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THE ABSENCE OF SPREAD OF ADAPTATION BETWEEN ROD PHOTORECEPTORS IN TURTLE RETINA

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SUMMARY

1. Adaptation by weak backgrounds and the spatial spread of desensitization between rods was studied in the snapping turtle retina, *Chelydra serpentina*. Intracellular membrane potentials were recorded from these photoreceptors in an eyecup preparation.

2. The kinetics and sensitivity of rod responses were changed significantly by large, very dim backgrounds. For the twenty-five most sensitive rods where the dark-adapted flash sensitivity, S_F^D, was greater than 1·0 mV/*Rh**, *Rh** being the number of effective photo-isomerizations per rod, the background intensity required to halve the amplitude of the linear range response averaged 0·21 *Rh** s^{-1}. The time-to-peak of the test responses was reduced up to 50 % by these dim backgrounds.

3. The desensitizing effects of full field backgrounds of various intensities on the responses to large test spots were measured. The dependence of incremental flash sensitivity, S_F, on background intensity, I_B, followed the form

$$S_F/S_F^D = 1/(1+(I_B/I_0)^{0.6}),$$

where I_0 is the background intensity which halved S_F^D. The same intensity dependence held for slit-shaped background fields that desensitized responses to small test spots.

4. The desensitizing effects of large, very dim flashed and continuous backgrounds took several seconds to appear and decay to dark levels. This in conjunction with the sparsity of photons suggests, that the desensitization from a single photo-isomerization can persist for several seconds.

5. A comparison of the desensitizing effects of spot and annular backgrounds revealed that small spot backgrounds superimposed on the centred test spots desensitized rods more effectively than annular fields. This finding held true even when annular patterns produced a greater maintained hyperpolarization in the rods. Thus, there was no unique relationship between desensitization and the steady maintained hyperpolarization evoked by a background field.

6. The dependence of adaptation on distance from the impaled rod was determined with slit-shaped background fields placed at different positions across the rod's receptive field.

7. The desensitizing effect of displaced slit stimuli was found to decline much more rapidly with distance than excitation. Displacing the slit by 20 μm from the centre reduced its desensitizing effect by more than 1 log unit. In contrast, excitation fell to about 80% at the same distance (λ ranging from 50 to 70 μm).

8. The fall off of desensitization with distance matched the calculated fall off with distance of light scatter from a slit.

9. No difference was noted in the kinetics of test responses in the presence of equally desensitizing, superimposed and displaced slits.

10. It is concluded that there is little spread of desensitization between rods. Thus, adaptation appears to be accounted for primarily on the basis of local photo-isomerizations in each rod outer segment.

11. The exceedingly low rates of photo-isomerizations required to halve sensitivity leads to the conclusion that a single photo-isomerization produces profound changes that can spread longitudinally along the whole outer segment. Therefore, adaptation cannot represent changes occurring locally within or near the disk absorbing the photon of light.

INTRODUCTION

The spatial summation, or pooling, of adaptive signals in the rod-mediated visual system has been studied in both lower and higher vertebrates including man. Examination of ganglion cell and psychophysical responses has shown that scotopic thresholds can be elevated by an adapting light falling on the retina at a spatially distinct location (Lipetz, 1961; Rushton, 1965*a*,*b*; Westheimer, 1965; Barlow & Andrews, 1967; Easter, 1968; Cleland & Enroth-Cugell, 1970; Enroth-Cugell & Shapley, 1973; Green, Tong & Cicerone, 1977; Tong & Green, 1977; Cicerone & Green, 1980). On the assumption that each photoreceptor acted as an independent light detector, many of the above investigators concluded that lateral adaptive effects occurred at sites proximal to the photoreceptors. It is now clear that in the retinas of some, and perhaps all species, the rods are electrophysiologically coupled to one another (Schwartz, 1975; Fain, 1976; Copenhagen & Owen, 1976*b*). Thus it is necessary to examine the proposition that lateral adaptive effects might, in whole or in part, be the result of adaptive signals spreading from one rod to another via the functional connexions between rods.

The experiments reported here were designed to answer the question of whether in the snapping turtle, an animal in whose retina the rod coupling has been well characterized, it is possible to demonstrate lateral adaptive interactions between rods.

METHODS

Retinal preparation

Rod photoreceptors in the eyecup preparation of the snapping turtle, *Chelydra serpentina*, were intracellularly impaled with fine, 2 M-potassium acetate filled glass micro-electrodes (200–400 MΩ) advanced from the vitreal surface of the retina. The isolated eyecup was 'cemented' with Ringer–agar gel to the top surface of an Ag–AgCl reference electrode within a semi-enclosed chamber. Moistened O_2 was blown gently into the chamber to maintain retinal metabolism and to keep the vitreal surface from drying out. All recordings were performed in a light-tight Faraday

cage at room temperature, 18–22 °C. Light stimulus patterns were projected onto the retina from an optical system mounted on benching located external to the cage. Further details of the electrodes, optical system and recording apparatus are available in previous publications (Copenhagen & Owen, 1976*a*, *b*). The dissection procedures were identical to those outlined in the previous work but, in addition, considerable caution was taken to ensure that the animal's eyes were fully dark adapted before decapitation and enucleation and that the dissection itself was performed rapidly under very dim red light.

Optics and micro-electrode positioning

The quality of the stimulus patterns was determined by measuring the spatial distribution of light with a photomultiplier tube having a circular entrance aperture of 5 μm diameter. Fig. 1*A* plots the photomultiplier output as the spot image was moved across the entrance pupil. The nominal half-width of the measured spot image, the distance from the peak to points where intensity was reduced to one-half of maximum, was measured to be 5 μm. Fig. 1*B* shows the photomultiplier output for scans across the spot and annular patterns used in some of the adaptation experiments.

The combined requirements for the maintenance of dark adaptation and image focusing necessitated the development of a refined method for alignment of electrodes. Prior to penetrating the retina with the micro-electrode, the eyecup chamber was covered with a removable opaque dish filled with saline. A 50 μm diameter stimulus spot was then focused on the top surface of the saline and the tip of the micro-electrode was positioned under visual control to penetrate the saline surface at the centre of the stimulus. In this way, the vertical position of the plane of focus was established and the tip of the micro-electrode and the stimulus spot were aligned laterally and vertically.

The micro-electrode was then withdrawn several hundred micrometers, the dish of saline was removed, and the retina raised to the level at which the electrode made electrical contact with the vitreous. The electrodes were then advanced through the vitreous and into the retina. Taking account of vitreal and retinal depths made it possible to penetrate rods at positions within 50 μm of the plane of focus of the stimulus spot, and within 30–40 μm of the centre of the spot. Since the measured depth of focus was ± 100 μm, the stimulus was thus brought into sharp focus on the rod outer segments.

An electrical readout of slit position was obtained from linear potentiometers attached to the X–Y stage of the slit holder. By displaying slit position on the x-axis of the storage oscilloscope and the intracellular voltage responses on the y-axis, it was possible to determine the centre of a rod's receptive field in 1–2 min. Digital read-outs of the slit position were recorded for more accurate plots of receptive field profiles.

Electronic recording and data analyses

Intracellular potentials were amplified by an FET-input pre-amplifier, displayed on a storage oscilloscope and recorded on an FM tape recorder (Racal). Response averaging was done off-line on a mini computer.

