects on the voltage-intensity relation for responses to 0.1-s flashes. The first effect is to compress the voltage-intensity curve vertically, i.e. to decrease the maximum amplitude of increment responses. This reduction of the voltage range available for increment responses is brought about by a combination of two phenomena: first, all background lights induce sustained hyperpolarizing potentials from which increment response rises; and second, bright backgrounds lower the saturation potential of the receptor.

The other major effect of steady backgrounds is to shift the voltage-intensity curve along the abscissa toward higher intensities. As a result of this shift, the voltage-intensity curve successively covers different ranges of light intensity. The receptor's instantaneous dynamic range for increment responses (i.e. from threshold to saturation) at all backgrounds tested is the same in extent

as that for responses from the dark-adapted receptor, i.e. about 4 log units. However, because of the successive shift of the voltage-intensity curve along the intensity axis caused by backgrounds of increasing intensity, the total range of light intensity over which the receptor can respond is very much enlarged. Incremental voltage-intensity curves were obtained in the normal preparation from at least three cells for each of the six backgrounds shown in Fig. 9. The positions and hence the extent of shift of the curves was in each case within 0.25 log units of the respective positions in Fig. 9. In the aspartate-treated eyecup, the voltage-intensity curves for increment responses were likewise found to become both compressed and shifted along the abscissa by backgrounds in a manner similar to receptors in the untreated eyecup. This shift of the receptor's instantaneous working range by backgrounds conforms with the notion originally derived from psychophysical experiments that visual adaptation represents a range-resetting phenomenon (Craik, 1938; Byzov and Kusnezova, 1971). It also shows that some of the range resetting in the visual system occurs already in the receptors.

Response Compression Vs. Shift of Instantaneous Range: Quantitative Considerations

The following section will analyze more quantitatively the relative extent to which compression of the voltage range and shift of the intensity range contribute to the changes in receptor sensitivity that are observed during light adaptation. The curves in Fig. 9 are plots of the expression $\Delta V/\Delta V_{\max} = \Delta I/(\Delta I + \sigma)$, which is identical in form with the function which describes responses of dark-adapted receptors to 0.1-s flashes (see above). ΔV is the peak amplitude of the increment response, measured from the steady level of membrane potential and ΔV_{\max} is its maximum value. ΔI is the intensity of the test flash which was superimposed upon the background I_B and which generated the increment response. ΔV_{\max} and σ are parameters which are a function of background intensity. In all states of light adaptation with or without aspartate, data points could be fitted with the above function using values of ΔV_{\max} and σ determined by the experiment. Thus, a complete quantitative description of the voltage-intensity relation for 0.1-s test flashes for any given state of adaptation can be provided by specifying the values of the parameters ΔV_{\max} and σ and the prevailing level of membrane potential.

The parameter ΔV_{\max} in such a description serves as a quantitative measure of the vertical compression of the voltage-intensity relation. Variation of ΔV_{\max} with background intensity is determined by the variation in the magnitude of the plateau potential and by the lowering of the saturation potential associated with any given background. The parameter σ is obtained experimentally as the intensity of a test flash which evokes an increment response of magnitude $0.5 \Delta V_{\max}$. σ is a direct measure of the lateral position of the

voltage-intensity curve in the semilogarithmic plot, and its variation with background intensity serves as a quantitative measure of the extent of adaptive lateral shift.

Fig. 10 displays the data from Fig. 9 in a double logarithmic plot and shows that for all backgrounds tested small amplitude responses were directly proportional to light intensity. This is evident in the graph by the fact that the initial segment of each voltage-intensity curve has a slope of one. The

