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THE EFFECTS OF BACKGROUND ILLUMINATION ON THE PHOTORESPONSES OF RED AND GREEN CONES

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SUMMARY

1. The photoresponses of light- and dark-adapted red and green cone photoreceptors were recorded intracellularly in the retina of the turtle, *Pseduemys scripta elegans*. Background illumination produced similar effects on both types of cones.

2. In response to the onset of a prolonged, steady background illumination the cone initially hyperpolarized to a peak which then sagged back to a steady-state polarization that was typically about one half the initial peak amplitude. This sag was observed for all backgrounds studied (dim as well as bright).

3. A resensitization was observed concomitantly with this sag; both the maximum increment and decrement responses grew in amplitude as light-adaptation proceeded. After about 2–3 min of background illumination, the amplitudes of these responses stabilized.

4. The dark-adapted cone produced graded responses to test pulses over a range of intensities spanning about 3·5 log units. The amplitudes of these responses were well fit by the relationship $V = I \cdot V_m/(I + \sigma)$.

5. After 2–3 min of background illumination, 500 msec test pulses either brighter or dimmer than the background intensity were substituted for the background. The light-adapted intensity–response curves constructed from this data were similar to the dark-adapted curve but were shifted horizontally and slightly vertically, so that they still spanned about 3·5 log units of intensity. Thus, in the light-adapted cone, graded responses were elicited by a range of bright test pulses which would have produced saturated responses when delivered to the dark-adapted cone.

6. The 'off response' observed at the offset of the background became faster as the background intensity was increased. It also became faster with time following the onset of any particular background intensity.

7. It was concluded that cone sensitivity during any state of light-adaptation is determined by two mechanisms; response compression resulting from the instantaneous non-linearity between 'internal transmitter' concentration and membrane potential and a more active 'cellular adaptation' mechanism which is manifest as a shift in the intensity–response curve. In the steady-state condition of light-adaptation, most of the sensitivity changes are a result of the cellular adaptation mechanism.

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8. Photopigment bleaching caused by the backgrounds, negative feed-back from horizontal cells and voltage dependent mechanisms in the cones could not account for this cellular adaptation. These effects of background illumination were interpreted in terms of the 'internal transmitter' hypothesis of phototransduction.

INTRODUCTION

Recent intracellular studies in the vertebrate retina have shown that background illumination causes large changes in the sensitivity of both rods and cones (Grabowski, Pinto & Pak, 1972; Normann & Werblin, 1974; Baylor & Hodgkin, 1974; Kleinschmidt & Dowling, 1975; Coles & Yamane, 1975; Fain, 1976). In these reports, the adaptation properties of rods have received the most attention. It is the cones, however, which seem to possess the more sophisticated adaptation machinery.

Two approaches have been used to study cone adaptation which have focussed upon different aspects of the phenomenon. Boynton & Whitten (1970) and Normann & Werblin (1974), relying mainly upon extracellular recordings from monkey and *Necturus*, respectively, have shown that *long term* (longer than 2 min) bright background illumination produces cone desensitization which is manifest as a shift in the dynamic range of the cone mass receptor response; light-adapted cones appear to be able to generate graded responses to graded intensities which would saturate their dark-adapted counterparts. Baylor & Hodgkin (1974), have recorded intracellularly from turtle cones and shown that under *short term* conditions of bright background illumination (from 0·6 to 1·2 sec), cone sensitivity is further significantly reduced by the instantaneous non-linearity between 'blocking particle' concentration and cone potential. These differences could be due to the obvious differences in adaptation time scales studied. They could also reflect differences in species or in the intracellular *vs.* the extracellular technique.

The experiments described in this and the following reports (Normann & Perlman, 1979) utilized intracellular recording techniques from turtle cones and horizontal cells, respectively, to study the effects of long term background illumination (from 2 to 6 min) on the polarization of these cells and their ability to respond to test pulses brighter or dimmer than the background. It will be shown that immediately following the onset of a bright background, the cone is initially saturated and cannot signal either a very bright or dim test pulse when substituted for the background. However, as light-adaptation proceeds the cone resensitizes. In the steady-state condition of light-adaptation, the cone photoresponse is characterized by an intensity–response curve that is shifted with respect to its dark-adapted curve. It will be shown that photopigment bleaching, voltage dependent membrane mechanisms, or negative feed-back from horizontal cells are not responsible for these changes.

The dark- and light-adapted responses are discussed in terms of the 'internal transmitter' hypothesis (Baylor, Hodgkin & Lamb, 1974). It is concluded that while there is an apparent increase in the rate of removal of the internal transmitter caused by increased background illumination, light-adaptation may also decrease the number of 'blocking particles' released per photon absorbed, or increase the dissociation constant between the blocking particles and the light modulated channels.

METHODS

Preparation and recording

Adult specimens of *Pseudemys scripta elegans* with shell lengths of 17–25 cm were used in these experiments. Recordings were made using the eyecup, prepared similarly to that described previously (Baylor & Hodgkin, 1973). The animal was decapitated and its head pithed, an eye was enucleated and the anterior hemisphere was dissected away. Excess vitreous humor was removed by snipping with scissors and draining with V-shaped pieces of tissue paper. The eyecup was mounted in a chamber which was enclosed except for a narrow slit through which entered the electrode and the stimulating light beams. Surrounding the eyecup, but not touching it, were layers of filter paper and cotton saturated with water which minimized retinal dehydration. A continuous stream of moistened 95% O_2, 5% CO_2 was directed into the chamber. The temperature was maintained at 18 °C using a Peltier device.

Electrodes were pulled on a Livingston-type micro-electrode puller from capillary tubing each of which had a small fibre fused to the inside wall. The electrodes, filled with 4 M-potassium acetate, had resistances of 100–300 MΩ. The electrodes, which were advanced through the retina from the vitreal surface, were positioned about half way between the optic disk and the edge of the eyecup and centred over a small spot of light produced by the photostimulator. The electrodes were connected to a negative capacitance preamplifier and all responses were tape recorded for later analysis. Cone and horizontal cell identification was based upon criteria of response kinetics, receptive field properties and spectral sensitivities (Baylor, Fuortes & O'Bryan, 1971).