Light calibration

Unattenuated test stimulus irradiances at the plane of the retina were 3×10^5 photons $\mu m^{-2} s^{-1}$ (525 nm) and $1{\cdot}5 \times 10^6$ photons $\mu m^{-2} s^{-1}$ (510 nm). Intensity measurements were performed with factory calibrated Model 40 × Optometer devices (Optics Technology). Cross checks with similar instruments used by Drs Denis Baylor (Stanford) and Kenneth Brown (UCSF) indicated an agreement of $\pm 5\%$ in the measurement of absolute irradiance. The effective collecting area of each rod was taken to be 13·6 μm^2 (Copenhagen & Owen, 1976*b*). This value assumed a rod cross-sectional area at the inner segment of 100 μm^2, an efficiency of 50% in the funnelling of light to the outer segment (O'Brien, 1951), an outer segment length of 18 μm, a specific axial density of rod pigment of 0·014 μm^{-1} at 520 nm (Liebman, 1972), and a quantum efficiency of bleaching of 0·62 (Dartnall, 1972).

Terminology

The terms *adaptation* and *desensitization* are used interchangeably in the text and refer to the reduction in response amplitude of a test stimulus caused by the application of background illumination to the retina.

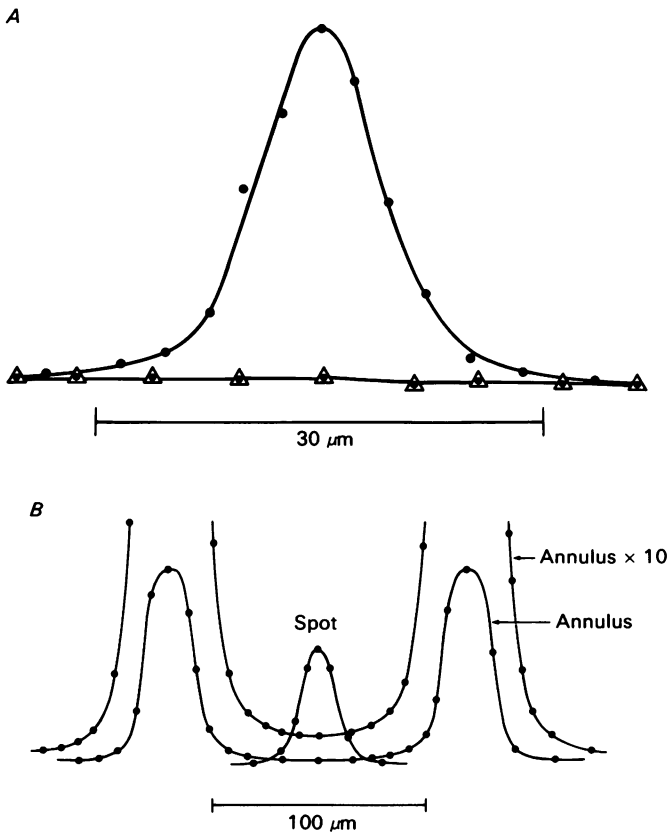

Fig. 1. Spatial profiles of test and adapting stimuli. *A*, the profile of the test spot measured at the retinal focal plane of the optical stimulator. Ordinate values denote the relative output current from a photomultiplier having a 5 μm diameter circular entrance pupil. The abscissa plots the position of the spot monitored as a voltage on a linear potentiometer attached to the aperture carrier. *B*, similarly determined plot of the spot and annular adapting patterns. The annular pattern is shown at 1 × and 10 × gain. Note that the annular pattern casts only 2·5 % as much of the light on to the centre point of its image as does the spot.

*Rh** is defined as the number of effective photo-isomerizations per rod in the retinal field being stimulated.

I_0 is defined as the background irradiance required to halve the dark-adapted flash sensitivity of a test stimulus (*Rh** s^{-1}).

RESULTS

Flash sensitivity

Turtle rods have receptive fields that cover a 200–250 μm diameter circular area of the retina (Copenhagen & Owen, 1976*b*). This receptive field is comprised strictly of summative inputs from neighbouring rods; no inhibitory surround nor antagonistic inputs from horizontal cells has ever been demonstrated. Thus, circular spots centred on an impaled rod and of diameter greater than 300 μm excite all the rods in the

receptive field comparably. Under these stimulus conditions, flash and background sensitivities were determined. Dark-adapted flash sensitivities were determined for forty rods by applying dim, brief (10–20 ms) stimuli that elicited responses whose amplitude varied linearly with light intensity. The flash sensitivity, S_F^D, is defined and computed as the peak amplitude of the response evoked by each effective photo-isomerization (mV/*Rh**).

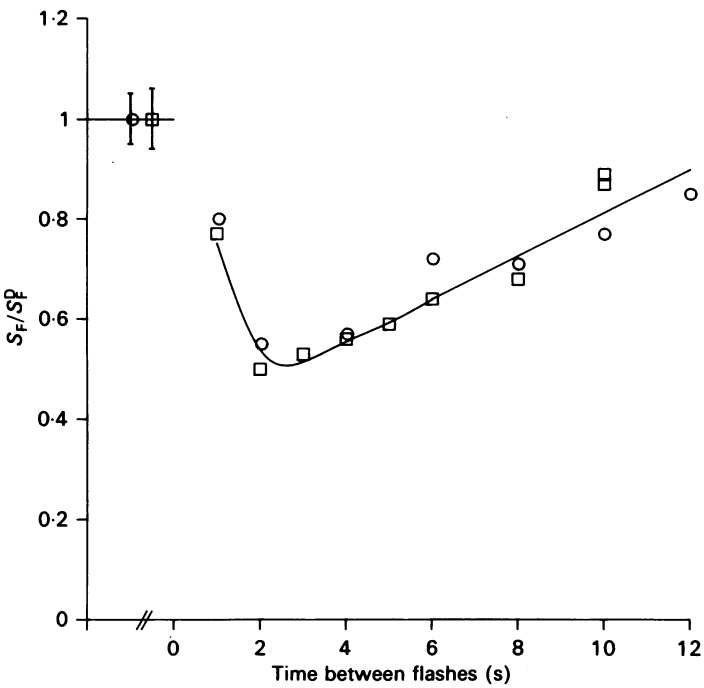

Fig. 2. Time course of desensitization. The net relative amplitude of full field test flashes (450 μm diameter, 15 *Rh** flash^{-1}, 510 nm) is plotted as function of delay from onset of conditioning flashes of identical parameters. S_F^D was 0·63 mV/*Rh** and 0·42 mV/*Rh** for these two rods. The resting potentials were −40, −41 mV respectively and the peak amplitudes of the conditioning flash responses were 6·2 mV and 4·9 mV.

To minimize interactions between successive stimuli, flashes were presented once every 15–30 s on to well dark-adapted eyecups. The interstimulus interval was chosen on the basis of two-flash experiments similar to those illustrated in Fig. 2. That is, a brief, dim conditioning flash was followed by a dim test flash presented at different delays. The incremental sensitivity, S_F, was determined by measuring the incremental hyperpolarization added by the test to the conditioning field response. In Fig. 2 S_F/S_F^D for each rod is plotted as a function of the delay between the two flashes. The response reduction caused by the conditioning flash took about 2 s to reach a peak and then approximately 15 s to subside. Thus, when allowing greater than 15 s for the recovery of sensitivity between single dim flashes, the mean S_F^D was found to be 1·73 mV/*Rh** with a range of 0·07–8·9 mV/*Rh** (n = 40). The highest values coincide with similar values measured in snapping turtle by Detwiler, Hodgkin & McNaughton (1980).

