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BACKGROUND AND BLEACHING ADAPTATION IN LUMINOSITY TYPE HORIZONTAL CELLS IN THE ISOLATED TURTLE RETINA

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SUMMARY

1. The effects of background illumination and bleached photopigment on luminosity type horizontal cells were studied in the isolated turtle retina.

2. Background illumination, which produced less than 60% bleaching, hyperpolarized and desensitized the horizontal cells to a degree which depended upon the background intensity. The desensitization of horizontal cells by these backgrounds is described by a Weber–Fechner type relationship. This desensitization primarily reflects the activation of a 'gain reduction' mechanism and cannot be accounted for by 'response compression'.

3. Following the termination of these backgrounds, horizontal cell sensitivity partially recovered but did not return to the pre-background, dark-adapted level. This desensitization was attributed to the presence of bleached photoproducts which were produced by the background exposure.

4. Application of very bright backgrounds caused the horizontal cells to initially hyperpolarize, and then to gradually depolarize towards the dark-adapted level along an exponential time course which appeared to reflect the decreased quantal catching associated with very high levels of photopigment bleaching.

5. From the time constant of the exponential decay of horizontal cell potential during the bright background illumination, the photosensitivity to bleaching of the cone photopigment was determined to be $4{\cdot}5 \times 10^7$ effective quanta (633 nm) μm^{-2}.

6. After termination of bright backgrounds which bleached more than 99% of the cone photopigment, the horizontal cell sensitivity increased linearly with time and after 25 min reached a level which was about 15% of the pre-background sensitivity.

7. Bleached photopigment reduces light sensitivity via at least two different mechanisms. For moderate degrees of bleaching ($< 95\%$), the presence of bleached photoproducts plays the major role in sensitivity control, producing a desensitization which is logarithmically related to the fraction of bleached pigment. During extensive bleaching ($> 99\%$), the contribution of reduced quantal catching to sensitivity control becomes apparent and produces an additional loss in sensitivity which is linearly related to the fraction of unbleached pigment present.

INTRODUCTION

The sensitivity of photoreceptors is reduced by continuous background illumination and by the presence of bleached photopigment. Background adaptation has been demonstrated extracellularly for monkey cones (Boynton & Whitten, 1970; Valeton & van Norren, 1983) and intracellularly in *Necturus* cones (Normann & Werblin, 1974) and turtle cones (Baylor & Hodgkin, 1974; Normann & Perlman, 1979*a*). Two desensitizing mechanisms are activated by background illumination. 'Gain reduction' is manifest as a lateral shift of the intensity–response curve along the log intensity axis. 'Response compression' contributes to sensitivity losses due to the monotonic reduction in the slope of the intensity–response curve at high intensity levels.

Two sensitivity-controlling mechanisms have been attributed to the presence of bleached photopigment. One produces desensitization which is a consequence of the reduced quantal catching associated with the reduction in the fraction of photopigment present. Such a mechanism has been demonstrated in the developing vertebrate retina (Witkovski, Gallin, Hollyfield, Ripps & Bridges, 1976) and in certain inherited retinal disorders (Ripps, Brin & Weale, 1978; Perlman & Auerbach, 1981). A different mechanism has been implicated to dominate visual sensitivity during dark adaptation after significant bleaching. This mode of desensitization results from the presence of bleached photoproducts and is characterized by a linear relationship between the log sensitivity loss and the fraction of bleached photopigment (Rushton, 1961; Dowling, 1963). This mechanism has been recently demonstrated in isolated rods by introducing a new locked analogue of 11-*cis* retinal which can bind to opsin but does not participate in phototransduction. Addition of this analogue to bleached rods reduced sensitivity losses from the degree determined by the 'log–linear' relationship to that expected from quantal catching considerations (Corson *et al.* 1989).

The relative contributions of background light and bleached photopigment to receptoral desensitization have been studied in the rod system (Pepperberg, Brown, Lurie & Dowling, 1978; Clack & Pepperberg, 1982; Pepperberg, 1984; Leibovic, Dowling & Kim, 1987). The roles of these mechanisms in controlling cone sensitivity deserve investigation because it is the cones which manifest the ability to operate over a wide range of ambient illumination (Baylor & Hodgkin, 1974; Normann & Werblin, 1974; Normann & Perlman, 1979*a*). However, the rapid regeneration of the cone photopigment in the turtle eyecup (Hodgkin & O'Bryan, 1977) has made such a study difficult in the conventional eyecup preparation.

This obstacle has been mitigated in this study by utilizing an isolated turtle retina preparation. The adaptation properties of the cone system have been inferred from intracellular recordings from L-type horizontal cells. It has been assumed, based upon previous work (Green, Dowling, Siegel & Ripps, 1975; Itzhaki & Perlman, 1987) that horizontal cell sensitivity merely reflects changes in the cone system under background illumination and during dark adaptation. We found that background adaptation in the isolated retina was very similar to that in the eyecup. Continuous illumination mainly reduced the gain of the phototransduction process. Bleached photopigment exerted a dual effect on cone sensitivity. For moderate degrees of

photopigment bleaching, log desensitization was linearly related to the fraction of pigment bleached. Extensive bleaching, on the other hand, produced a linear desensitization which could be accounted for by reduced quantal catching.

METHODS

The isolated turtle retina preparation

Most of the experiments were performed on the isolated retina preparation of the turtle *Pseudemys scripta elegans*. The technique for preparing the isolated retina has been described in detail elsewhere (Perlman, Normann, Chandler & Lipetz, 1990). After decapitation and pithing, the turtle head was wrapped in a moist paper towel and refrigerated at 10 °C for a period of 4–8 h to loosen the adhesion between the retina and the pigment epithelium and to allow the separation of the retina with minimal damage to the outer segments. The following procedures were performed under very dim illumination. After the refrigeration period, the eye was enucleated and hemisected. The vitreous humour was carefully removed from the posterior half of the eye. The eyecup was then bisected along the vertical meridian (just adjacent to the optic disk), and again along the horizontal meridian (parallel to the visual streak) to produce four pieces of about 4 × 4 mm. One of these pieces was placed vitreal side down on a 1 cm diameter piece of Millipore filter (0·45 μm pore size). The sclera, choroid and pigment epithelium were then detached by peeling the sclera back, leaving the retina attached to the filter paper with photoreceptors up.

Scanning electron microscope

The effect of the isolation procedure on the anatomical integrity of the preparation was examined using scanning electron microscopy. Isolated retinas were fixed for 4 h in 2% paraformaldehyde and 2% gluteraldehyde in 0·1 M-sodium phosphate buffer. They were then washed in 0·1 M-sodium phosphate buffer, pH 7·3, twice for 10 min. Following dehydration in a graded series of ethanols, 50, 70, 80, 95 and 100%, they were dried by the critical point drying method in a Polaron Critical Point Drier, using carbon dioxide as the transition fluid. Specimens were sputter-coated with gold and photographed in a JEOL JSM-35 scanning electron microscope at 15 kV.

The scanning electron micrograph shown in Fig. 1 was taken from a retina which had been used for some of the physiological experiments described in the results section. Over much of the retina, the isolation procedure provided a preparation devoid of pigment epithelium, yet left the photoreceptor outer segments completely intact.