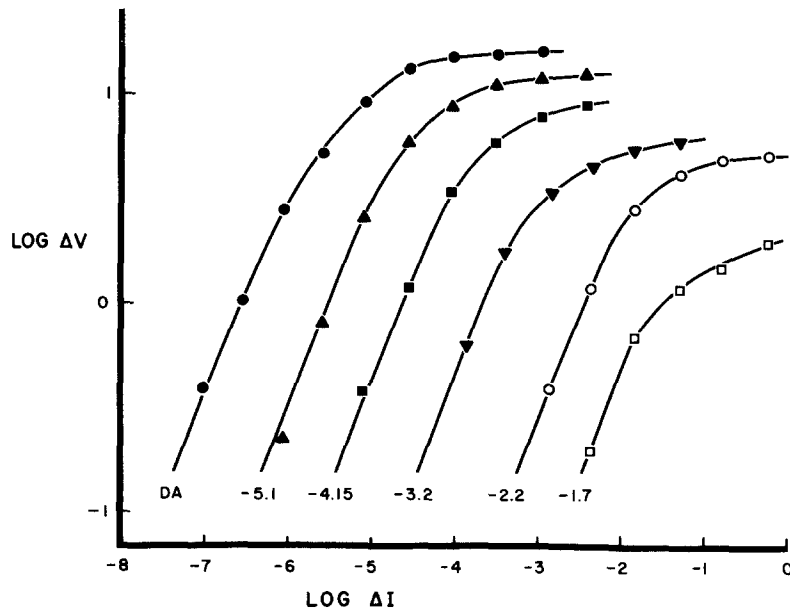


FIGURE 10. Voltage-intensity data from the two receptors in Fig. 9 displayed in a double logarithmic plot. The ordinate plots peak amplitude of responses in millivolts. Peak amplitudes of increment responses were measured from the prevailing steady level of membrane potential. The initial segment of all curves has a slope of 1, indicating linearity of small amplitude responses with light intensity in the dark-adapted state and for all backgrounds studied.

different positions of the curves for the different backgrounds indicate greatly differing values for the constant of proportionality between voltage and intensity for small amplitude responses. Since the linear region of the voltage intensity curve is described by the expression $\Delta V = \Delta V_{\max} \cdot \Delta I / \sigma$, the constant of proportionality is given by $\gamma = \Delta V / \Delta I = \Delta V_{\max} / \sigma$. γ has the dimension of sensitivity and could for example be expressed in units of millivolt response per photon incident or absorbed. Sensitivity, however, is usually defined as the reciprocal of threshold which in turn is defined as the energy required to produce a response of constant small magnitude. Since it is more useful for comparative receptor physiology to define sensitivity as magnitude of re-

sponse per unit of light intensity (e.g. per photon absorbed), we will use γ as a measure of receptor sensitivity and we will call it the "linear incremental sensitivity." With this definition of receptor sensitivity, it is possible to determine quantitatively from the experimental data the relative contribution which voltage range compression (measured by ΔV_{\max}) and resetting of intensity range (measured by σ) make to receptor sensitivity changes during light adaptation.

The experimental data in Fig. 9 show that a background of $\log I_B = -4.15$ reduced ΔV_{\max} roughly twofold with respect to the dark-adapted state, and that it shifted the voltage-intensity relation to the right by about 1.5 log units, i.e. by a factor of about 30. This particular background, therefore, reduced the sensitivity about 60-fold with respect to the dark-adapted sensitivity, two-fold through compression of the incremental voltage range and 30-fold through lateral shift. A background of $\log I_B = -3.2$ compressed the voltage range three-fold and shifted the response characteristic to the right by about 2.2 log units (a factor of about 160), the total reduction of sensitivity being roughly 500-fold. In the other receptor plotted in Fig. 9 (open symbols), a background of $\log I_B = -1.7$ compressed the voltage range about 10-fold and shifted the response characteristic by 3.5 log units to the right from its dark-adapted position, causing a total sensitivity reduction by a factor of roughly 30,000.

Fig. 11 illustrates graphically the absolute contribution of voltage compression and shift of intensity range to receptor adaptation. Data points on the curve labeled σ are the average values of thresholds that would have been measured if the voltage-intensity curve had merely been shifted to the right without concomitant voltage compression. The points were obtained by linearly expanding the experimental incremental voltage-intensity curves along the voltage scale such that their ΔV_{\max} was equal to the dark-adapted value and by then determining the light intensity that would have elicited a 0.5-mV criterion response from that curve. This intensity was called threshold and is plotted against the background intensity. Data points on the curve labeled ΔV_{\max} are average values of thresholds which we would have measured if the voltage-intensity curve had not been shifted from its dark-adapted position but merely been compressed to the extent actually observed for the different backgrounds. Threshold criterion for this curve, too, was a response of 0.5-mV peak amplitude. Addition of the log threshold changes produced by voltage compression and by range resetting yields a curve (not shown in Fig. 11) which is superimposable upon the increment threshold function shown in Fig. 12. Fig. 11 demonstrates that for all backgrounds the sensitivity change was derived primarily from the lateral shift of the voltage-intensity curve (i.e. from the change in σ) rather than from the compression of the voltage range (i.e. from the change in ΔV_{\max}). In other words, response compression