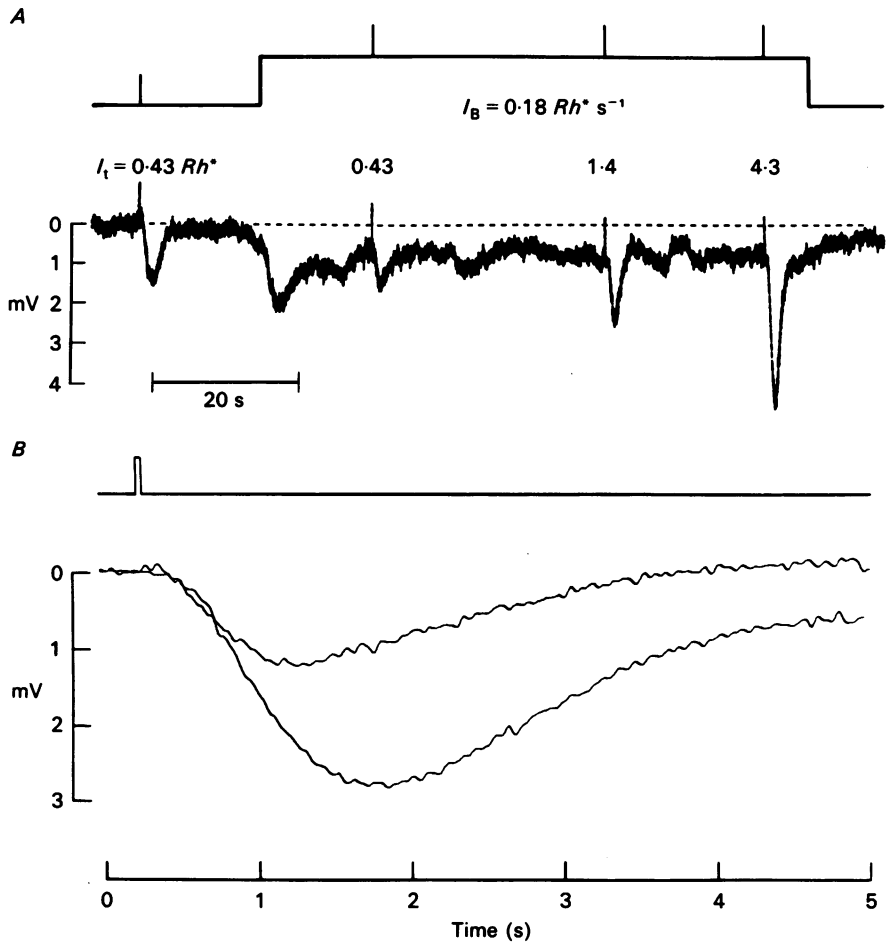

Fig. 3. Effects of background illumination on test spot responses. *A*, the intracellular potential recorded from a rod. The test stimulus response (525 nm, 13 ms, 0·43 *Rh** flash^{-1}, 750 μm diameter) is shown before (S_F = 3·7 mV/*Rh**) and 16 s after the onset (S_F = 1·5 mV/*Rh**) of the background field (500 nm, 0·18 *Rh** s^{-1}, 600 μm diameter). Two subsequent test spot flashes, 0·5 and 1·0 log units brighter, are also shown. The dotted line denotes the dark-adapted membrane potential and the vertical lines before the hyperpolarizing test responses are a 1 mV calibration pulse. *B*, averaged test responses before and during the background. In the dark, the test flash sensitivity was 5·8 mV/*Rh**. The traces are averages of six interleaved control/adaptation sequences. After a background containing 0·2 *Rh** s^{-1} was applied, the flash sensitivity was 2·5 mV/*Rh**. There were 35 s between successive 15 s adaptation periods. The test flashes were presented 10 s before and 10 s after the onset of the background. I_t refers to the number of effective isomerizations per rod per flash and I_B is the background irradiance.

Most experimental results covered in the remainder of this paper were obtained from rods having $S_F^D \geqslant 1$ mV/*Rh**.

Adaptation with full field backgrounds

The effects of full field backgrounds on flash sensitivity were investigated. These results enable one to characterize the intensity dependence and temporal behaviour

of adaptation in the turtle rods and allow comparison of these rods' properties to those of other species.

Fig. 3 illustrates the protocol for determining effects of dim backgrounds on test flashes. Basically, test flashes were presented in the dark and on weak background fields. Sufficient time was allowed between stimuli to ensure complete recovery of sensitivity between each experimental run. The recording in Fig. 3*A* illustrates the rod potential recorded for a single background presentation. A test flash (0·43 *Rh** rod^{-1} $flash^{-1}$, 525 nm, 750 μm diameter) was presented before the onset of the background (0·18 *Rh** rod^{-1} s^{-1}, 500 nm, 600 μm diameter) and then again 16 s after its onset. Two subsequent test flashes, two and four times brighter, were also presented on the background. These records, illustrating the desensitization caused by the dim background, show that, in the dark, the large test spot elicited a 1·6 mV response (S_F^D = 3·7 mV/*Rh**) whereas with a dim full field background, the same stimulus evoked a 0·65 mV response (S_F = 1·5 mV/*Rh**). Increasing the intensity of the two subsequent test flashes (by factors of 3 × and 10 ×) increased the response to 1·8 mV and to 4·2 mV, respectively. Thus, the dark-adapted flash sensitivity was more than halved by a background which itself hyperpolarized the rod by approximately 0·8 mV. This desensitization was produced by a background that was estimated to elicit only one photo-isomerization per 5·5 s in each rod (See Methods).

Fig. 3 shows also that these dim desensitizing backgrounds modified the kinetics of the test flash response. This is illustrated more clearly in Fig. 3*B*, where, on an expanded time scale, the superimposed, averaged test flash responses from another rod are shown before and during background illumination. The dark-adapted flash sensitivity for this rod was reduced from 4·0 mV/*Rh** to 1·7 mV/*Rh** by the dim background which produced 0·2 *Rh** s^{-1}. The time to peak was shifted from approximately 1·5 s to 1·0 s. The increase in time to peak is qualitatively similar to that in toad rods (Fain, 1976; Capovilla, Cervetto & Torre, 1983) and agrees with similar findings in turtle rods (Baylor & Hodgkin, 1974; Detwiler *et al.* 1980).

The desensitizing effects of the adapting backgrounds did not manifest themselves instantaneously. As Fig. 2 shows, it took several seconds for the desensitization of the test responses to build up after the brief conditioning flash. Fig. 4 illustrates for three different rods the time course of the desensitization to steps of light. The background was cycled on and off and the test stimulus was flashed at different times after the onset of the background. The incremental flash sensitivity, S_F, was measured by subtracting the amplitude of the response to the background from the peak response elicited by the test stimulus superimposed on the background. The points plotted to the left give the mean and standard error of the normalized values of S_F^D. The points to the right of zero time plot S_F/S_F^D and show the time course of desensitization. The continuous curve drawn through the points is a simple exponential decay with a time constant of 6 s. Thus, under these conditions, steady-state desensitization is not achieved for periods $\leqslant$ 10 s.

Steady-state adaptational effects were determined by measuring test responses at least 12 s after the onset of a background. A criterion adaptation level in each rod was quantified by establishing the magnitude of background irradiance required to halve S_F^D. This minimum adaptation level is termed I_0 and is expressed in effective photo-isomerizations per second per rod (*Rh** s^{-1}). The backgrounds used in any

particular experiment varied from 0·11 to 3·5 Rh^* s^{-1} (average 0·54) for the forty rods used in this study. Later analysis of these recordings revealed that while the average reduction in flash sensitivity (S_F/S_F^D) was 0·48, the actual reduction varied from 0·22 to 0·71. Although it was possible to obtain I_0 for individual rods by linear interpolation from the above experiments, it was deemed more appropriate to determine the functional dependence of incremental sensitivity on background intensity, so that I_0 could be calculated more precisely.