The superfusion system

The superfusion system consisted of a constant perfusion pressure system connected to a small calibre inlet tube. The inlet tube was positioned directly above one corner of the retina to ensure adequate superfusion of the preparation. The flow rate was about 0·6 ml min^{-1}. The superfusion solution was composed as follows (concentrations in mM): NaCl, 110; KCl, 2·6; $MgCl_2$, 2; $CaCl_2$, 2; D-glucose, 10; $NaHCO_3$, 22. The pH of the solution was maintained at 7·5 by constant bubbling with a 95% O_2, 5% CO_2 gas mixture.

The photostimulator and recording system

A two-channel photostimulator originating from a common light source was used in this study. The spectral content and intensity of each beam were independently controlled. For both test and background channels, large-field diffuse retinal illumination was used to cover the entire receptive fields of L-type horizontal cells. White light stimuli of 500 ms duration were used to investigate the effects of backgrounds and bleaching on horizontal cells. Accordingly, sensitivity values, determined from the peak amplitude of linear range photoresponses (no larger than 1·5 mV), are given in terms of step sensitivities. While monochromatic light is typically used to monitor sensitivity changes in retinal cells, white light was used in this study, as has been used by others (Hodgkin & O'Bryan, 1977), in order to generate background illuminations of sufficient intensity to produce substantial photopigment bleaching while still being able to evoke incremental responses on top of the bright backgrounds. For the goals of this study, the need for high intensities outweighed the differential excitation of white light on the red and green cones. The white test and background intensities were

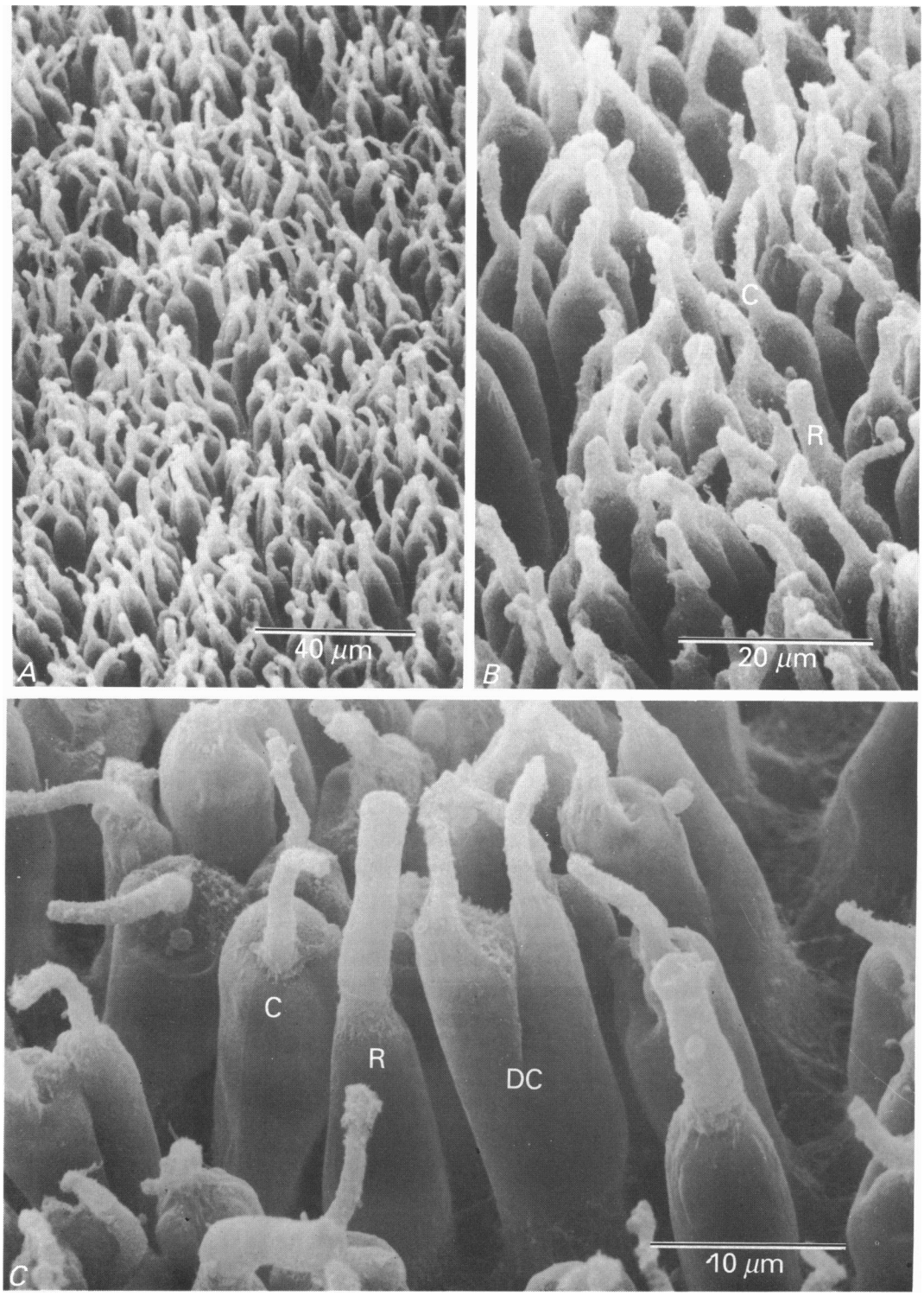

Fig. 1. For legend see facing page.

expressed in terms of effective quanta of 633 nm. This was done by recording the intensity–response curves of several L-type horizontal cells using calibrated (PIN10 photodiode, United Detector Technology) monochromatic (633 nm) light stimuli, originating from the test channel. Then, white light stimuli from the test and background channels were used to generate similar curves. Generally, the shapes of the intensity–response curves for the 633 nm and the white test flashes were identical, but were displaced along the log intensity axis. The extent of the displacement was used to calibrate the intensity of the white light in terms of effective quanta (633 nm) s^{-1} μm^{-2}.

The intracellular microelectrodes were pulled on a modified Livingston electrode puller and had resistances of about 200 MΩ when filled with 3 M-potassium acetate. The membrane potential and photoresponses of retinal neurones were monitored with an oscilloscope and a paper recorder and recorded on an FM tape-recorder. Off-line analysis and plotting were done on a small computer system.

RESULTS

Dim to moderate background illumination

Light adaptation

The effects of background illumination on luminosity type horizontal cells were determined from the photoresponses, elicited by a sequence of light stimuli of different intensities, superimposed upon backgrounds of different intensities. A continuous recording from one horizontal cell studied under three different background exposures is shown in Fig. 2. Intensity–response measurements were performed prior to, during and immediately after the application of each background. The time course of light adaptation and of the recovery from the background light was followed by applying light stimuli of constant intensity at the beginning and end of the background exposure (the responses bounded by two arrow-heads). For the three background intensities shown in Fig. 2, the horizontal cell exhibited a transient hyperpolarization at background onset which was followed by a gradual sag back to a 'steady' level which was about one-half of the amplitude of the transient hyperpolarization seen at background onset. Similar findings were seen previously in cones and in L-type horizontal cells in the turtle eyecup preparation (Normann & Perlman, 1979*a*, *b*; Itzhaki & Perlman, 1987).