Photostimulator

The photostimulator used in this study contained two beams of light originating from a single light source (General Electric, Quartzline 45W). The two beams were focused upon the retina after being combined with a beam splitting prism. Both beams were shuttered with a single balsa wood vane fixed to a rotary stepping motor so that, depending upon the state of activation of the motor, light was incident upon the retina from either the test channel or the background channel. The vane was positioned in the two beams to make a monotonic transition in retinal illumination as the beam was switched between channels. The time required to switch between channels was about 5 msec. With this type of photostimulator, test flashes of any intensity (either brighter or dimmer than the background) could be substituted for a wide range of background intensities.

The intensity, colour, and size of the image produced by each beam was independently controlled by neutral density and interference filters and by an iris diaphragm interposed in each optical pathway (the diameter of the illuminated area could be varied from 0·3 to 3·2 mm). *X-Y* manipulators attached to each diaphragm were used to adjust the position of the two images so that the spot illuminated by each beam could be centred on the impaled cell. The emission spectrum of the light source was determined at the retinal plane using a calibrated photodiode (United Detector Technology, Inc., Santa Monica, Model No. 1223) and narrow band interference filters which spanned the visible spectrum in 20 nm steps. The flux of unattenuated light, determined by multiplying the emission spectrum of the light source at each measured wavelength by the spacing between measured wave-lengths and the normalized red cone action spectrum (Baylor & Hodgkin, 1973), was $6{\cdot}4 \times 10^{15}$ effective quanta (640 nm) sec^{-1} cm^{-2} for the test channel and $9{\cdot}1 \times 10^{15}$ effective quanta (640 nm) sec^{-1} cm^{-2} for the background channel. Unless otherwise specified, all intensities described in the text refer to the number of log units of attenuation produced by the neutral density filters interposed in each beam. White light was used in all the adaptation experiments described in this report.

RESULTS

Cone hyperpolarization produced by constant illumination

The cones in the dark-adapted turtle respond in a graded manner when stimulated with light pulses of graded intensities (Baylor & Fuortes, 1970). For very bright stimuli, the cone response saturates and exhibits a maximum amplitude regardless

of the intensity of the test flash (Baylor & Fuortes, 1970; Baylor *et al.* 1974). Various levels of steady white illumination (3·2 mm spot diameter) were shone upon the dark-adapted retina to determine the behaviour of the cone under these conditions. Fig. 1*A* shows typical responses to four background illuminations of different intensities (each approximately 10 times the intensity of the preceding intensity) in one particularly stable cone. In response to these backgrounds, the cone initially hyperpolarized to a peak and then sagged towards a steady-state that remained relatively constant as long as the retina was illuminated.

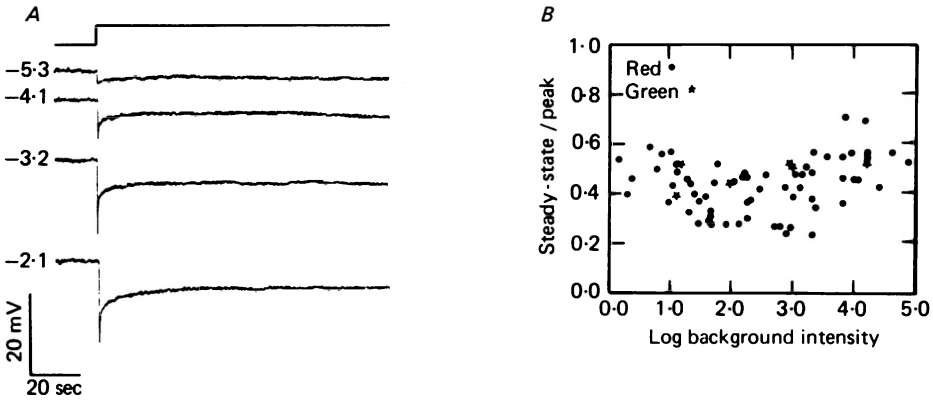

Fig. 1. The effect of steady illumination on cone polarization. *A*, red cone responses to four different levels of background illumination. The numbers to the left of each response describe the attenuation of the background beam in log units. *B*, ratio of the amplitudes of the steady-state and peak components of red and green cone responses (measured from the dark-adapted potential recorded prior to the background onset) for sixty-four background intensities. The scale on the abscissa was defined as log background intensity–log threshold intensity, where log threshold intensity was the intensity of the test pulse (in log units of attenuation) which elicited a response in the dark-adapted cone that was 10% of the maximum response.

Fig. 1*A* illustrates an interesting feature of light-adaptation; for any intensity of steady background illumination used in these experiments (the total range of background intensities investigated spanned 5 log units above threshold), the final steady level of hyperpolarization was about one half the amplitude of the initial peak. This observation, made in twenty-five different cones, is summarized in Fig. 1*B* where the ratio of steady polarization to peak polarization in both red and green cones is plotted as a function of the log of the effective background intensity (log background–log threshold, where log threshold was defined as the stimulus which evoked a peak response which was 10% of the maximum peak response). Some cones were sufficiently stable to allow a number of different background intensities to be studied. For the sixty-four background intensities illustrated in Fig 1*B*, the mean ratio of plateau to peak response was $0{\cdot}44 \pm 0{\cdot}11$ (S.D.). This sag from peak to plateau has been briefly described in the cones of *Necturus* (Normann & Werblin, 1974) and turtle (Baylor & Hodgkin, 1974; Simon, Lamb & Hodgkin, 1975).

The cone 'off response' during background illumination

As the intensity of a supersaturing stimulus is increased the response of the dark-adapted cone is prolonged but does not change in amplitude (Baylor & Fuortes, 1970; Baylor *et al.* 1974). The lowest trace in Fig. 2 illustrates this with the response of a dark-adapted cone to a 500 msec pulse of $-1{\cdot}06$ log units intensity. For this bright intensity, the 'off response' appeared biphasic. Immediately upon termination of the test pulse, the cone potential fell only slightly for the first 0·5 sec and then began to recover towards its dark-adapted resting potential. The kinetics of the 'off response' have been extensively described for the dark-adapted cone (Baylor *et al.* 1974).