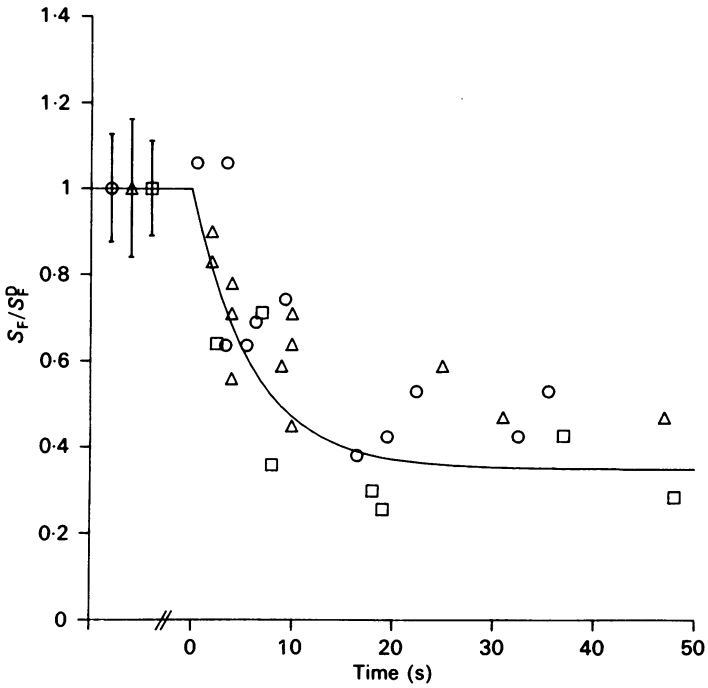

Fig. 4. Time course for the onset of adaptation. The ratio S_F/S_F^D of full field test stimuli (450 μm diameter, 510 nm) is plotted as a function of delay from onset of a full field background stimulus (450 μm diameter, 510 nm). Results from three different rods are shown. S_F is calculated as mV/Rh^* using the net peak amplitude of the test response as shown in the inset. The three rods exhibited the following values for S_F^D and I_0: 5·2 mV/Rh^* 0·07 Rh^* s^{-1}; 0·42 mV/Rh^* 0·79 Rh^* s^{-1}; 0·23 mV/Rh^* 1·4 Rh^* s^{-1}. The continuous line is a simple exponential function having a time constant of 6 s.

Effect of background lights

The intensity of an adapting background was systematically varied and flash sensitivities were measured. These results are plotted in Fig. 5. To establish the trend of the data, measurements from five rods were superimposed by translocation along the abscissa; the degree by which each data set was shifted is shown in the Figure legend. Sensitivity was not found to decrease with background intensity according to Weber's law (the dotted line in the Figure) but rather more closely obeyed the relationship:

$$\frac{S_F}{S_F^D} = \frac{1}{1+(I_B/I_0)^{0·6}}. \quad (1)$$

Hence, I_0 was related to the sensitivity reduction that a particular background produced by

$$I_0 = I_B((S_F/S_F^D)^{-1} - 1)^{0.6}. \quad (2)$$

Using the above relationship, the measured reductions in flash sensitivity were used to compute I_0 for the forty rods used in this study. The mean value for I_0 was 1·0 *Rh** s^{-1} (range 0·05–16 *Rh** s^{-1}). If one eliminates the four cells with $I_0 > 2{\cdot}5$, the mean value for I_0 drops to 0·28 *Rh** s^{-1}.

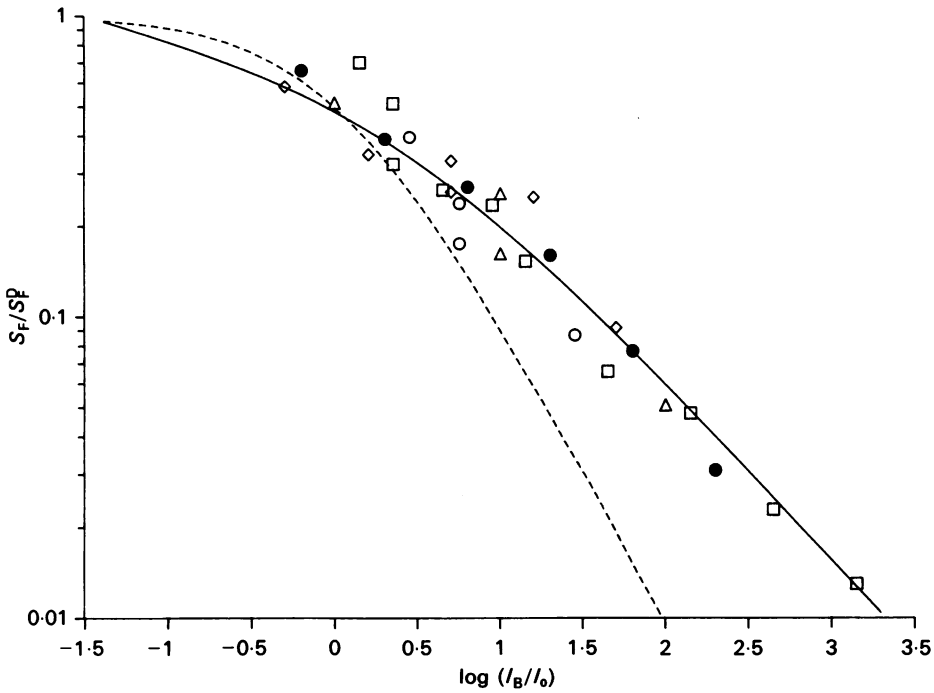

Fig. 5. Shape of the background *versus* intensity relationship. Full field test and background stimuli were used to determine incremental sensitivities, S_F/S_F^D for the five rods are as follows: △ −2·1 mV/*Rh**, ○ −5·9 mV/*Rh**, □ −0·63 mV/*Rh**, ◇ −0·93 mV/*Rh**, ● −0·57 mV/*Rh**. Each set of points has been translocated along the abscissa to align the value of I_0 with 0 relative log background. The actual calculated values of I_0 are: △ −0·36 *Rh** rod^{-1} s^{-1}, ○ −0·086 *Rh** rod^{-1} s^{-1}, □ −0·18 *Rh** rod^{-1} s^{-1}, ◇ −0·16 *Rh** rod^{-1} s^{-1}, ● −0·40 *Rh** rod^{-1} s^{-1}. The continuous curve represents eqn. 1 and the dashed line a Weber–Fechner relation.

The inverse of I_0, which is a measure of adaptation sensitivity, is plotted against flash sensitivity in Fig. 6. The data in Fig. 6 shows a considerable degree of variability in the relationship between S_F^D and $1/I_0$. A linear regression fit to the data showed that $1/I_0 = 3{\cdot}03\ S_F^D \pm 1{\cdot}78$ with a correlation of 0·535. This result might tend to suggest that the flash sensitivity and the background intensity level which minimally adapts can be independently determined within a rod.

The very low rate of photo-isomerizations required to significantly desensitize the rods leads one to hypothesize that either the desensitizing effects of a photo-isomerization can spread to neighbouring rods or else desensitization from a single photo-isomerization persists within a rod for periods in excess of several seconds.