From the amplitudes of the constant-intensity responses, it is clear that the horizontal cell does not simply summate quanta from the background and test flashes: the potential level reached at the peak of the response elicited by the test stimulus becomes smaller in the presence of the background. Background experiments were conducted on seventeen L-type horizontal cells in fourteen isolated retina preparations. In all cases, results similar to those shown in Fig. 2 were observed.

A better appreciation of the effects of background illumination on the dynamic range of the cell may be obtained by plotting the photoresponse peak amplitude as a function of the log of the stimulus intensity, for each series of responses. Such

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Fig. 1. Scanning electron micrographs of an intact region of the isolated turtle retina preparation. *A*, large areas of the preparation have distinct outer segments. *B*, at higher magnification, rod (R) and cone (C) outer segments can be distinguished. *C*, at still higher magnification, the morphological properties of single cones (C), rods (R) and double cones (DC) can be appreciated.

intensity–response curves, measured in the dark-adapted state and at three different levels of background illumination, are shown in Fig. 3. The data points describe the peak polarization achieved during a response relative to the dark-adapted level as a function of the total illumination incident upon the retina at the time the response

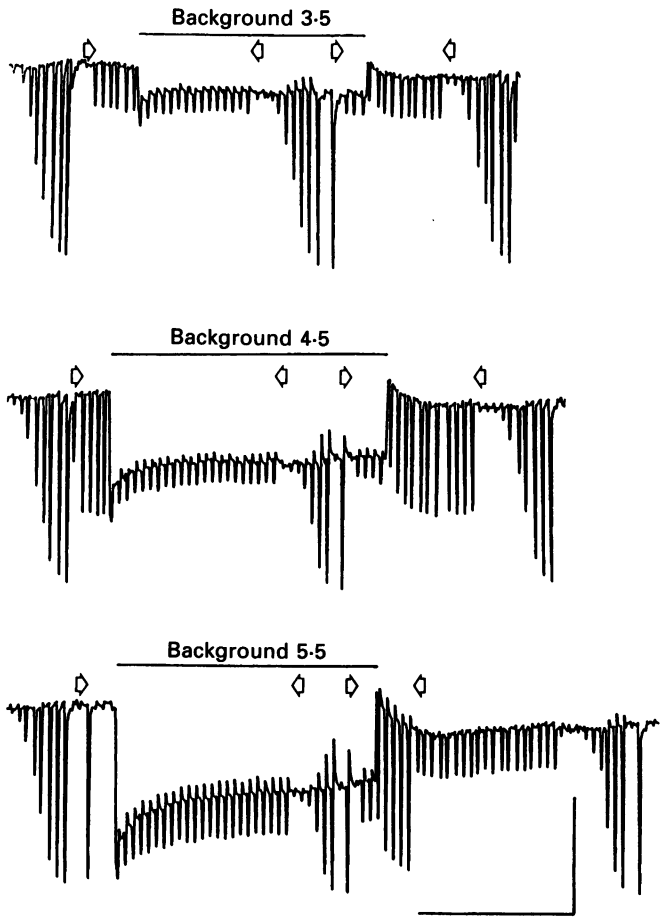

Fig. 2. The effects of background illumination on an L-type horizontal cell in the isolated turtle retina. Intensity–response series were measured prior to, during and after each background exposure. For each background experiment, light stimuli of constant intensity were applied at 3 s intervals. These stimuli were delivered only during the time periods bounded by the two arrow-heads at the beginning and at the end of each background. The figure is a continuous record from one cell but has been divided into three parts for clarity. The intensity of each background is given in log effective quanta (633 nm) s^{-1} μm^{-2}. Calibration bars: vertical, 30 mV; horizontal, 1 min.

was recorded (background + test stimulus). Plotting intensity–response curves in this fashion provides the best indication of the effects of background illumination on horizontal cell function (Normann & Perlman, 1979*c*; Valeton, 1983; Hemila, 1987). The symbols in this figure represent the actual data points while the continuous curves are plots of a modified Michaelis–Menten type relationship (Naka & Rushton, 1966):

$$V_I = V_{max} I^n / (I^n + \sigma^n), \quad (1)$$

where V_I is the amplitude of the response elicited by a stimulus intensity, I; V_{max} is the maximal response elicited by a supersaturating stimulus in the dark-adapted state; σ is the semi-saturating stimulus intensity and n defines the steepness of the

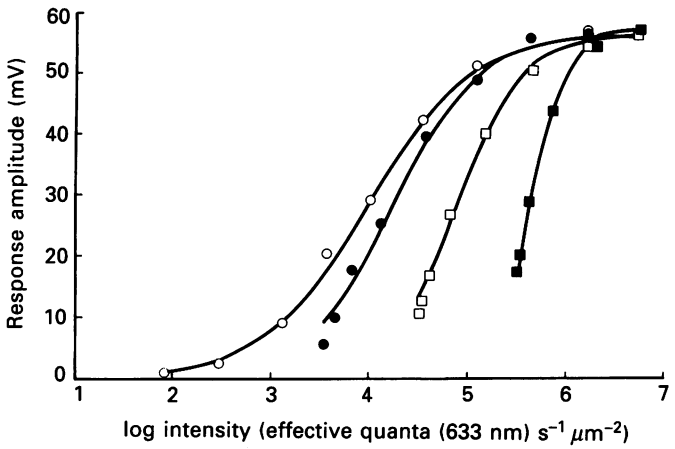

Fig. 3. Intensity–response curves measured in the dark-adapted state prior to any background application (○) and during illumination with backgrounds of the following log intensities (effective quanta (633 nm) s^{-1} μm^{-2}): 3·5 (●); 4·5 (□) and 5·5 (■). The peak amplitude achieved by each response was measured relative to the pre-background 'dark' potential. The intensity used to elicit each response is the total illumination incident upon the retina (background + stimulus intensity). The continuous curves through the data points were derived from eqn (1). The background intensities (in log units) and the parameters which characterize these curves, n (steepness) and log σ (where σ is the semi-saturating stimulus intensity), are: dark adapted, 0·8 and 4; background, 3·5, 1 and 4·25; background, 4·5, 1·3 and 4·9; background, 5·5, 2·4 and 5·65. I_B and σ are given in effective quanta (633 nm) s^{-1} μm^{-2}.

intensity–response curve. The values of n and σ are functions of the background intensity as indicated in the figure legend. The steepening of the horizontal cell intensity–response curve with increased background intensity, expressed by the increase in the value of n, is one feature which distinguishes the light adaptation properties of cones from horizontal cells, as has been noted previously in the turtle eyecup (Normann & Perlman, 1979*a*, *b*). This difference prevents comparison of the entire dynamic range between cones and horizontal cells but does not affect comparisons of sensitivity data (Itzhaki & Perlman, 1987).

The data in Fig. 3 clearly demonstrate the lateral shift of the horizontal cell intensity–response curve along the log intensity axis (increase in σ) during background illumination. This process reflects the activation of a 'gain reduction' mechanism within the photoreceptors which occurs even when the retina is separated from the pigment epithelium.