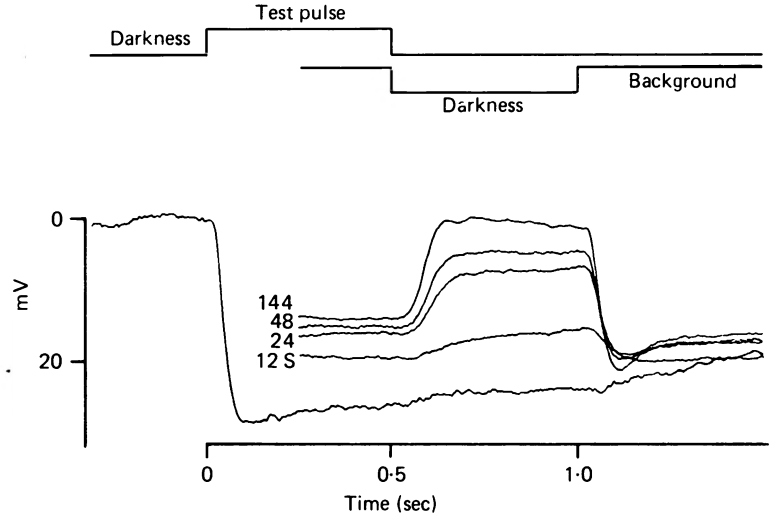

Fig. 2. The cone 'off response' at various times after the onset of a $-1{\cdot}06$ log unit background illumination. The bottom trace shows the red cone response to a $-1{\cdot}06$ log unit test pulse 500 msec long (upper stimulus monitor). The four traces above the bottom trace are (from the bottom to the top) responses to 500 msec pulses of darkness (lower stimulus monitor) at times 12, 24, 48 and 144 seconds after the onset of the $-1{\cdot}06$ background intensity. The stability of this and subsequent recordings was such as to allow accurate determinations of the d.c. level throughout this experiment. The position of each trace reflects the d.c. level of the cone (relative to the level prior to the onset of the background) at each time the response was recorded.

Unlike the dark-adapted case, under conditions of bright background illumination, cones were able to generate fast and large off responses. In Fig. 2 are shown the responses of the same cone to 500 msec pulses of darkness at various times (indicated by the numbers to the left of each trace) after the onset of a background of $-1{\cdot}06$ log units intensity. In this and in subsequent experiments, the d.c. stability of the recordings was such as to allow reliable determinations of the cell potential (relative to its value in the dark-adapted state) at all times following the onset of the background. Therefore, in this and in all subsequent figures, all responses are shown with d.c. fidelity preserved. Shortly after the onset of the background, the cone was capable of producing only a very small off response but as light-adaptation proceeded, the cone generated progressively larger and faster off responses. After a few minutes of

background illumination, the amplitude of the off response usually exceeded by a few millivolts the amount of steady hyperpolarization produced by the background (Figs. 5 and 6). Speculations about the implications of this overshoot of the dark-adapted level will be discussed later.

The cone increment response during background illumination

Simultaneously with the growth of the off response, the responses to test pulses brighter than the background became larger as light-adaptation proceeded. Cone responses to unattenuated test flashes at various times (shown to the left of each trace) after the onset of a −1·06 log unit background are shown (again with d.c.

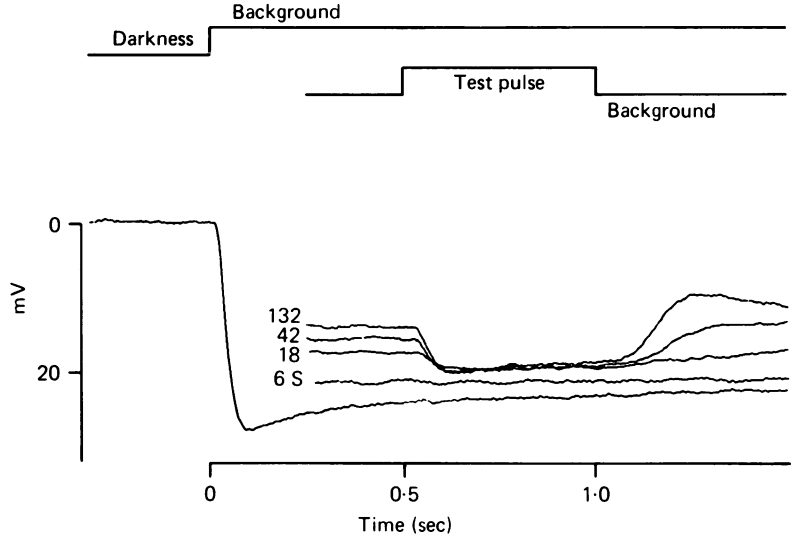

Fig. 3. The cone increment response at various times after the onset of a −1·06 log unit background illumination. The bottom trace shows the red cone response to the onset of a −1·06 log unit background (upper stimulus monitor). The 4 traces above the bottom trace are (from the bottom to the top) responses to 0·0 test pulses 500 msec long (lower stimulus monitor) at times 6, 18, 42 and 132 secs after the onset of the −1·06 log unit background. The position of each trace reflects the d.c. level of the cone at each time the response was recorded.

fidelity preserved) in Fig. 3. Also shown in Fig. 3 is the initial response to the −1·06 log unit background. Immediately upon application of a bright background, the cone was saturated and test pulses of the brightest intensity available could not elicit any increment response. As light-adaptation progressed, the cone potential began to fall, the cone came out of saturation and increment responses could be elicited; the greater the fall of cone hyperpolarization, the larger the amplitude of the increment response.

The similarity in the time courses of the sag of the cone membrane potential, and the growth in amplitude of both the increment and decrement responses recorded as light-adaptation proceeded is further emphasized in Fig. 4. The curves of Fig. 4, which were constructed from the same cell whose responses were illustrated in Figs. 2

and 3, show that as light-adaptation to bright backgrounds proceeds, the cone resensitizes (measured in terms of responses amplitude for a given pulse intensity). The fall in cone polarization illustrated here and in Fig. 1*A* might reflect an unblocking of light-modulated channels in the outer segment caused by the light-adaptation mechanism. This notion is strengthened by the observed simultaneous growth of the increment responses; channels which have become unblocked by the mechanism of light-adaptation can be blocked again by a sufficiently bright test pulse.