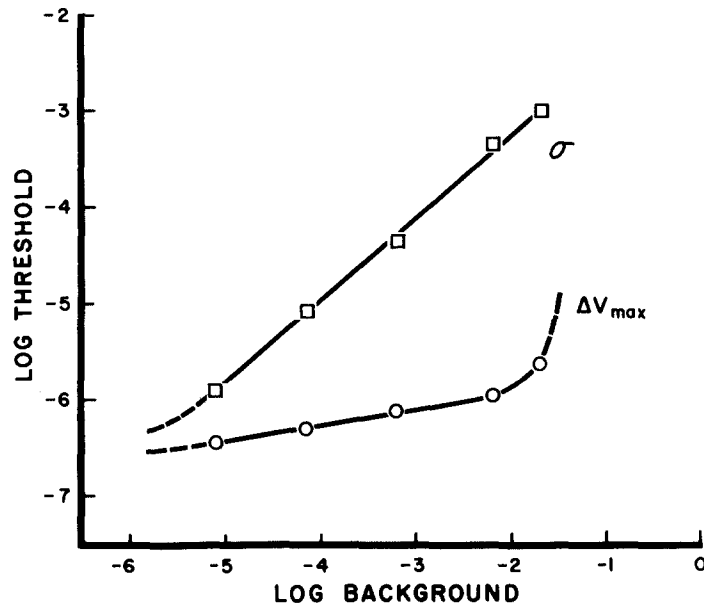


FIGURE 11. Extent of threshold change due to voltage compression (curve labeled ΔV_{\max}) and to shift of the voltage-intensity function (curve labeled σ) during light adaptation. Data points are average values from at least three cells for each background. See text for details.

(i.e. the change in ΔV_{\max}) contributes only in a minor way to the receptor sensitivity reduction that occurs in light adaptation.

INCREMENT THRESHOLD FUNCTION Psychophysical results on sensitivity changes in light adaptation are customarily displayed in the form of an increment threshold function. This function is a plot of the logarithm of the increment threshold against the logarithm of background intensity. Fig. 12 shows the increment threshold function for gecko photoreceptors obtained by selecting an increment response of 0.5-mV amplitude as a criterion of threshold. Closed circles show average values of threshold from several cells for each background in the normal preparation. Bars indicate the range of threshold values found in these cells. For a range of about 3.5 log units of background intensity, data points fall on a straight line which has a slope of 1 indicating that gecko photoreceptors over that range of backgrounds adapt to light in conformity with Weber's law. This experimental generalization states that threshold varies in direct proportion with background intensity. Under the condition that the response chosen as a criterion of threshold lies within the linear response range of the receptor, threshold is simply inversely related to sensitivity. This condition was fulfilled for the results displayed in Fig. 12. Thus, the sensitivity of gecko photoreceptors varies in inverse proportion with background intensity over a range of about 3.5 log units. With very bright backgrounds (greater than $\log I_B = -2.2$) thresholds increased

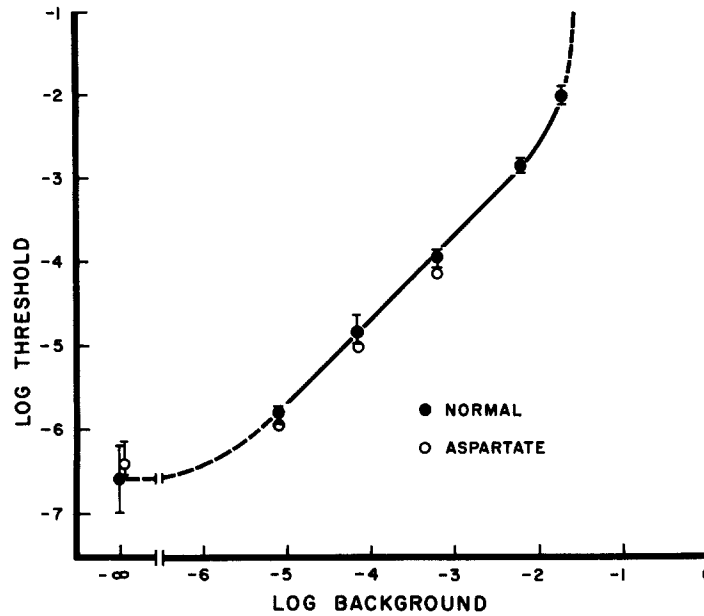


FIGURE 12. Increment threshold function of *Gekko gekko* photoreceptors in the normal and in the aspartate-treated preparation. Threshold criterion was a 0.5-mV incremental response to a 0.1-s test flash superimposed upon each background. The ordinate plots the log of intensity which evoked such a response. The abscissa plots the log of background intensity. Data points for each background are average values of threshold from several cells both in the normal and in the aspartate-treated preparation. (Normal DA, 14 cells; $\log I_B = -5.1$, 5 cells; $\log I_B = -4.15$, 8 cells; $\log I_B = -3.2$, 4 cells; $\log I_B = -2.2$, 2 cells; $\log I_B = -1.7$, 2 cells; Aspartate DA, 7 cells; $\log I_B = -5.1$, -4.15 , and -3.2 , 3 cells each). Bars indicate the range of threshold values found for the various backgrounds in the receptors in the normal preparation. Increment thresholds under aspartate were generally slightly lower than normal. Data points can be fitted with a straight line of slope of 1 over a range of about 3.5 log units indicating that *Gekko gekko* photoreceptors adapt to backgrounds over that range in conformity with Weber's law under both experimental conditions.

more rapidly than background intensity, and between $\log I_B = -1.7$ and $\log I_B = -1.1$ gecko photoreceptors saturated. As noted earlier this saturation was not associated with amplitude saturation of the receptor as the plateau membrane potential induced by such a background was still several millivolts less hyperpolarized than the dark-adapted saturation potential.