Bleaching adaptation

Since the isolated retina preparation is virtually devoid of pigment epithelium (Fig. 1), the regeneration of bleached cone photopigment, as will be shown later,

proceeds at a very slow rate and represents the synthesis of only a very small fraction of the photopigment. Thus, the application of any background light is expected to produce a certain degree of bleaching which will persist even after the termination of the background illumination. The effect of a given amount of photopigment bleaching can be assessed from the intensity–response curve recorded in darkness

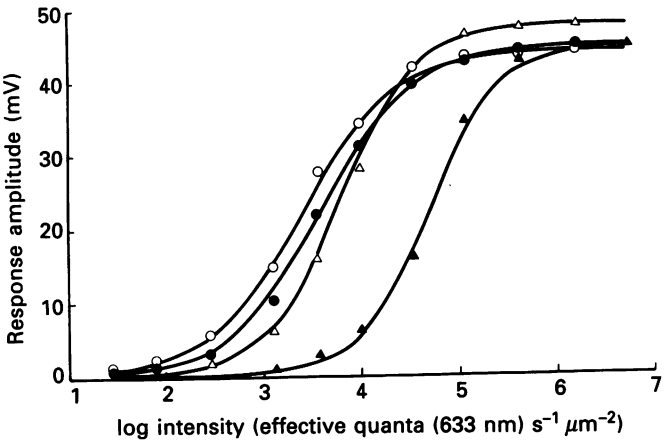

Fig. 4. The effects of partial bleaches on an L-type horizontal cell. Intensity–response curves were measured in darkness prior to any background application (○) and after termination of exposures to backgrounds of the following intensities (in log effective quanta (633 nm) s^{-1} μm^{-2}): 3·5 (●); 4·5 (△) and 5·5 (▲). The continuous curves through the data points were derived from eqn (1). The background intensities (in log units) and the parameters which characterize these curves, n and log σ, are: dark adapted, 0·9 and 3·4; background, 3·5, 0·9 and 3·6; background, 4·5, 1·1 and 3·8; background, 5·5, 1·2 and 4·7. The units of I_B and σ are effective quanta (633 nm) s^{-1} μm^{-2}.

after the termination of the background exposure. A family of such curves, recorded from one horizontal cell, is shown in Fig. 4. The curves were measured under dark-adapted conditions prior to any background illumination (○), and after termination of approximately 2 min background exposures of the following intensities (given in log effective quanta (633 nm) s^{-1} μm^{-2}): 3·5 (●), 4·5 (△) and 5·5 (▲). It is clear from these curves that the presence of bleached photopigment, just like background illumination, desensitizes the horizontal cell and shifts its intensity–response curve to the right along the log intensity axis. Also, like background illumination, the greater the amount of bleached photopigment present, the steeper is the intensity–response curve. The smooth curves in Fig. 4 are plots of the modified Naka–Rushton relation (eqn (1)) with the values of n and σ given in the figure legend. It should be noted that these curves have not been normalized and therefore there is some variation in the maximum response recorded during the 20 min time course of the experiment.

Bright background illumination

The effects of a very bright background light (log intensity of 6·5 effective quanta (633 nm) s^{-1} μm^{-2}) on a horizontal cell in the isolated turtle retina are shown in Fig. 5. These effects are clearly distinct from those observed with backgrounds of lesser

intensity (Fig. 2). The application of the bright background elicited a transient hyperpolarization of large amplitude. However, unlike the findings seen with less intense backgrounds, the horizontal cell potential slowly repolarized towards the pre-background level despite the continual presence of the bright background. After termination of the bright background, the membrane potential exhibited a small (1–3 mV) transient depolarizing change which recovered to a level very close to that measured just before turning off the background. Such overshoots above the 'dark' level at background offset were more apparent with dim to moderate backgrounds (Fig. 2) and were also noted in the turtle eyecup (Normann & Perlman, 1979*b*).

Prior to and throughout the background exposure, test stimuli of fixed intensity were applied at 3 s intervals to follow the time course of responsivity changes (the responses bounded by two arrow-heads). Although the amplitudes of the increment responses to the test stimuli initially increased, the absolute potential level attained by the peak response gradually decreased. This observation may reflect the action of light-adaptation mechanisms similar to those described before. As the background exposure continued, the amplitude of the increment responses started to decrease and, in some cells, they almost disappeared.

The step sensitivity was very low immediately after turning off the bright background and only bright light stimuli could elicit measurable responses. With prolonged times in darkness, a significant recovery of sensitivity occurred. The lower part of Fig. 5 shows intensity–response series recorded at various times (denoted adjacent to the traces) after turning off the background. In each series of responses, the far right response was elicited by an unattenuated light stimulus. The responses to its left were evoked by stimuli of lesser intensities separated from each other by approximately 0·5 log units. Bright background experiments were performed in nine horizontal cells and they all responded in similar fashion to the one described in Fig. 5. However, only five of these were sufficiently stable throughout the experiment to allow monitoring of the effect of the background and also to follow partial recovery after its termination. The data from these five cells are presented here.

In contrast to the effects of the bright background on horizontal cells in the isolated retina (Fig. 5), the same background light (log intensity of 6·5 effective quanta (633 nm) s^{-1} μm^{-2}) produced a different effect on horizontal cells studied in the conventional turtle eyecup prepared according to previously described procedures (Baylor & Hodgkin, 1974; Normann & Perlman, 1979*a*). The results of such an experiment are shown in Fig. 6. The horizontal cell responded with a transient hyperpolarization to background onset and then recovered to a 'steady' level which was maintained as long as the background was on. The increment responses elicited by bright light stimuli (the responses bounded by two open arrow-heads) gradually grew in amplitude reflecting the activation of light-adaptation mechanisms. After termination of this background exposure, the cell was desensitized compared to the pre-background state but rapidly recovered as indicated by the responses to test stimuli of fixed moderate intensity (the responses bounded by two filled arrow-heads). Within 3 min in darkness, recovery was complete and the intensity–responses series recorded at that time was indistinguishable from the one measured prior to the application of the background. Similar results were observed in ten horizontal cells studied in six eyecup preparations.

Schnapf & Baylor (1988) have noted an exponential decrease in the membrane current of isolated monkey cones during exposure to bright background illumination. They attributed this observation to a decrease in quantal catching associated with substantial bleaching of the cone photopigment. Similarly, the differential effects of the bright background on horizontal cell membrane potential, illustrated in Figs 5 and 6, can also be accounted for by the substantial bleaching of photopigment in the isolated retina compared to that in the eyecup.