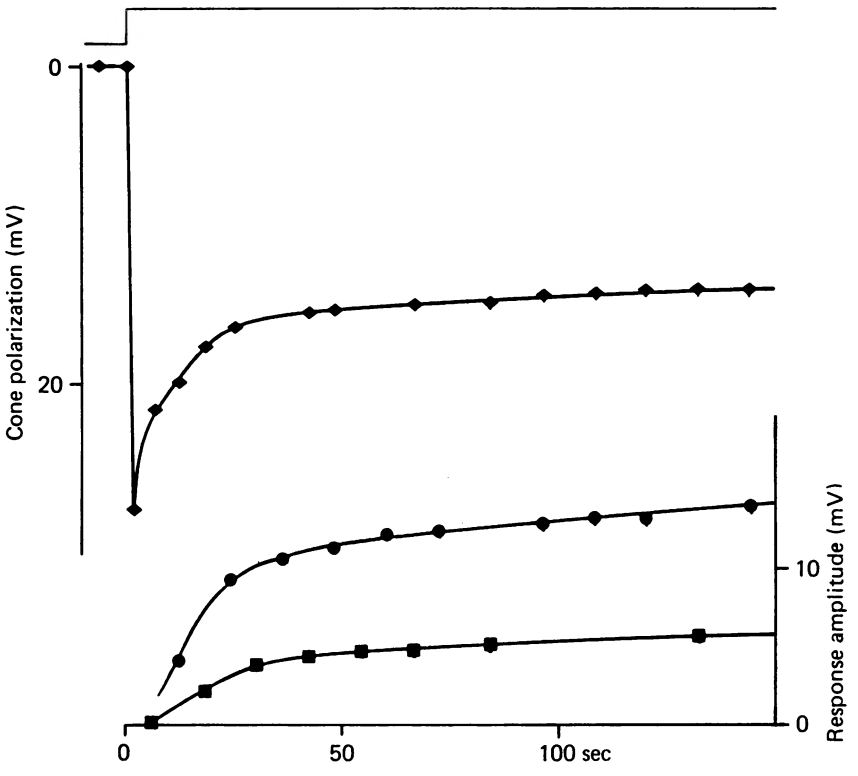

Fig. 4. The sag of the cone polarization (diamonds) and the growth in the amplitude of the cone increment responses (squares) and off responses (circles) as a function of time after the onset of a −1·06 log unit background (stimulus monitor). The data in this Figure was obtained from the responses of Figs. 2 and 3.

The effect of long term background illumination on the dynamic range of the cone

The responses of light-adapted cones to graded increment and decrement test pulses around each background were also recorded. The experimental protocol used in these experiments is illustrated by the typical recording shown in Fig. 5. The upper trace shows the light intensity incident upon the retina on a log scale and the lower trace the cone potential. The responses of a dark-adapted cone to a graded series of test pulse intensities (hereafter called an intensity–response series) was first recorded. A given background was then turned on and left uninterrupted for at least 2 min (an interval chosen to insure that the cone had reached a steady-state of light-adaptation) after which test pulses either dimmer or brighter than the background were substituted for the background every 6 sec and the light-adapted

intensity–response series recorded. In many instances the light-adapted intensity–response series was repeated, after which the background illumination was terminated. Intensity–response series were then repeatedly performed to verify that the cell condition had not changed during the experiment.

Typical responses recorded following this protocol are shown in Fig. 6. The dark-adapted intensity–response series is shown in Fig. 6*A*. Each test pulse in this series was approximately 0·5 log unit more intense than the preceding intensity (see Figure legend). Threshold for this cell (in terms of a measurable response) was between −6·5 and −6·0 log units while saturation (defined as the minimum test intensity which would elicit a maximum amplitude in the peak of the response) was

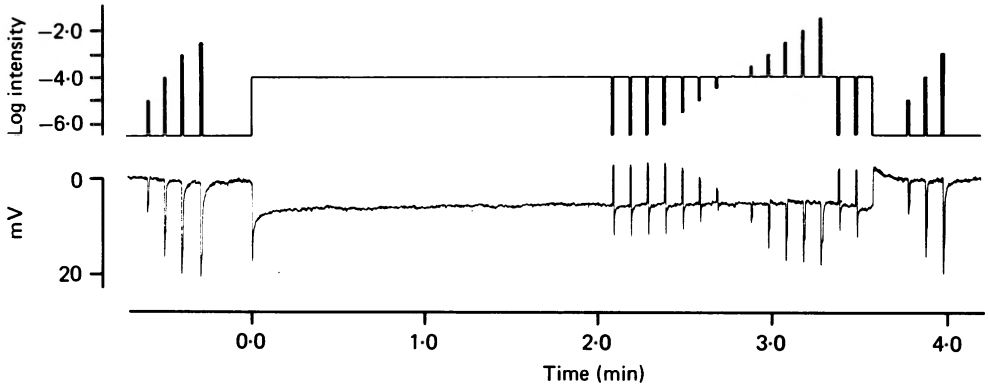

Fig. 5. Experimental protocol for light-adaptation experiments. The top trace shows intensity of the light incident upon the retina on a log scale. The lower trace is the cone polarization in response to the stimulus protocol.

around −2·5 log units; a total dynamic range of about 3·5–4 log units. Fig. 6*B* and *C* show (with d.c. fidelity maintained) the responses of the same cone to 500 msec increment and decrement test pulses recorded at −4·15 and −2·0 log unit backgrounds. The dotted line shows the dark-adapted resting potential recorded before the onset of the background. This cone, when light-adapted to the −4·15 log unit background produced graded responses over the range of intensities from −5·5 to −1·5 log units and when light-adapted to the −2·0 log unit background produced graded responses from about −4·0 to −0·5 log units.