Open circles in Fig. 12 show average values of threshold from receptors in the aspartate-treated preparation. As this figure shows, receptor threshold after aspartate treatment also increased in direct proportion to background intensity, but for any given background they were slightly lower than those of normal receptors. This can be explained by the slightly larger sensitivity of receptors in the presence of aspartate, deriving from greater values of ΔV_{\max} .

In summary, normal receptor responses and responses of receptors in the aspartate-treated eyecup exhibit similar adaptation characteristics for all aspects of light adaptation for which they were compared. This includes the general characteristics of background responses and of increment responses, the adaptational changes in the voltage-intensity function for increment responses, and the increment threshold function. The only consistent differences between response properties under the two experimental conditions were in response wave-forms and amplitudes. Aspartate always abolished the fast initial transient observed in normal receptor responses, and response amplitudes generally were larger in the presence of aspartate than in the normal preparation.

Dark Adaptation

We investigated dark adaptation in individual gecko photoreceptors in untreated preparations after an exposure of the dark-adapted eyecup to the brightest light available from the test beam of the photostimulator ($\log I_T = -0.25$) for 1–3 s. Fig. 13 shows the receptor response to such a stimulus.

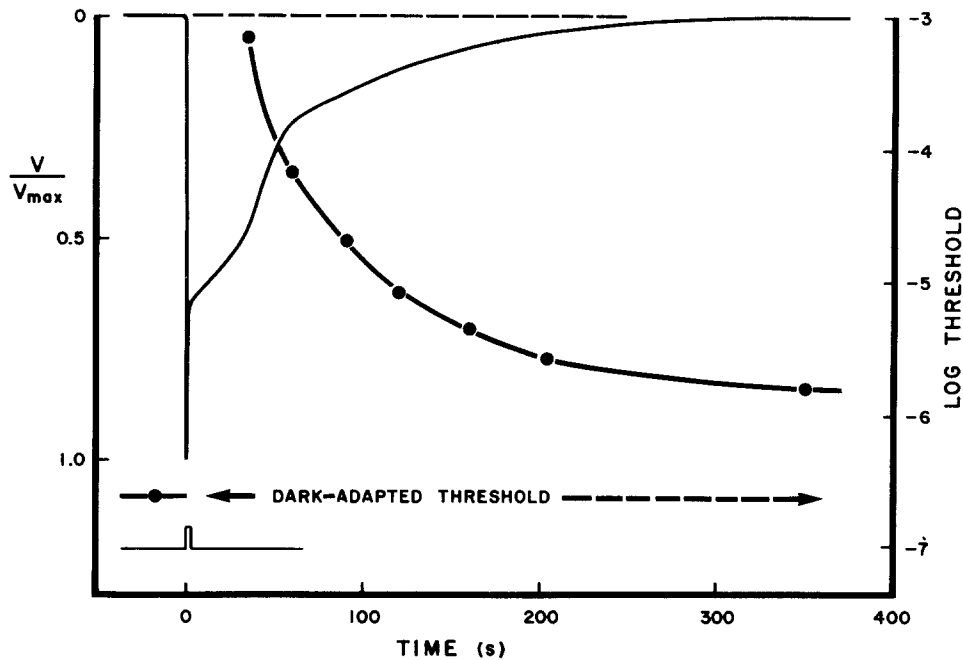


FIGURE 13. Comparison of the temporal change of normalized membrane potential (left ordinate scale) with the temporal change of threshold (right ordinate scale) for the receptor whose voltage-intensity curves are shown in Fig. 14. The dark-adaptation curve was constructed from the measured voltage-intensity curves by determining the test flash intensity which would produce a 0.5-mV threshold response at the times indicated. Threshold was still elevated by about 0.8 log units above its dark-adapted value at a time when the membrane potential had fully returned to its dark-adapted level.

Initially the cell was maximally hyperpolarized but some membrane potential recovery occurred virtually instantaneously (i.e. within about 100 ms). Thereafter, the membrane potential more slowly receded towards base line and reached its original dark value within 3–5 min after the adapting stimulus. The recovery of membrane potential after a bright 1–3-s exposure typically had the three distinct phases shown in Fig. 13.