Although the results from the two-flash experiments in Fig. 2 were consistent with the latter proposition the possibility of contributions from laterally directed desensitization was investigated.

Spot and annular adapting stimuli

If adaptation were dependent on stimulation of neighbouring rods, an annular pattern should accentuate these lateral desensitizing effects. Furthermore, if laterally flowing signals generated by photo-isomerizations in neighbouring rods controlled

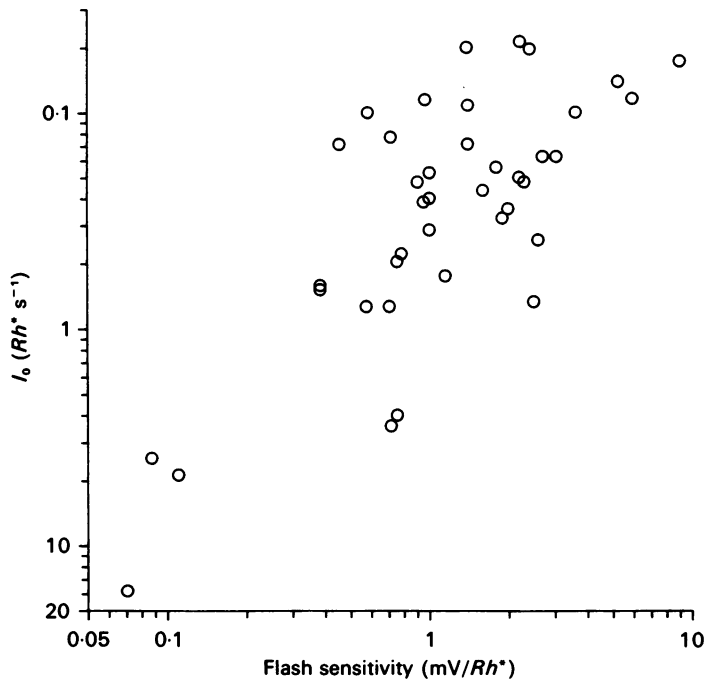

Fig. 6. Relationship between dark-adapted flash sensitivity and adaptability. Adaptability is represented by plotting I_0 in Rh^* s^{-1} on a negative logarithmic scale. Results from forty rods are included.

sensitivity, it might be expected that there would be a strong correlation between membrane potential in the rod and incremental sensitivity. Thus, the adaptive effects and membrane potentials induced by concentric and annular backgrounds were compared.

The records in Fig. 7 illustrate the effects of spot and annular backgrounds on flash sensitivity. A small test spot (10 μm diameter) centred on the rod was used to probe its sensitivity. A small superimposed spot (25 μm diameter) and a concentric annulus (120 μm i.d., 170 μm o.d.) were used as adapting fields. The top trace shows the timing of the background and test stimuli. The middle trace shows first the response of the test spot on a darkened background, then the onset of the annulus followed by the response of the test spot at three different intensities and finally the return to dark-adapted conditions. The annular illumination produced a peak hyperpolar-

ization of 5 mV which sagged to a steady-state level of approximately 2 mV. The annulus reduced the test spot response by about a third, relative to the control. Responses comparable in amplitude to those from the control test spot were elicited with test intensities approximately two times brighter. The lowest trace shows that the concentric adapting spot elicited a smaller, less transient response than the annulus yet produced a much greater desensitization. For this case, the test spot

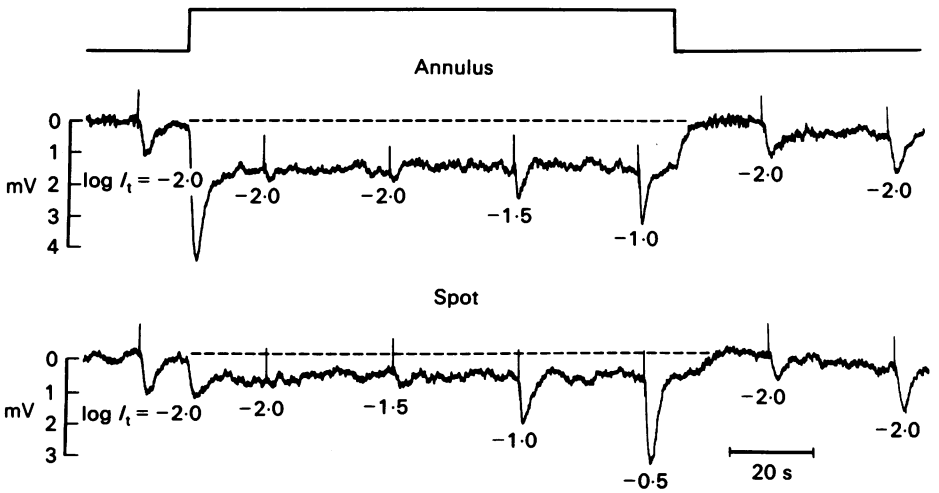

Fig. 7. Comparison of desensitization produced by annular and spot light patterns. The top trace shows timing of background. The middle trace shows the effect of an annular background and the bottom trace the effect of a small spot background. A test spot (10 μm diameter, 525 nm) was flashed before and during presentation of background patterns (annulus: 170 μm o.d., 117 μm i.d., 525 nm, and spot: 27 μm diameter, 500 nm).

intensity had to be increased by ten times to elicit responses of control amplitude. Similar results were obtained in five other rods. These results demonstrate that the hyperpolarization evoked by the background is not a unique indicator of desensitization. The photo-isomerizations produced by the spot, which falls on the impaled rod and its immediate neighbours, more effectively desensitized the rod than those produced by the annulus, which illuminates distant neighbouring rods. The question of whether the weak desensitizing effects of the annulus resulted from signals which spread laterally across the retina or from light spreading to the impaled rod is unresolved by these experiments.

Spatial spread of adaptation

Measurements of the spatial weighting functions for the spread of adaptation and excitation were obtained with a narrow slit. Excitation receptive field profiles were measured in the usual manner by flashing a slit at various positions across the retina and determining the dependence of peak response amplitude on position. For each of the twenty rods tested, this relationship was satisfactorily fitted by a simple exponential having a length constant of 50–70 μm, which agrees well with previous results in turtle (Detwiler *et al.* 1979; Copenhagen & Owen, 1980).

The spread of adaptation was investigated by probing the impaled rod's sensitivity with a 25 μm test spot centred in the rod's receptive field and measuring the desensitization produced by an adapting slit imaged at various distances from the test spot. This protocol is illustrated in Fig. 8 which shows the membrane potential shifts evoked by the adapting slit and the responses to single presentations of the centred test flash. In the upper trace, the hyperpolarization on the left shows