These data illustrate three features of cones which had experienced long term light-adaptation. First, the cone was able to produce graded responses to graded increment and decrement stimuli. Secondly, the total range of intensities over which graded responses could be obtained in the light-adapted state (measured in terms of log units of intensity) was about the same as it was in the dark-adapted state, about 3·5 log units. Thirdly, the total potential excursion which the light-adapted cone could achieve(the amplitude of the maximum increment response plus the amplitude of the maximum decrement response) was about the same as the potential excursion of the dark-adapted cone (the amplitude of a saturated response in the dark-adapted retina).

These features of the light-adapted cone are better illustrated in Fig. 7 where the

potential at the peak of the cone response, measured from the dark-adapted resting potential (defined as 0), is plotted as a function of the log of the test intensity which elicited each response. The data shown in Figs. 6 and 7 are from two different cones. The steady-state potential produced by each background is shown as the horizontal line which intersects each curve. Each continuous curve in this and in all subsequent Figures was drawn from a template describing the function

$$V = \frac{I \cdot V_m}{I + \sigma}, \tag{1}$$

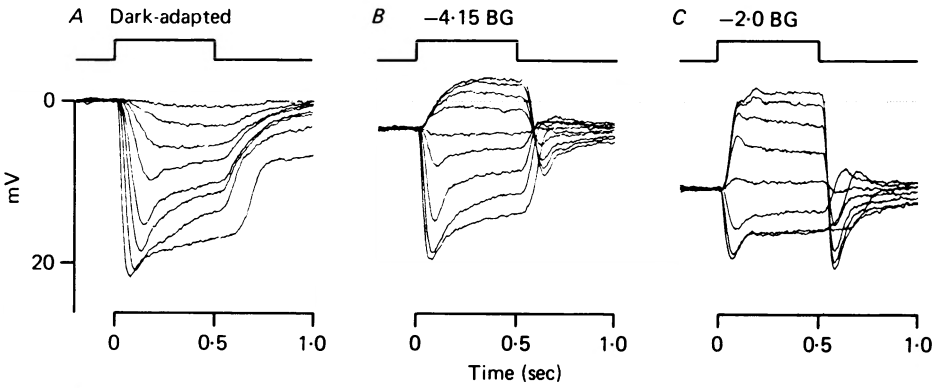

Fig. 6. Effects of background illumination on red cone photoresponses. *A*, dark-adapted photoresponses to 500 msec pulses of −6·4, −5·7, −5·1, −4·6, −4·0, −3·7, −3·2 and −2·6 log unit intensity. *B*, photoresponses from retina light adapted to −4·15 log unit background (BG). Test pulse intensities were darkness, −6·4, −5·7, −5·1, −4·6, −4·0, −3·7, −3·2, −2·6 and −2·1 log units. *C*, photoresponses from retina light-adapted to −2·0 log unit background. Test pulse intensities were darkness, −4·1, −3·7, −3·2, −2·6, −2·1, −1·6, −1·1 and −0·5 log units. The steady-state potential of the cone measured at each background relative to its value prior to the onset of the background (dotted line in this Figure) is the difference between the potential before the responses and the dotted line.

where V is the peak amplitude of the cone response produced by each intensity I, V_m is the maximum response and σ is the intensity which produces a response whose peak is $0{\cdot}5\ V_m$. The curve (Baylor & Fuortes, 1970) was fitted by eye to the data for the best fit. The data summarized in this Figure are from one particularly stable cone in which the effect of four different backgrounds could be studied (the cell was held for 1 hr, 15 min). In the twenty-two other cones studied using this protocol, from one to four backgrounds were used before the cell response began to deteriorate, but in each case, the cell behaved similarly to the two shown in Figs. 6 and 7. There was some variability in the behaviour of each light-adapted cone; some cones produced smaller increment but larger decrement responses than those illustrated in Figs. 6 and 7. This variability is also reflected in the data of Fig. 1*A*, showing in different cones the extent of the sag from peak to plateau recorded under background illumination.

Fig. 7 best illustrates the main effects of long term light-adaptation of cones. Background illumination shifted the dark-adapted intensity–response curve described by eqn. (1) horizontally (and slightly vertically) along the log intensity

axis. A bright background (− 2·0 log units) which produced a saturated dark-adapted response caused such extensive shifting of the cone intensity–response curve (approximately 2·5 log units) that fully graded responses could be recorded around

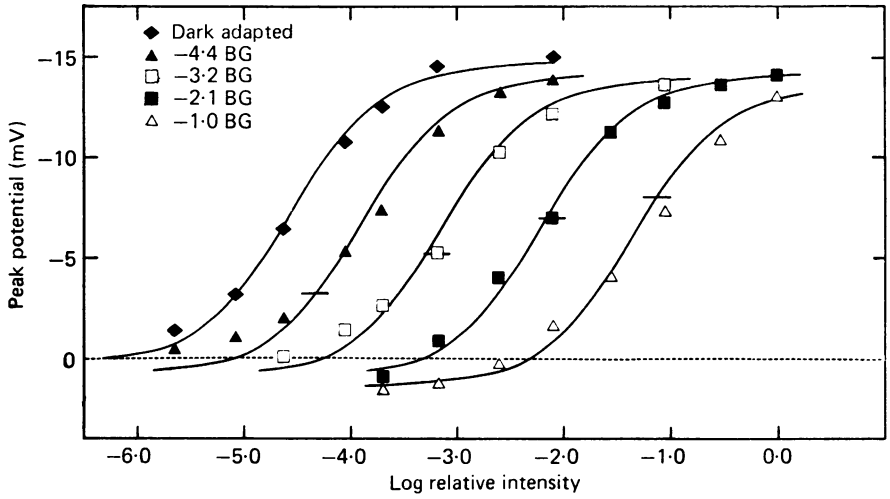

Fig. 7. Dark- and light-adapted intensity–response curves recorded in a red cone. The peak of either the increment or decrement response, measured from the dark-adapted potential recorded before the background onset (dashed line in this Figure) is plotted as a function of the log of the test pulse intensity which elicited each response. The steady hyperpolarization produced by each background is given by the intersection of the intensity-response curve and the small horizontal line (test pulses of these intensities, when substituted for the backgrounds (BG) elicited no responses). The continuous curve was drawn from a single template which describes the function $V = I \cdot V_m/(I + \sigma)$ (see text).

this originally saturating background. The total potential excursion which could be achieved by the light-adapted cone was independent of the background intensity used. Finally, the dynamic range of the cones (the range of intensities over which graded responses could be elicited) was about 3·5 log units, and was independent of the state of adaptation of the cones.