Voltage-intensity curves for responses to 0.1-s test flashes were obtained during the recovery of membrane potential and thereafter when the membrane potential was virtually stationary. The responses rose from the changing or steady level of membrane potential and their peak amplitude was measured from that level. Voltage-intensity curves obtained in this way could be fitted satisfactorily with the function $\Delta V/\Delta V_{\max} = I/(I + \sigma)$. ΔV_{\max} and σ in this case were a function of time after the adapting stimulus. Fig. 14 shows such a set of voltage-intensity curves obtained from the receptor in Fig. 13 during the first few minutes of dark adaptation. Since the curves had to be obtained in quick succession, only the lower portion of the full "instantaneous" voltage-intensity curve was determined. Voltage-intensity data are plotted on log-log coordinates, and they show that small amplitude responses are linear with light intensity throughout the course of dark adaptation. The sensitivity of the receptor increased greatly during dark adaptation as is indicated in Fig. 14 by the successive shift of the voltage-intensity curve to the left along the abscissa. On several occasions we also observed that after a strong adapting exposure, the saturation potential of the receptor was initially several millivolts less hyperpolarized than the dark-adapted saturation potential.

Dark-adaptation curves displaying threshold as a function of time after the adapting stimulus were constructed from these voltage-intensity curves. Fig. 13 also shows the dark-adaptation curve obtained from the receptor whose response is plotted in that figure, and it compares the temporal change of threshold with the concurrent change of membrane potential. Similar data were obtained from three other receptors. After the initial transient there was a fairly rapid recovery of both sensitivity and membrane potential which lasted for about 5 min. This rapid phase of dark adaptation brought the threshold to within about 0.8 log units of its dark-adapted value, while the membrane potential over that period returned to its original dark-adapted level. In no case was it possible to study the full extent of dark adaptation in a single receptor because cells either deteriorated or were lost before they had again fully dark adapted. However, at the time at which the membrane potential had reached the original dark-adapted level, threshold was always still elevated and was only slowly decreasing, indicating the beginning of a slow phase of dark adaptation.

In all cells studied, dark adaptation after our adapting procedure showed these two phases and the membrane potential changed substantially and

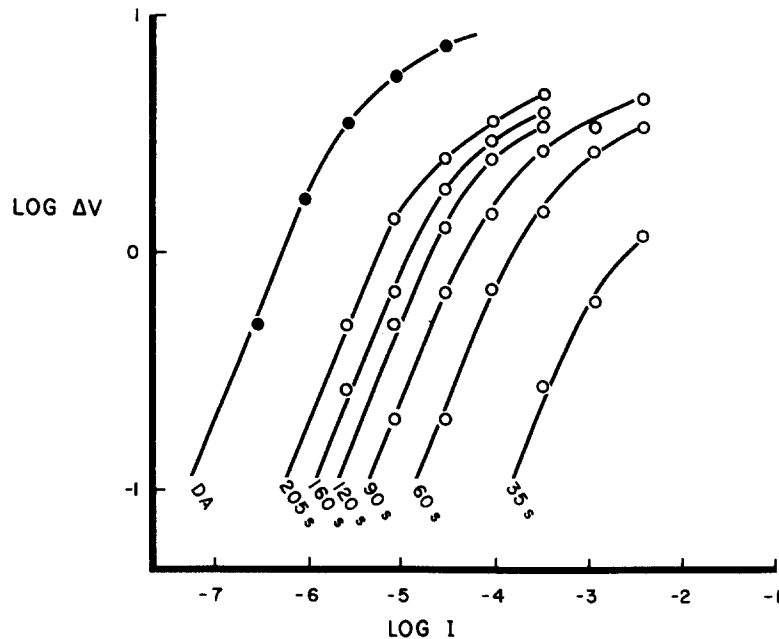


FIGURE 14. Voltage-intensity data from the receptor in Fig. 13 for responses to 0.1-s test flashes given to the cell at various times after the adapting stimulus. The ordinate plots the logarithm of the peak amplitude of responses (in millivolts) measured from the prevailing level of membrane potential. The abscissa plots the logarithm of test flash intensity. Interstimulus intervals during measurement of the curves were a few seconds, and numbers at the bottom of each curve indicate the times after the adapting stimulus at which the first (lowest intensity) test flash was given for each particular curve.

roughly in parallel with the threshold *only* during the fast phase of dark adaptation. During the slow phase there was no clearly measurable change of membrane potential. A similar parallelism between return of membrane potential and decrease of log threshold during the initial (fast) phase of dark adaptation was observed by Grabowski et al. (1972) for the rods of the axolotl after a 50% bleach. The presence of two phases of dark adaptation after our adapting flash suggests that the adapting exposure bleached a significant fraction of the receptor's pigment content and that the fast and slow phase of dark adaptation in gecko photoreceptors correspond to "neural" and "photochemical" adaptation (Dowling, 1963, Dowling and Ripps, 1970).