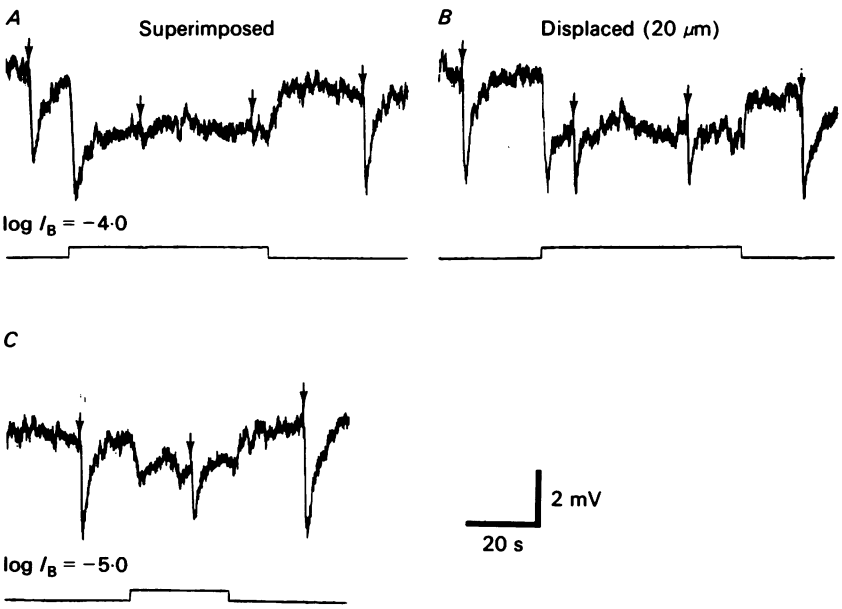

Fig. 8. Effects of superimposed and displaced slits on test spot responses. All flash responses are to a 25 μm diameter spot centred on the impaled rod. *A*, initial control test response is followed by superimposed slit (log I = −4·0, 510 nm) during which two test responses are observed. After termination of the slit, another control test spot was flashed. *B*, same protocol as *A* except the slit was displaced 20 μm from the centred position. *C*, same protocol as *A* except centred slit intensity was reduced by 1 log unit (log I_B = −5·0).

the response to the centred test spot (25 μm diameter). Twenty seconds after the onset of a superimposed slit the same test spot elicited a greatly reduced response (18% of control). The trace on the upper right shows that the same adapting slit displaced 20 μm elicited approximately the same initial transient and following steady-state hyperpolarization, yet it reduced the test spot responses to a lesser degree; the test spot amplitude was approximately 60% of control. These results show once again that desensitization spreads over a shorter lateral extent than excitation.

In order to obtain a quantitative measure of the spatial extent of desensitization, the slit-induced adaptation at each position was expressed in terms of the equivalent superimposed background intensity. That is, we determined the intensity of a superimposed slit that would adapt the test spot response by the same amount as the off-centre slit.

This procedure is illustrated in the bottom trace of Fig. 8, which shows test

responses when the intensity of the superimposed slit was reduced by 1 log unit. The reduction in the control response was approximately 50%. Since displacing the slit by 20 μm and dimming the slit by a log unit produce equivalent effects on flash sensitivity, we conclude that the adaptational sensitivity had decayed by a factor of about 10 at a distance of 20 μm from the impaled rod. Thus, by using two sets of measurements: the slit-induced reductions at different positions and the sensitivity

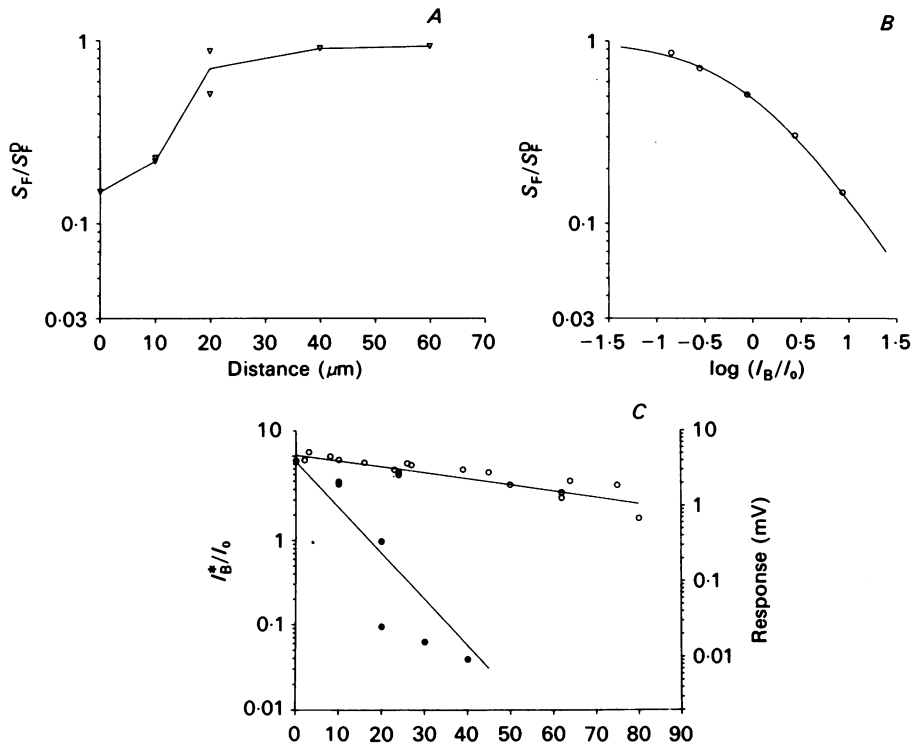

Fig. 9. Derivation of equivalent adaptation produced by displaced slits. *A*, ratio of S_F/S_F^D for test spot (25 μm diameter, 525 nm) as function of slit position (5 μm width, 525 nm). *B*, ratio of S_F/S_F^D for same test spot as function of the intensity of a superimposed slit. *C*, filled circles show equivalent background (I_B^*/I_0) calculated by concatenation of each data point in Fig. 9*A* with curve in Fig. 9*B*. Straight line drawn through solid points is simple exponential having an 8 μm length constant. Open circles show peak response amplitudes elicited by same slit flashed at different positions across rod's receptive field. Straight line is simple exponential having a 55 μm length constant.

reductions produced by a superimposed slit at several intensities, it was possible to infer the functional relationship between slit position and equivalent background intensity, I_B^*/I_0.

This analysis for data obtained at a number of different displacements is illustrated, for another rod, in Fig. 9. That is, Fig. 9*A* plots the relative reduction in flash sensitivity for a constant intensity adapting slit moved laterally across the rod's receptive field. Fig. 9*B* plots the relative reduction in flash sensitivity as a function of the intensity of a superimposed slit. The filled circles in Fig. 9*C* were