Light-adaptation of the green cone

The results described above were obtained from red absorbing cones. On only two occasions were green absorbing cones impaled with sufficient stability to allow the light-adaptation properties of this cell type to be determined. All aspects of the green cone responses were similar to the red cone responses but were somewhat slower (Baylor & Hodgkin, 1973). Background illumination produced effects in the green cones which were similar to those observed in the red cones. The intensity-response series of a green cone in its dark-adapted state is shown in Fig. 8*A*, around a − 5·1 log unit background in Fig. 8*B* and around a − 3·3 log unit background in Fig. 8*C*.

Fig. 9 shows the intensity–response curves measured from these responses. As in Fig. 7, the continuous curves were drawn from the template described by eqn. (1) and fitted to the data by eye. The green cone, like the red cone has a dynamic range

of about 3·5–4 log units which is independent of the state of adaptation and which is shifted along the log test-intensity axis by background illumination.

Since this complete shifting of the entire dynamic range of the cell was observed for both red and green cones, it is suggested that this is a property related to the structure of the photoreceptor, not to the photopigment contained therein.

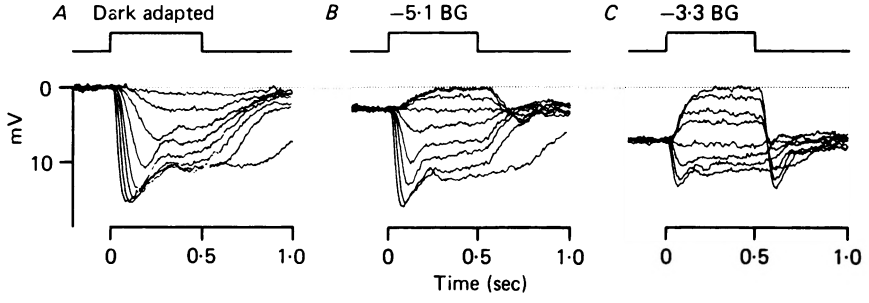

Fig. 8. Effect of background illumination on green cone photoresponses. *A*, dark-adapted responses to 500 msec pulses of −6·3, −5·6, −5·0, −4·5, −4·0, −3·6, −3·0 and −2·1 log units intensity. *B*, responses from retina light-adapted to −5·1 log background. Test pulse intensities were darkness, −6·3, −5·6, −5·0, −4·5, −4·0, −3·6, −3·0 and −2·1 log units. *C*, responses recorded from retina light-adapted to −3·3 log unit background. Test pulse intensities were darkness, −4·5, −4·0, −3·6, −3·0, −2·6, −2·1 and −1·0 log units. The steady-state potential of the cone measured at each background (BG) relative to its value before the onset of the background (dotted line in this Figure) is the difference between the potential before the responses and the dotted line.

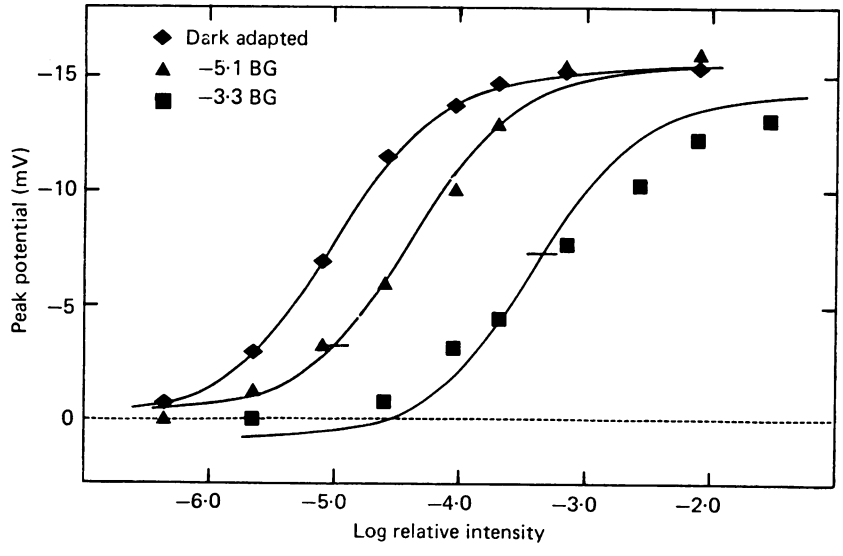

Fig. 9. Dark- and light-adapted intensity–response curves in a green cone determined as described in Fig. 7. The continuous curve was drawn from a single template describing the function $V = I \cdot V_m/(I+\sigma)$ (see text). The poor agreement between the data points recorded around the −3·3 log unit background and the continuous curve is probably due to a gradual deterioration of the cell; the control intensity–response series recorded after this background (BG) was smaller than the intensity–response series recorded before the background onset.

Unfortunately, neither blue absorbing cones nor rods were impaled in the turtle retina during the course of these experiments so this generalization could not be further tested.

Effect of spot size on light-adaptation of cones

Horizontal cells in the turtle retina have been shown by Baylor *et al.* (1971) and O'Bryan (1973) to be both presynaptic as well as postsynaptic to cones, with the horizontal cell to cone interaction being inhibitory. The sag from peak to steady-state hyperpolarization (Fig. 1) and the shift of the light-adapted intensity–response curve (Figs. 7 and 9) observed during background illumination could result from this feed-back pathway.