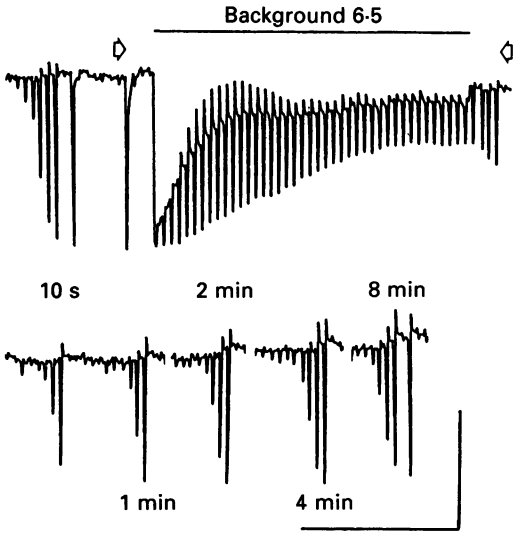

Fig. 5. The effects of a bright background (log intensity of 6·5 effective quanta (633 nm) s^{-1} μm^{-2}) on an L-type horizontal cell in the isolated turtle retina. Bright test flashes of constant intensity were delivered at 3 s intervals to follow the progression of the background effect on the horizontal cell responsiveness (bounded by the two arrow-heads in the upper part of the figure). The recovery in darkness from the bright background is shown in the lower part of the figure. Intensity–response series were monitored at different time intervals as indicated adjacent to each series. In each series, the far right response was elicited by the brightest (unattenuated) light stimulus. Each response to its left was evoked by stimuli of lesser intensity separated by about 0·5 log units from each other. Calibration bars: vertical, 30 mV; horizontal, 1 min.

In the isolated turtle retina, the rate of repolarization of horizontal cell potential during the bright background (log intensity of 6·5 effective quanta (633 nm) s^{-1} μm^{-2}) also followed an exponential time course as illustrated in Fig. 7. Data from five different horizontal cells (represented by different symbols) are shown in this figure. Each set of data has been arbitrarily shifted vertically relative to each other for figure clarity. The data points describe the log of the membrane potential (relative to the post-background dark level) measured at various times after the onset of the background. In some of the experiments (three out of five), where long-duration exposures to bright background were applied, the membrane potential just prior to turning off the background was virtually identical to the post-background level. Therefore, the post-background 'dark' potential was chosen as a reference level in all the experiments to allow determination of the time constant also in cases where the background duration was limited and the membrane potential did not return to the

expected 'dark' level. The straight lines through the data points were derived using a linear regression procedure. The average time constant (±S.D.) calculated for the five horizontal cells shown in Fig. 7 was 14·3±2·6 s.

Dark adaptation in the isolated retina

The recovery of the horizontal cell sensitivity in the dark following the bright background exposure (lower part of Fig. 5) can be better appreciated by plotting the

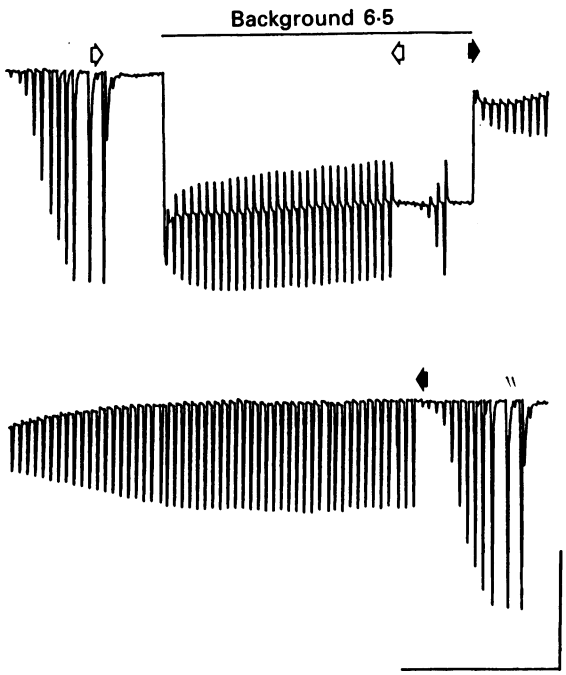

Fig. 6. The effects of a bright background (6·5 log effective quanta (633 nm) s^{-1} μm^{-2}) on an L-type horizontal cell in the turtle eyecup preparation. The desensitizing effect of the background was followed with bright light stimuli applied every 3 s during the time period bounded by two open arrow-heads. The recovery from the background was followed by light stimuli of moderate intensity delivered during the time period bounded by the two filled arrow-heads. Calibration bars: vertical, 30 mV; horizontal, 1 min.

intensity–response curves recorded at different time intervals after termination of the bright background exposure. These curves, shown in Fig. 8, were measured immediately after impaling the horizontal cell prior to any background application (○), just prior to the application of the bright background (●) and at different time intervals after its termination (△, 10 s; ▲, 2 min; □, 5 min; ■, 10 min and ◆, 20 min). The continuous curves are modified Naka–Rushton relationships (eqn (1)).

The difference between the first dark-adapted curve (○) and the one measured just prior to the application of the bright background (●), probably reflects pigment bleached by previous applications of less intense backgrounds. Therefore, the rate of recovery in the dark after the termination of the bright background light exposure was related to the sensitivity of the cell measured just prior to its application as shown in Fig. 9 for five different horizontal cells (described by different symbols).

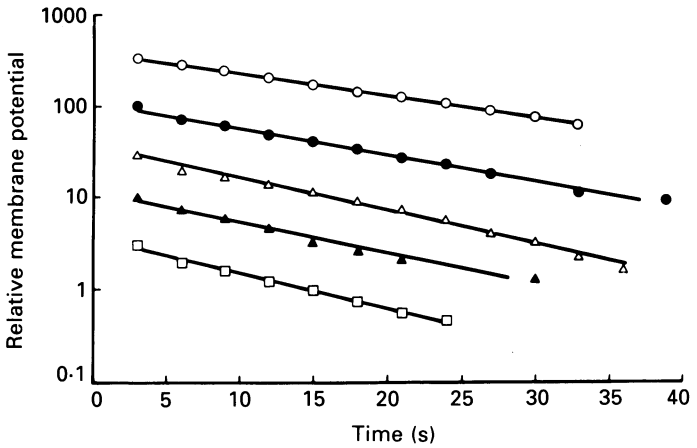

Fig. 7. The effects of a bright background (6·5 log effective quanta (633 nm) s^{-1} μm^{-2}) on the membrane potential of five L-type horizontal cells (described by different symbols) in the isolated turtle retina. The open circles describe the time course of repolarization during exposure to the bright background of the horizontal cell shown in Fig. 5. The data points for each cell have been shifted vertically for clarity. The membrane potential during the background was measured relative to the post-background level. The straight lines were fitted by a linear regression procedure to each set of data points.

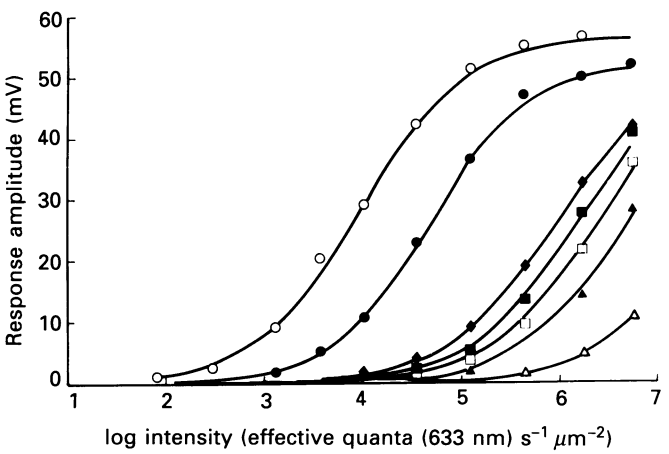

Fig. 8. The recovery of an L-type horizontal cell from the bright background exposure as reflected in the intensity–response curves. The data were measured prior to any background application (○), just before the exposure to the bright background (●) and at different time intervals after termination of the background light: 10 s (△); 2 min (▲); 5 min (□); 10 min (■) and 20 min (◆). The data were fitted with eqn (1) and the parameters which characterize the curves, n and log σ, were: dark adapted, 0·8 and 4·0; pre-bleach, 0·9 and 4·7; 10 s, 0·8 and 7·5; 2 min, 0·8 and 6·7; 5 min, 0·8 and 6·4; 10 min, 0·8 and 6·2; 20 min, 0·8 and 6·0. The units of σ are effective quanta (633 nm) s^{-1} μm^{-2}.