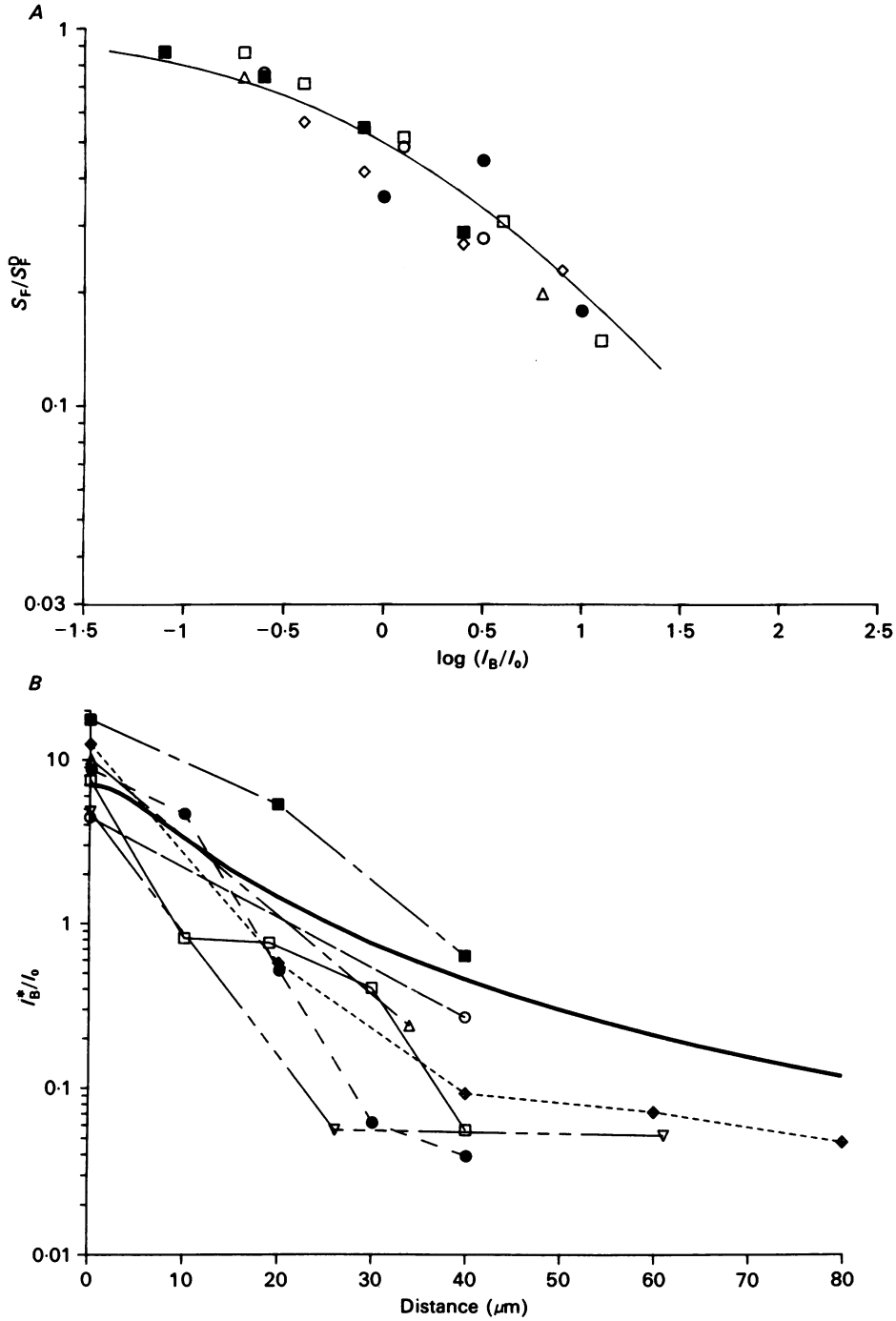

Fig. 10. Adaptation by slits. *A*, effect of a slit background on incremental sensitivity. Data points are shown from experiments using 5 μm diameter test spots and 3·5 μm slits and 25 μm diameter test spots and 5 μm slits. The data from six rods have been translocated along the abscissa to align the relative values of I_0. The continuous curve plots eqn. (1). *B*, spatial dependence of adaptation. The decline of equivalent background

obtained from the data in 9*A* and 9*B*, as described above, and show the equivalent background intensity for comparable desensitizations at each slit position.

The continuous line fitted to the filled circles is an exponential decay having a space constant of 8 μm. The open circles in Fig. 9*C* show the space constant of summation for the same rod. The line through the data points corresponds to an exponential decay with a space constant of 55 μm. These data indicate that the effective space constant for summation of excitatory signals is at least five times greater than that for desensitization.

Fig. 10*B* shows all of our data on the spatial decay of slit-induced adaptation. It was difficult to hold cells long enough to obtain as complete data as that shown in Fig. 9. Consequently we have pooled data on the effects of slit backgrounds. The dependence of S_F on background intensity for centred slits is shown in Fig. 10*A* for six rods. The continuous line through the data points is eqn. (1). It should be noted that these data indicate that S_F has the same dependence on I_B for slit backgrounds as for full field backgrounds (Fig. 5). Moreover, since these data can be satisfactorily fitted by eqn. 1, it is possible to use this relationship to obtain the following:

$$\frac{I_B^*}{I_0} = (r^{-1}-1)^{\frac{5}{3}}, \tag{3}$$

where $r = S_F/S_F^D$. In Fig. 10*B*, I_B^*/I_0 for seven rods are plotted as a function of the distance of the adapting slit from the centre of each rod's receptive field. As with the data in Fig. 9*C*, the adaptive effects of displaced slits declined rapidly over the first 10–20 μm of displacement.

To test the proposition that the spatial profile of adaptation might be due to scatter, an estimate of light scattered from the slit onto the impaled rod was derived as a function of its position. The slit image was measured and digitized (see Methods). This image was convolved with a line spread function for the turtle retina which was derived from the theoretical point spread function calculated by Copenhagen & Owen (Fig. 12, 1976*b*). Replotting their point scatter data we found it to be well fitted by the function

$$A(r) = \frac{b^2}{(b^2+r^2)^{\frac{3}{2}}}, \tag{4}$$

where r is radial distance and $b = 9{\cdot}0$ μm. A linear system with a point spread function of this form will have a line spread function of the form

$$A(x) = \frac{b^2}{b^2+x^2}, \tag{5}$$

where x is linear distance and A is the relative amplitude of light scatter (Jones, 1958).

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as a function of the distance of the slit from the impaled rod is shown. The points were obtained from seven rods using eqn. (3) and measurements similar to those in Fig. 9*A*. The continuous curve plots the calculated values of light intensity scattered from a translocated slit onto 5 μm diameter circle at centre of receptive field. The image of the slit profile, measured with 5 μm entrance pupil on photomultiplier, was convolved numerically with the line spread function for retinal scatter, eqn. (6), to obtain the profile at the photoreceptor level. The curve was shifted to fit the cluster of points at 0 μm.

To obtain the light spread from the slit stimulus at the level of the rods, $A(x)$ was convolved with the measured slit image. The continuous line in Fig. 10*B* shows the results of the convolution. The decline of adaptive sensitivity with distance is consistent with what one might expect if sensitivity was only affected by the light scattered onto the impaled rod.

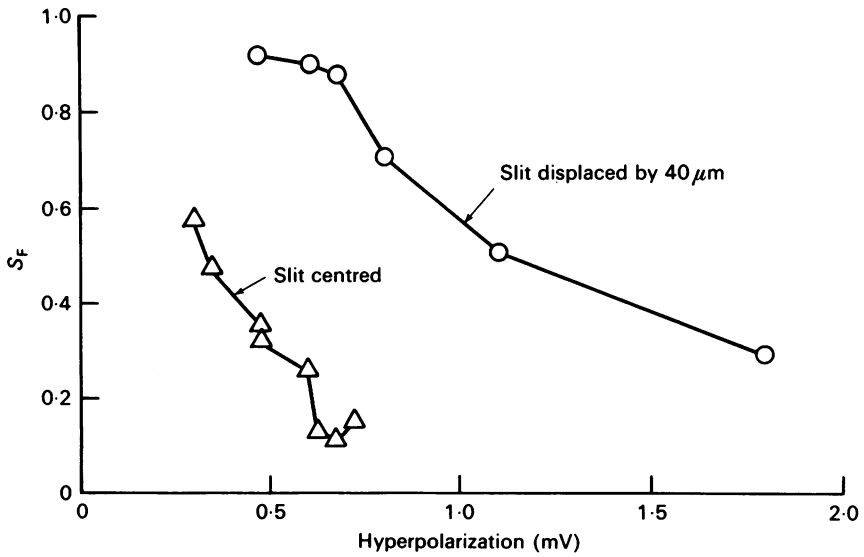

Fig. 11. Flash sensitivity and membrane potential. Normalized flash sensitivity is plotted against the steady-state hyperpolarization evoked by a centred adapting slit (△) and one displaced 40 μm (○). Flash responses were obtained with a 25 μm, 510 nm stimulus in the centre of the rod receptive field (same cell as in Fig. 9). The intensity of the slit was varied over a range of 2 log units.