An experimental technique that isolates cones from horizontal cell feed-back is to use small spots of light which provide large cone excitation but only small amounts of horizontal cell excitation. The effects of small diameter (0·31 mm) spots for both the background and the test pulses on the light–adaptation properties of three cones was

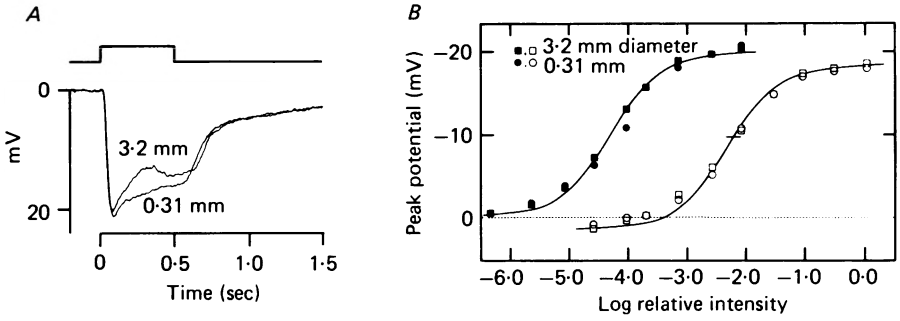

Fig. 10. The effect of circular spot diameter on the light-adaptation of a red cone. *A*, saturated responses of a cone to constant intensity test pulses of 0·31 mm and 3·2 mm diameter. *B*, intensity–response curves recorded in the dark-adapted retina (filled symbols) and around a −2·1 log unit background intensity (open symbols) for spots of 0·31 mm (circles) and 3·2 mm (squares) diameter. The continuous curve was drawn from a single template describing the relation $V = I \cdot V_m/(I+\sigma)$ (see text).

investigated and typical results are shown in Fig. 10. Fig. 10*A* shows superimposed responses to test pulses of the same intensity but of 0·31 and 3·2 mm diameters. The horizontal cell feedback onto this cone is evident in the greater sag in the response to the 3·2 mm pulse. Fig. 10*B* shows intensity–response curves measured from responses elicited by both 3·2 mm and 0·31 mm spots in the dark-adapted retina and around a −2·1 log unit background. Again, the continuous curves were drawn from a template described by eqn. (1) and fitted to the data by eye. The large and small spot intensity–response curves are very similar in terms of both the dynamic range of the cell and in the shift of the curve with background illumination. It is, therefore, concluded that horizontal cell feed-back onto cones plays little or no role in the long term adaptation properties of the cone.

DISCUSSION

The data described in the previous section, and that of Baylor & Hodgkin (1974) provide a relatively complete intracellular description of the effects of background illumination on turtle cone photoresponses. These studies point out two basic effects of backgrounds on cone responses; one due to the instantaneous non-linearity in the relationship between blocking particles released by light and sodium channels (hereafter called response compression, Kleinschmidt & Dowling, 1975) and one due specifically to the adaptation machinery which is manifest as a shift in the steady-state intensity–response curve (hereafter called cellular adaptation).

The contribution of each of these mechanisms to the cone behaviour depends upon the background intensity and the length of time that the background has been shone upon the retina. For very short periods of bright background illumination, Baylor & Hodgkin (1974) have shown that response compression can be a significant source of cone desensitization. However, as light-adaptation proceeds, the cone polarization falls, reducing the extent of response compression, and the cone actually resensitizes to a certain degree (Fig. 4). Under steady-state conditions of background illumination, the cone potential was never more than half the peak potential (Fig. 1). Response compression under these conditions would cause a cone desensitization of no more than 0·6 log units (Baylor & Hodgkin, 1974, eq. 8). Therefore, response compression plays only a minor role in determining steady-state cone sensitivity.

It is in the steady-state that the main effects of cellular adaptation are seen; bright backgrounds cause extensive shifting of the dark-adapted intensity–response curve (by 3–4 log units, Figs. 7 and 9) which reflects large reductions in cone sensitivity. Normann & Werblin (1974) derived the conditions which define a Weber's Law type of desensitization; the slope of the intensity–response curve (measured on a semi-log plot as in Figs. 7 and 9) measured around each background must be constant. The curves of Figs. 7 and 9 approximately meet these conditions showing that Weber's Law describes cone desensitization. Despite these large sensitivity changes, the cellular adaptation mechanism is beneficial in causing the dynamic range of the cone to be centred approximately around the average background intensity.

Possible mechanisms of cellular adaptation which will be considered in the remainder of this report are (*a*) decreases in quantum catching due to photopigment bleaching, (*b*) negative feed-back from horizontal cells onto the cones, (*c*) voltage dependent properties of the cone plasma membrane, (*d*) changes in the rate of production or removal of an internal transmitter, released by light, which blocks sodium channels and (*e*) changes in the dissociation constant describing the reaction between this transmitter and the sodium channels. Certainly additional mechanisms (or more specific ones) could be proposed but the results pertain mainly to the above possibilities.

Photopigment bleaching

Light quanta incident upon the retina can only contribute to cone excitation if they are absorbed by the cone photopigment. Since background illumination of the retina bleaches some of the photopigment, it could be argued that the decrease in quantum catching resulting from losses of photopigment could be sufficiently large to account

for the adaptation effects described earlier. The background intensity which bleaches about 50 % of the photopigment has been determined by calculation based on the estimates of photopigment photosensitivity and rates of regeneration. It has also been measured directly using spectrophotometry on the retina of the toad, *Bufo marinus*.

The calculation of bleaching levels was made assuming a simple photochemical reaction for the bleaching of photopigment (Alpern, 1971) as shown below

$$-\frac{dp}{dt} = \frac{Ip}{Q_e} - \frac{1-p}{t_0}. \tag{2}$$

where p = fraction of unbleached pigment; I = bleaching intensity; Q_e = the reciprocal of photosensitivity and t_0 = time constant of pigment regeneration. When eqn. (2) is set equal to zero (after a steady state has been reached), it can be reduced to the simple relationship

$$P = \frac{I_0}{I_0 + I}, \tag{3}$$

where $I_0 = Q_e/t_0$ is the intensity required to bleach 50 % of the photopigment in the steady-state. I_0 was calculated using the following estimates: $Q_e = 8 \times 10^{15}$ quanta cm^{-2} and $t_0 = 100$ sec obtained from turtle ERP measurements (Hodgkin & O'Bryan, 1977). Substituting these values yields an intensity required for a 50 % bleach of 8×10^{13} quanta sec^{-1} cm^{-2}. These calculations in conjunction with the calibration of the unattenuated intensity of the light source ($9{\cdot}1 \times 10^{15}$ effective quanta (640 nm) sec^{-1} cm^{-2}) lead to the estimate that a background intensity of $-2{\cdot}0$ log units would produce a steady-state bleach of about 50 %. Thus, the desensitization due to the decrease in quantum catching caused by a $-2{\cdot}0$ log unit background would only produce a shift of the cone intensity–response curve along the log intensity axis of about 0·3 log unit.