DISCUSSION

The experiments described in this paper provide further evidence that responses of vertebrate photoreceptors display adaptation characteristics. In addition, our experiments on the aspartate-treated eyecup of the gecko show that these characteristics derive from intrinsic receptor processes and not from horizontal cell activity. Aspartate treatment alters the waveform of

intracellularly recorded receptor responses, perhaps indicative of the loss of horizontal cell feedback onto the receptors, but it does not modify the adaptation characteristics of receptor responses in any major way.

Adaptation to light in gecko photoreceptors has the following three characteristics. First, after the initial rapid hyperpolarization at the onset of a background light, the membrane potential of the receptor gradually recovers with time in the light. With moderate and bright backgrounds this recovery process brings the receptor below the saturation level which, if maintained, would completely inactivate the cell. The membrane potential never recedes all the way back to the dark-adapted resting potential in the light, rather, it stabilizes at a plateau of hyperpolarization whose magnitude is graded with background intensity (Fig. 6).

Second, background lights shift the instantaneous voltage-intensity curve of the receptor along the intensity scale, thus providing an extension of the range over which the receptor can respond. As shown in Fig. 11, by far the greater part of receptor adaptation (i.e. change in linear incremental sensitivity) derives from this shift of the voltage-intensity curve. Only a small part of receptor adaptation can be attributed to the response compression that results from the maintained hyperpolarization of the receptor in the light.

Third, bright backgrounds lower the saturation potential of the cell. This lowering of the saturation potential in combination with the maintained (plateau) membrane potential in the light acts to constrict the voltage range over which incremental responses can be generated. The brightest backgrounds employed ($\geq \log I_B = -1.1$) compress the incremental voltage range to such an extent that incremental responses can no longer be elicited and the receptor appears saturated. Under this condition, thresholds are infinite even though the membrane potential is still at a level about 5 mV less hyperpolarized than the dark-adapted saturation potential.

Dark adaptation after a strongly adapting flash displays similar characteristics. For example: (a) after the adapting exposure, the membrane potential is initially elevated and returns to the dark-adapted level relatively slowly; (b) the voltage-intensity curve is shifted to the right along the intensity axis and only gradually shifts back towards its dark-adapted position; and (c) the saturation potential of the receptor is lowered for some time after bright light adaptation.

Under all conditions of light and dark adaptation investigated, response amplitudes were related to test flash intensity by the function $\Delta V/\Delta V_{\max} = \Delta I/(\Delta I + \sigma)$. Although the parameters ΔV_{\max} and σ varied widely, depending on the state of adaptation, small amplitude responses were *always* linear with light intensity. From these findings we can characterize receptor adaptation in the main as a resetting of the instantaneous intensity range over which the receptor can respond. This range adjustment is associated with

considerable changes in the receptor's linear sensitivity which could be expressed for example in terms of millivolts of response per photon absorbed.

A further important observation is that receptor sensitivity and membrane potential can vary independently. For example, the level of membrane potential changes very little with backgrounds brighter than about $\log I_B = -3.0$ (see Figs. 6 and 9). Sensitivity, however, continues to decrease substantially with these brighter backgrounds (see Fig. 12). Further, during the slow stage of dark adaptation, thresholds continue to fall at a time when there is no observable change of membrane potential. Even under conditions during which both sensitivity and membrane potential are changing, there is no unique relationship between these two quantities. Fig. 15 shows this for both light and dark adaptation. Thresholds for a given state of adaptation are plotted against the value of membrane potential prevailing for that condition of adaptation. The upper curve shows data from two cells during the course of dark adaptation after a 2-s exposure to $\log I = -0.25$. Thresholds at a given time of dark adaptation were obtained from voltage-intensity curves of the kind displayed in Fig. 13 and were plotted against the prevailing