The line through the data points was derived by a linear regression procedure which is satisfactorily ($R = 0{\cdot}979$) described by the equation:

$$S_t/S_{\text{pre}} = 0{\cdot}00648t + 0{\cdot}00002, \quad (2)$$

where S_t and S_{pre} are, respectively, the step sensitivities of the horizontal cell measured

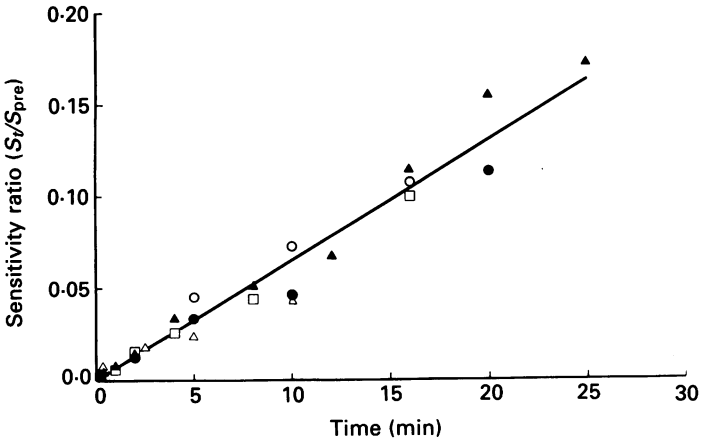

Fig. 9. The recovery of horizontal cell sensitivity to light after the exposure to bright background illumination. Step sensitivity was measured from the peak amplitude of small (less than 1·5 mV) photoresponses. The data points describe the sensitivity loss, relative to the pre-bleach value, S_t/S_{pre}, at different time intervals after termination of the bright background. Different symbols describe different cells studied in different experiments. The straight line was derived by a linear regression procedure.

at time t (in minutes) after the termination of the bright background exposure and prior to its application. The data illustrated in Figs 8 and 9 indicate that pigment regeneration occurs in the cones of the isolated turtle retina but it occurs at a very slow rate: 25 min after the termination of the bright background, sensitivity has increased to a level that is about 15% of the value measured prior to the exposure to the background.

Photoresponse kinetics

Several sensitivity-controlling mechanisms operate in the cones, and each may dominate under specific adaptation conditions. Under conditions when reduced quantal catching is readily apparent, it is expected that light stimuli, adjusted to produce equal quantal absorption, will elicit photoresponses which will match in terms of both amplitude and kinetics. Such a match between two photoresponses would not be expected if other sensitivity-controlling mechanisms, which involve the phototransduction process, dominate.

Figure 10*A* compares two responses recorded at different times following the termination of a bright background exposure. One response was evoked by a stimulus intensity of 6·23 log effective quanta (633 nm) s^{-1} μm^{-2} which was delivered after 8 min in darkness. The other response was evoked by a stimulus of 5·64 log

effective quanta (633 nm) s^{-1} μm^{-2} delivered after 20 min in darkness. These two responses match almost perfectly in terms of amplitude and kinetics even though they were recorded at different states of dark adaptation. In contrast, responses which were measured in the dark, following exposures to different backgrounds of

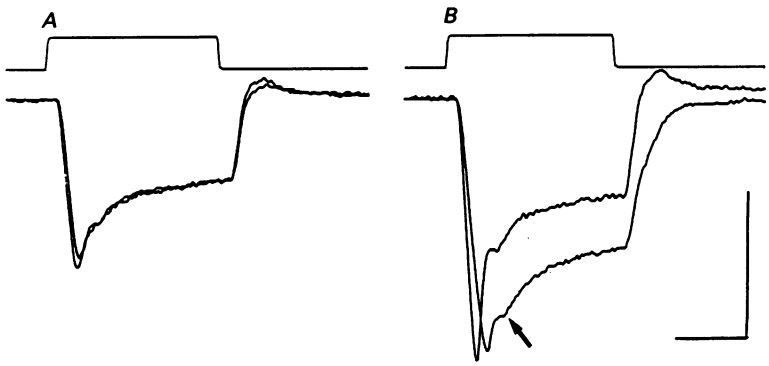

Fig. 10. Comparisons of photoresponses with similar peak amplitudes, recorded under different bleaching conditions. *A*, photoresponses recorded in darkness during the recovery from a bright background exposure (6·5 log effective quanta (633 nm) s^{-1} μm^{-2}). One response was elicited by a stimulus intensity of 6·23 log units 8 min after termination of the background. The other response was evoked by a stimulus intensity of 5·64 log units delivered 20 min after the background. *B*, photoresponses recorded in darkness after exposure to backgrounds of moderate intensities. One response was recorded after a background of 3·5 log units (arrow) and the other after a background of 5·5 log units. Calibration bars: vertical, 20 mV; horizontal, 200 ms.

moderate intensity, could not be made to match in terms of both amplitude and kinetics as shown in Fig. 10*B*. The traces are responses recorded after background intensities of 3·0 (arrow) and 5·5 log effective quanta (633 nm) s^{-1} μm^{-2}. These two responses match in peak amplitude but differ significantly in terms of kinetics. The response recorded after the brighter background is characterized by faster temporal characteristics.

DISCUSSION

The data described in this study were obtained from L-type horizontal cells in the isolated turtle retina and were used to assess the relative contribution of the different sensitivity-controlling mechanisms to the cone system. One difference between these two cell types is the steepening of the intensity–response curve under light-adapted conditions which was observed in turtle L-type horizontal cells and not in the red cones (Normann & Perlman, 1979*a*, *b*). However, it has been shown previously for the skate and turtle eyecups that the sensitivity to light of L-type horizontal cells basically reflects events occurring in the photoreceptors under light-adaptation conditions and during the recovery from photopigment bleaching provided that full-field diffuse retinal illumination is used (Green, Dowling, Siegel & Ripps, 1975; Itzhaki & Perlman, 1987). Therefore, sensitivity data from horizontal cells can be used to draw inferences about adaptive mechanisms operating within the cones.