Photopigment bleaching by the background illumination was also measured directly by following the changes in the optical density of the isolated retina before and at various times throughout the continuous exposure of the retina to a given background intensity using the techniques described in appendix 1 of Normann & Werblin (1974). Unfortunately, measurements made on the isolated turtle retina were not successful, probably because most of the measuring light passed between the small cone outer segments. A rough estimate of the bleaching efficiency of the background channel was obtained by following the bleaching kinetics in an isolated toad retina. This measurement also showed that a background intensity of around $-2{\cdot}0$ log units was required to produce a 50 % bleach within 2 min. Because pigment regeneration may be reduced (or even eliminated) in the isolated retina, this $-2{\cdot}0$ log unit value is regarded as a lower limit of the 50 % bleaching intensity. The agreement of these two techniques allows the confident conclusion that a decrease in quantum catching produced by photopigment bleaching plays very little role in the light-adaptation of the cone over the range of background intensities considered in this study.

Negative feed-back from horizontal cells to cones

A major role of horizontal cell feed-back to cones in the light-adaptation of cones is unlikely in the light of the results of Fig. 10. Small and large spots of light elicit

responses with different kinetics but do not affect the light-adaptation properties of the cones. Further, if horizontal cell feed-back caused the sag from peak to plateau (and if saturation was due to blocking of all light modulated channels) then the cone should still appear saturated even though it had sagged from peak to plateau. Fig. 4 shows that this was not the case. Also, the kinetics of this feed-back scheme have been shown by Baylor *et al.* (1971) to be fast, too fast to account for the relatively slow fall of cone hyperpolarization as light-adaptation proceeds (Figs. 1 and 4).

Voltage dependent conductance in light-adaptation

Vertebrate photoreceptors have been shown to have voltage dependent membrane mechanisms (Baylor *et al.* 1974; Werblin, 1975). It has been suggested that in rods (Schwartz, 1976) and in cones (Baylor *et al.* 1974) this mechanism causes the initial hyperpolarization to sag back to the plateau level. The sag in cone hyperpolarization from peak to steady-state (Figs. 1 and 4) might therefore be a consequence of such a voltage dependent mechanism. While experiments directed specifically at this mechanism have not been performed, a major contribution to cone adaptation by this mechanism is unlikely for reasons similar to those used in the previous section.

The data presented in this study, however, support the conclusions of Baylor *et al.* (1974), concerning the presence of a voltage dependent mechanism in the cone plasma membrane. The highest level of cone hyperpolarization was produced by bright test pulses delivered to the dark-adapted retina; very bright pulses delivered to the light-adapted retina elicited hyperpolarizations which generally were smaller than this level (Figs. 3, 6 and 7). In a similar manner, the largest depolarized level attained by the cone was not its dark-adapted level but that produced by steps of darkness delivered to the light-adapted retina (Figs. 6, 7 and 8). These results are consistent with a conductance monotonically related to membrane potential; hyperpolarization produces a decrease in potassium conductance or an increase in sodium conductance. This interpretation is consistent with the observation that the total potential excursion of the cone is independent of the state of adaptation of the cell.

Interpretation of cone adaptation in terms of the 'internal transmitter hypothesis'

Fuortes & Hodgkin (1964) suggested that the excitation of *Limulus* photoreceptors resulted from an 'internal transmitter', released by light, which modulated the ionic permeability of the plasma membrane. This scheme was adapted for vertebrate photoreceptors by Baylor & Fuortes (1970), Hagins (1972) and later Baylor *et al.* (1974). The light-adaptation data presented here was interpreted in terms of this internal transmitter hypothesis using the following simple set of assumptions which are consistent with the hypothesis (Baylor *et al.* 1974). Quantum absorption produces 'blocking particles' which block light modulated channels; the blocking particles are removed (or inactivated) by some mechanism, and the interaction of the blocking particles with the channels is described by equilibrium kinetics with a given dissociation constant.

Under these assumptions, the sag from peak to steady-state observed during background illumination, and the concomitant growth of both the increment and decrement responses, reflects unblocking of light modulated channels due to either a decrease in the concentration of blocking particles or an increase in the dissociation

constant of the reaction between the blocking particles and the channels or both. The fall in the concentration of blocking particles can be attributed to either or both an increase in the rate of removal (or inactivation) of blocking particles or a decrease in the number of particles produced per photon absorbed.

If cellular adaptation was a result of a decrease in the sensitivity of the production mechanism or an increase in the dissociation constant of the reaction between the blocking particles and the light modulated channels, identical effects would obtain. Both mechanisms could account for the shifting of the cone intensity–response curves caused by background illumination but neither would have an effect on the response kinetics.

However, the light-adapted cone responses to 500 msec pulses of darkness (Figs. 6 and 8) show that there was an increase in the speed of the off response as the background intensity was increased. Further, Fig. 2 shows that the darkness response became faster as light-adaptation proceeded. Such changes in the response kinetics could result from an increase in the rate of removal (or inactivation) of blocking particles. However, depending upon the relative values of the rates of blocking particle production and removal, increases in the latter might or might not have consequences on the kinetics of the falling phase of the photoresponse. Thus, large increases in the rate of removal could cause extensive reductions in blocking particle concentration (and, hence, extensive shifts in the intensity–response curve) but have only small effects upon response kinetics.

We conclude, therefore, that in terms of the internal transmitter hypothesis, the sag from peak to steady-state (and the concomitant growth of the increment and decrement responses), and the shifting of the cone intensity–response curves caused by background illumination is a result of unblocking of light modulated channels. These changes are due, at least in part, to an increase in the rate of removal of blocking particles. Decreases in the sensitivity of the blocking particle production mechanism or increases in the dissociation constant between blocking particles and light modulated channels could also contribute to these changes.

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