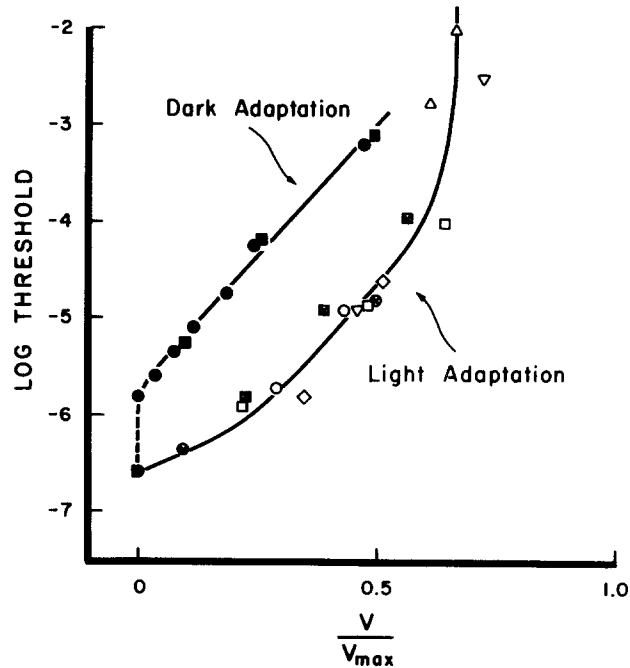


FIGURE 15. Relationship between sensitivity and membrane potential for the two conditions of light adaptation and of dark adaptation after a strongly adapting flash. The curve pertaining to dark adaptation was drawn through data points from two cells and the curve pertaining to light adaptation through data points from seven cells. See text for details.

value of membrane potential, normalized as the fraction of the maximum hyperpolarization obtained from the dark-adapted cell. The lower curve shows the relation between increment threshold and normalized steady membrane potential during light adaptation. Data points for this curve were derived from measurements on seven cells. Fig. 15 shows that although log threshold varies with the level of membrane potential over a substantial range during both light and dark adaptation, the relationship between these two quantities is different for the two conditions. From these results, it is clear that receptor sensitivity cannot be uniquely set by the level of membrane potential. Rather, it appears that receptor sensitivity is controlled at least in part by a stage of visual transduction *preceding* the generation of membrane voltage change (see also Dowling and Ripps, 1972 and Fein and DeVoe, 1973).

Comparative Aspects of Receptor Adaptation to Light

It has long been believed that rods generally saturate in bright background light but that cones do not. Electrophysiological studies have, for the most part, confirmed this notion. For example, a lack of saturation in background light has been demonstrated for the cones of the macaque monkey (Boynton and Whitten, 1970), carp (Witkovsky et al., 1973), mudpuppy (Normann and Werblin, 1974), and turtle (Baylor and Hodgkin, 1974). On the other hand saturation has been found to occur in the rods of the rat (Green, 1971, 1973; Penn and Hagins, 1972), mudpuppy (Normann and Werblin, 1974), gecko (this paper), and marine toad (Fain, 1974). The only known exception to the above generalization are the rods of the skate which continue to give incremental responses even with backgrounds which bleach more than 90% of the rhodopsin in the receptors (Dowling and Ripps, 1972). The skate, however, is unique in that it has an all-rod retina, with only one kind of rod (Dowling and Ripps, 1970). If the skate is to continue to see in bright light, mechanisms must exist to prevent saturation of its sole kind of visual cell. All the other species thus far studied, with the exception of the gecko, have cones as well as rods, and above rod saturation cones can mediate visual responsiveness. In the gecko, the smaller rod (see Methods) may have cone-like adaptation properties and may function at light levels above saturation of the larger rod (Dodt and Jessen, 1961). We do not yet know if rods in all species saturate at the same absolute level of background intensity (as measured in photons absorbed per rod per second). However, all seem to saturate with background fields that bleach only trivial fractions of the visual pigment.

Receptors that do not saturate (cones and skate rods) all adapt to backgrounds by shifting their incremental voltage-intensity function to encom-

pass ranges of higher light intensity (Boynton and Whitten, 1970; Dowling and Ripps, 1972; Normann and Werblin, 1974). Although it has recently been proposed that much of the change in sensitivity of such photoreceptors during light adaptation derives directly from bleaching of visual pigment (Boynton and Whitten, 1970), substantial shifts of the voltage-intensity curve occur already at background intensities which do not bleach a significant fraction of the photopigment (Dowling and Ripps, 1972, Normann and Werblin, 1974). Only at the very brightest backgrounds is enough pigment bleached away to lower the receptor's quantum catching efficiency. Increment thresholds of nonsaturating receptors follow Weber's law over the entire range of backgrounds (Boynton and Whitten, 1970; Dowling and Ripps, 1972; Witkovsky et al., 1973; Normann and Werblin, 1974; Baylor and Hodgkin, 1974).