Background adaptation

The effects of background illumination of dim to moderate intensities on the horizontal cells in the isolated turtle retina were very similar to those described in the eyecup preparation (Normann & Perlman, 1979*b*; Itzhaki & Perlman, 1987). The

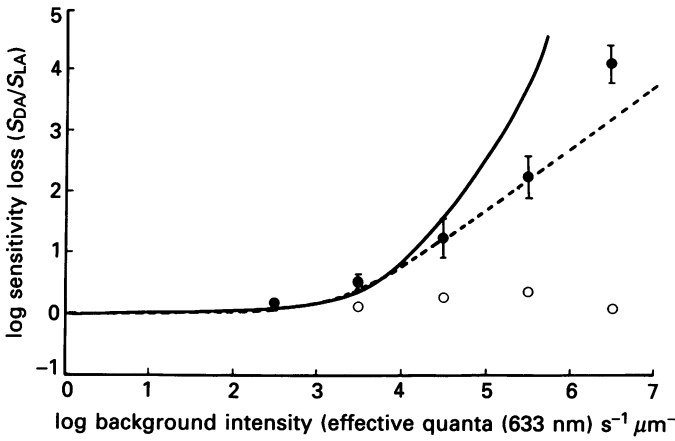

Fig. 11. Background adaptation of L-type horizontal cells in the isolated turtle retina. Filled circles describe the mean ± S.D. of sensitivity loss (above the dark-adapted level) induced by each background. The dashed curve is a Weber–Fechner type relationship. The continuous curve represents the sensitivity loss expected if 'response compression' was the dominant sensitivity-controlling mechanism. Open circles are the actual contribution of 'response compression' to sensitivity losses calculated from the experimental findings for each background (eqn (3)). S_{DA} and S_{LA}: dark- and light-adapted step sensitivities.

intensity–response curves (plotted semilogarithmically), recorded during background illumination, shift laterally towards higher intensities and become steeper. To evaluate the desensitizing effects of background illumination on L-type horizontal cells in the isolated turtle retina, step sensitivities were calculated from the peak amplitude of small photoresponses. The filled circles in Fig. 11 describe the average loss of step sensitivity (relative to the pre-background dark-adapted state) as a function of log background intensity. Background adaptation (up to an intensity of 5·5 log effective quanta (633 nm) s^{-1} μm^{-2}) is satisfactorily described by a Weber–Fechner type relationship (Fig. 11, dashed curve), similar to the one described for the turtle cones in the eyecup (Baylor & Hodgkin, 1974; Normann & Anderton, 1983). Thus, we can conclude that background adaptation of the cone system does not depend upon interactions between the cones and the pigment epithelium. The desensitizing effect of the brightest background (log intensity of 6·5 effective quanta (633 nm) s^{-1} μm^{-2}) is larger than predicted from the Weber–Fechner relationship (Fig. 11). This deviation reflects the contribution of bleached pigment to the sensitivity loss.

Two sensitivity-controlling mechanisms have been considered to operate in the vertebrate photoreceptors during background illumination (Baylor & Hodgkin, 1974; Normann & Perlman, 1979*c*; Valeton & van Norren, 1983; Green, 1986;

Hemila, 1987). 'Response compression' reflects movement of the operating point along a single intensity–response curve to portions of the curve where the slope is smaller. 'Gain reduction' is expressed as a lateral shift of the intensity–response curve along the log intensity scale. The data illustrated in Fig. 3 clearly demonstrate the contribution of 'gain reduction' to sensitivity control in the turtle cone system. In order to estimate the contribution of 'response compression' alone to background desensitization, we have postulated a hypothetical photoreceptor which is characterized by a single intensity–response curve regardless of the ambient illumination. Such a cell will require stronger stimuli for the generation of a criterion increment response because its operating point is set by the 'steady' hyperpolarization to regions of the curve which have a shallower slope. Assuming a Naka–Rushton type of relationship for the intensity–response curve (eqn (1)) with $n = 1$ and a constant σ, the desensitizing action of 'response compression' can be derived (Baylor & Hodgkin, 1974; Dawis, 1978). This theoretical curve (Fig. 11, continuous curve) fits the experimental data only over a very narrow range of background intensities. As the background intensity is made brighter, its desensitizing effect increases rapidly and deviates extensively from the Weber–Fechner law.

The actual contribution of 'response compression' to the sensitivity losses can be evaluated from the following equation (Baylor & Hodgkin, 1974; Normann & Perlman, 1979*c*; Valeton & van Norren, 1983):

$$S_{DA}/S_{LA} = [V_{max}/(V_{max} - V_{ss})]^2, \tag{3}$$

where S_{DA} and S_{LA} are the dark- and light-adapted step sensitivities, V_{max} is the maximal response amplitude that can be recorded from the cell and V_{ss} is the 'steady' membrane potential measured during the background light relative to the dark-adapted level. These calculated values have been plotted in Fig. 11 (○). It can be clearly seen that 'response compression' provides a negligible contribution to background adaptation in the isolated turtle retina.

Based on these considerations, it is concluded that 'gain reduction' is the major contributor to background adaptation of the cone system in the isolated turtle retina as has been previously shown in the turtle eyecup (Baylor & Hodgkin, 1974; Normann & Perlman, 1979*a*). This mechanism appears to be inherent to the cone photoreceptors as it is independent of a close association between the retina and the pigment epithelium.

Bleaching adaptation

The isolated retina preparation has allowed us to investigate the contribution of bleached photopigment to cone desensitization. Sensitivity losses measured after background exposures (Figs 3 and 7) were attributed to the presence of bleached photopigment. The fraction of pigment bleached by a background exposure can be calculated from the following equation (Alpern, 1971):

$$P_t = P_0 \exp(-I_B t/Q_e), \tag{4}$$

where P_0 and P_t are the fractions of unbleached pigment present at time = 0 and at time = t after turning on a background light of intensity I_B. Q_e is the photosensitivity

to bleaching of the photopigment. Equation (4) can be used only if pigment regeneration is negligible during this period. This assumption is supported by the sensitivity data measured in darkness during the recovery from the effects of the bright backgrounds (Figs 8 and 9). These data indicate that, unlike previous reports on frog cones (Goldstein, 1967; Hood & Hock, 1973), the regeneration of the cone photopigment in the turtle retina strongly depends on the pigment epithelium and is very slow in the isolated retina preparation.

In order to estimate the bleaching effectiveness of the backgrounds used in our study, we need to know the value of the photosensitivity to bleaching, Q_e. This parameter can be derived from the exponential decrease in the horizontal cell membrane potential observed during exposure to bright backgrounds (Fig. 7), assuming that it faithfully reflects the cone membrane potential. A similar analysis has been used to evaluate Q_e from the exponentially decreasing membrane current of monkey cones (J. L. Schnapf, personal communication).

The voltage response of a cone photoreceptor reflects the extent of quantal absorption by the photopigment. For backgrounds of dim to moderate intensity, the fraction of unbleached photopigment remains close to 1 and therefore the number of quanta absorbed depends mainly upon the intensity of incident light. Under these conditions, the 'steady-state' response to a background light increases and sensitivity decreases as the background intensity is raised (Baylor & Hodgkin, 1974; Normann & Perlman, 1979*a*; Itzhaki & Perlman, 1987). However, for very bright backgrounds, while the voltage response to the background monotonically decreases the sensitivity also decreases (Fig. 5). This observation reflects a substantial decrease in the probability of quantal absorption associated with extensive bleaching of the photopigment.

The exact relationship between the voltage response of a cell and background intensity has not been measured here. It was reported for turtle cones and horizontal cells (Normann & Perlman, 1979*a*, *b*) that the steady-state response to prolonged (1–2 min) background exposures was roughly half of the transient response to background onset. Since the transient response to background onset followed the Naka–Rushton relationship (eqn (1)), the steady-state response can be approximated by the equation

$$V_{BSS} = V_{BSS,max} I^n_{B,eff}/(I^n_{B,eff} + \sigma^n_B), \quad (5)$$

where V_{BSS} is the 'steady-state' hyperpolarization induced by a background of effective intensity $I_{B,eff}$, $V_{BSS,max}$ is the maximal degree of hyperpolarization measured in the presence of a bright background (roughly 1/2 V_{max} measured with a bright saturating light stimulus). σ_B is a constant and n defines the steepness of the curve. In eqn (5), $I_{B,eff}$ is used rather than I_B to emphasize the concept that quantal absorption determines excitation and not the incident quanta.

For dim to moderate backgrounds, bleaching is negligible, therefore, $I_{B,eff}$ remains constant throughout the background exposure and is linearly related to I_B. However, for bright backgrounds, when substantial bleaching occurs, $I_{B,eff}$ changes with time depending upon the fraction of unbleached pigment present (Clack & Pepperberg, 1982; Pepperberg, 1984). The fraction of unbleached pigment, P_t, present at time t after turning on the background light is given by eqn (4). Therefore,

$$I_{B,eff} = I_B P_0 \exp(-I_B t/Q_e). \quad (6)$$

Substituting eqn (6) into eqn (5), setting $P_0 = 1$ and assuming that when substantial bleaching starts to occur $I_{B,eff}$ is smaller than σ_B we get

$$V_{BSS} = V_{BSS,max} I_B^n (\exp(-I_B t n/Q_e))/\sigma_B^n. \quad (7)$$

Equation (7) predicts an exponential decay of the voltage response during the application of a bright background which bleaches a substantial amount of photopigment. This prediction has been borne out in Fig. 7. From eqn (7), the time constant, τ, of the exponential decay in potential is given by:

$$\tau = Q_e/(I_B n). \quad (8)$$

The value of n has not been determined in this study. However, from a previous report (Normann & Perlman, 1979*a*), n for the transient response of turtle cones was 1. Since the 'steady-state' potential produced by a background illumination was about one-half of the initial peak, we assumed a value for n of 1.

From the time constant of the decay in membrane potential measured in the presence of the bright background (14·3 s) and the intensity of this background we have derived a value for Q_e of $4{\cdot}5 \times 10^7$ effective quanta (633 nm) μm^{-2}. This value is close to the values calculated from the early receptor potential measurements from red cones in the turtle eyecup ($8{\cdot}0 \times 10^7 \pm 2{\cdot}2 \times 10^7$ effective quanta (644 nm) μm^{-2}; Hodgkin & O'Bryan, 1977). Thus, despite the simplifying assumptions made here, the value calculated for the photosensitivity to bleaching is in close agreement with values derived from more direct measurements.

The filled circles in Fig. 12 show the relationship between the fraction of pigment present after each background exposure (calculated from eqn (4) with $Q_e = 4{\cdot}5 \times 10^7$ effective quanta (633 nm) μm^{-2}) and the loss in sensitivity (relative to the pre-background values). The continuous curve describes the expected sensitivity loss due to the reduced probability of quantal absorption by the remaining photopigment. For bleaches of up to 60% and most likely as high as 95%, the loss in sensitivity is greater than expected based on quantal absorption considerations. Under these conditions, a different mechanism, associated with bleached photopigment, must determine the cone sensitivity. Rushton (1961) and Dowling (1963) demonstrated that the log of sensitivity loss was linearly related to the fraction of bleached photopigment present during dark adaptation. This 'log–linear' relationship is described by:

$$\log(S_{pre}/S_{post}) = K(1-P), \quad (9)$$

where S_{pre} and S_{post} are respectively the step sensitivities measured before and after the background exposure. P is the fraction of unbleached pigment remaining after termination of the background light and K is a proportionality constant. In order to estimate the value of K for turtle red cones, we have performed a linear regression on the data of Fig. 12 which reflect less than complete bleaching conditions. A value of 2·1 was thus obtained (dashed line in Fig. 12).

Rods and cones in different species seem to obey the relationship described in eqn (9) under most circumstances but they differ in the value of K. Some typical values for K reported in the literature are: 3 for human cones (Rushton, 1966); 19 and 12

for human rods (Rushton, 1966; Alpern, 1971); 6 for rat rods (Dowling, 1963); 5 for skate rods (Dowling & Ripps, 1970) and 6 for cat rods (Ripps, Mehaffey & Siegel, 1981).

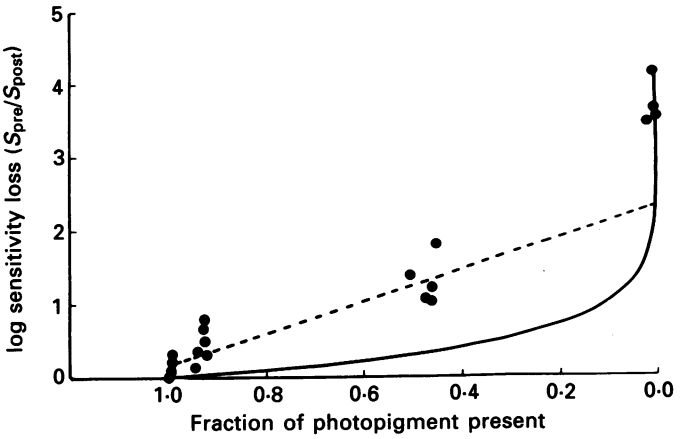

Fig. 12. Bleaching adaptation of L-type horizontal cells in the isolated turtle retina. Each data point represents the dependence of sensitivity loss on the fraction of unbleached photopigment. The bleaching effect of each background was calculated from eqn (4). The continuous curve describes the expected loss of sensitivity from the reduction in quantal catch. The dashed line satisfies eqn (9) with $K = 2{\cdot}1$. S_{pre} and S_{post}: step sensitivities measured before and after background exposure.

When bright backgrounds, which produced substantial bleaches (more than 99%), were applied, the loss of sensitivity was larger than expected from the log–linear relationship. The additional desensitization is likely to be due to reduced quantal catching. The role of quantal catching, made apparent during strong bleaching, is also supported by the kinetics of the photoresponses recorded at different times during the recovery from the bright bleaching exposure (Fig. 10). Identical photoresponses were elicited by different stimulus intensities at different times indicating an identical degree of cone excitation. Thus, the two conditions differed mainly with regards to quantal catching.

This study examined the relative contribution of different adaptive mechanisms to light sensitivity of the cone system in the turtle retina and tested the involvement of the pigment epithelium in these mechanisms. We have shown that regeneration of the cone pigment in the isolated turtle retina proceeds at a very slow rate, contrary to previous reports from the frog retina (Goldstein, 1967; Hood & Hock, 1973). Background illumination of dim to moderate intensities desensitizes the cone system mainly by activating a 'gain reduction' mechanism in the cone phototransduction process. This adaptive mechanism is inherent to the cones and is independent of the pigment epithelium. Sensitivity loss due to bleached pigment is produced via two different mechanisms. Under low to moderate levels of bleaching (less than 95%), the bleached photoproducts produce desensitization which is logarithmically related to the degree of bleaching. With extensive bleaching (more than 95%), the contribution of reduced quantal catching becomes apparent.

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