Among the saturating (rod) receptors, there appear to be two types, those that adapt significantly and those that show very little if any adaptation to background light. By a receptor that adapts we mean a receptor which shifts its incremental voltage intensity function significantly in response to background fields. The rods of the gecko and of the marine toad (Fain, 1974) belong to this group. Both shift their voltage-intensity function by about 4 log units before they saturate, and their increment threshold function follows Weber's law for at least 3 log units. The rods of the rat, and the mudpuppy, on the other hand, appear to be essentially nonadapting receptors. They do not significantly reset their operating range in response to backgrounds, and they are therefore limited to the narrow dynamic range of their dark-adapted voltage intensity function. Further, their increment threshold function is nonlinear. It never follows Weber's law and increment thresholds rise for only 2-3 log units before the receptor saturates.

Implications for Receptor Mechanisms

Recent work on vertebrate photoreceptors has led to the suggestion that light causes the release of a substance from the saccules of the outer segment that diffuses to the plasma membrane of the outer segment and blocks the flow of Na^+ ions across that membrane (Baylor and Fuortes, 1970; Hagins, 1972). Such a suggestion is particularly appropriate for the rods in which the saccules containing visual pigment are separate from the plasma membrane. It has been proposed that Ca^{2+} is the blocking substance, and a number of studies are compatible with this suggestion (e.g. Yoshikami and Hagins, 1973).

The present experiments emphasize the need for a mechanism to remove and sequester such a blocking substance from the plasma membrane in continuous light. They also indicate some of the properties of such a mechanism. For example, for a receptor to continue to function in the presence of a

steady background light, the blocking substance must be removed from the plasma membrane at a rate at least equal to the rate of release of the substance from the saccules. In the gecko, the partial return of membrane potential in the light, with the concomitant increase in responsiveness of the receptor (Fig. 7), suggests that initially in steady light the uptake mechanism removes the blocking substance *faster* than it is released from the saccules.

Some of the major differences in adaptation properties of receptors in various species could be explained by differences in the behavior of the unblocking mechanism. For example, in the nonadapting, saturating rods of the rat and mudpuppy, it would appear that the rate of removal of blocking substance from the plasma membrane in steady light *never* exceeds its rate of release from the saccules. Thus there is no recovery of membrane potential in the light, and the receptor saturates within a few log units of absolute threshold (Penn and Hagins, 1972; Normann and Werblin, 1974). This happens through saturation of the membrane potential when all Na⁺ channels in the outer segment are blocked. In adapting receptors such as bufo and gecko rods, on the other hand, the partial recovery of membrane potential in the light serves to increase receptor responsiveness, and it allows the receptors to function at backgrounds that otherwise would cause voltage saturation. It is interesting to note that the kinetics of the process of membrane recovery in the light appears to differ substantially between species. In the *Gekko gekko*, the recovery process, accompanied by increase of receptor response amplitude, takes between 5 and 30 s to occur. In the skate on the other hand, return of responsiveness of the receptor potential in the light takes many minutes (Dowling and Ripps, 1972). Indeed with the very brightest lights, increment responses from skate receptors increase in amplitude for nearly 1 h.

The present experiments also show that receptor sensitivity changes during adaptation are not closely dependent on the level of membrane potential (Fig. 15). This finding suggests that the mechanism regulating sensitivity changes of the receptor is located distal to the plasma membrane, and we propose that the greater part of the sensitivity changes observed in receptors relate to the amount of blocking agent released from the saccules upon light stimulation. On this view, the amount of blocking substance released per visual pigment molecule bleached is a constant fraction of the amount of substance contained in the saccules. Further, in steady light the blocking substance is depleted from the saccules and the extent of depletion is intensity dependent. Thus, more visual pigment must be activated during light adaptation to cause the release of a given (e.g. threshold) number of blocking molecules. As a result, the sensitivity of the receptor decreases in the light, and the voltage-intensity curve is shifted towards higher intensities.

If this suggestion is correct, a further requirement is that a fraction of the blocking substance must be sequestered in the light and not returned immediately to the saccules, for if the latter were true, sensitivity and membrane potential would be closely linked. Rather, much of the blocking substance removed from the plasma membrane in the light must be temporarily stored in a nonreleasable form and returned to the intrasaccule space only during dark adaptation. Part of that return, namely the replenishment related to the sensitivity increase during "photochemical" dark adaptation, would appear to depend on and to be paced by the resynthesis of visual pigment (Rushton, 1961; Dowling, 1963; Dowling and Ripps, 1970).

A final comment concerns the "saturation" of gecko rods, which we have shown occurs when the membrane potential is substantially below the maximum voltage elicited with light in the dark-adapted retina. An important, unanswered question is whether under such bright light conditions the unsaturated level of steady membrane voltage indicates that a number of Na^+ channels in the outer segment plasma membrane are still open. This would suggest that the saccules are failing to release any blocking substance under such conditions. An alternate possibility is that all of the Na^+ channels are closed but that the lowered membrane potential results from another mechanism, such as a conductance change in the inner segment or the activation of an electrogenic pump in the cell.

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