Because of the electronically mediated interactions between rods, the steady-state response in any rod is the summation of polarizations from neighbouring rods. If desensitization is mediated primarily by photo-isomerizations in the outer segment of each rod, then the polarization should not be strictly correlated with desensitization, as shown in Figs. 7 and 8. The data in Fig. 11 further illustrates the independence of these two parameters. Using measurements from the rod in Fig. 9, the normalized reduction in flash sensitivity, S_F/S_F^D, is plotted against steady-state hyperpolarization evoked by a centred and displaced slit. Although stronger backgrounds produced increased hyperpolarization and greater reduction in sensitivity for both slit positions, a much larger hyperpolarization was induced by the displaced slit for comparable reductions in sensitivity.

If rod desensitization is determined by events restricted to the outer segment it should not matter whether one adapts with a superimposed or displaced stimulus. When a rod is desensitized equally, the kinetics of the test responses should be comparable. In Fig. 12*A* and *B*, this prediction is tested. In Fig. 12*A*, S_F/S_F^D and time-to-peak of the test response are plotted at several background intensities and displacements for two rods. At every position of the background slit, an increase

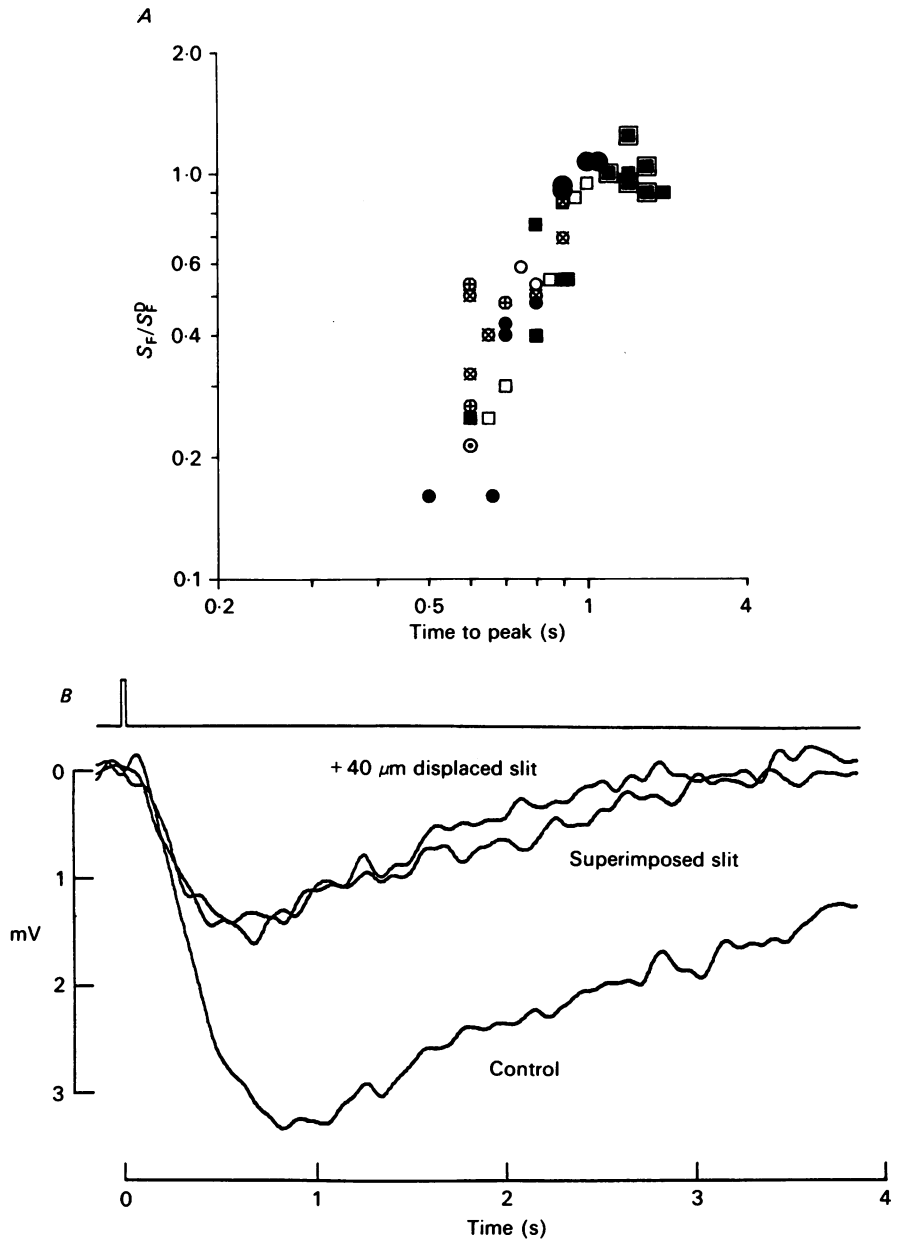

Fig. 12. Comparison of response kinetics using superimposed and displaced adapting slits. Centred test spot (25 μm diameter, 10 ms duration) responses are shown at several adapting slit intensities and positions. *A*, time-to-peak, *T*, is plotted against S_F/S_F^D for two rods (circles and squares) which were adapted with a slit in various positions. Slit intensities were varied up to 2 log units at each position. Straight line is drawn by eye through data points. Controls ▣, ⊙; superimposed ●, ■; 20 μm displaced slit ⊗; 40 μm displaced ○, □; 60 μm displaced ⊕; 80 μm displaced ⊙. *B*, test spot responses (5 μm diameter, 20 ms duration) in presence of superimposed adapting slit (log $I_B = -5{\cdot}0$), displaced slit (+40 μm, log $I_B = -3{\cdot}5$) and no slit. Each tracing is average of five responses.

in intensity shortened the time-to-peak and reduced S_F. The relationship between sensitivity and speed was the same, independent of whether superimposed or displaced slits were used. This provides additional evidence for a single mechanism controlling the incremental sensitivity and the shortening of the time-to-peak. Fig. 12*B* shows averaged responses from another rod in the absence of any adapting field and in the presence of a superimposed ($\log I_B = -5{\cdot}0$) and a displaced slit-shaped adapting field ($\log I_B = -3{\cdot}5$). When the slit intensities were adjusted to reduce the peak of the flash responses equally, as shown here, the rising phase, time-to-peak, and return to base line appear very similar at both positions. A similar equality of the effects of adaptation on the kinetics of test responses was found to hold true for concentric spot and annular adapting fields adjusted to produce comparable desensitizations.

DISCUSSION

Minimum adaptation level

The present experiments demonstrate that, with full field illumination, a few photo-isomerizations in each rod can significantly reduce sensitivity. For the twenty-five rods with $S_F^D \geqslant 1$ mV, the average background illumination required to halve S_F^D was 0·21 *Rh** s^{-1}. I_0 has been determined in several studies of amphibian rods. Hemila (1977) reported that a background photo-isomerization rate of 0·45 s^{-1} elevated threshold responses by a factor of 3–4 in the aspartate isolated receptor potential of frog retina. This value is close to that reported here. Baylor, Matthews & Yau (1980) and McNaughton, Yau & Lamb (1980) reported an I_0 of 4–7 *Rh** s^{-1} for isolated rod outer segments in *Bufo marinus*. This value for *Bufo* rods agreed with that reported by Fain (1977) who recorded from eyecups. Given the difference in experimental protocols, recording methods and possible difference in degree of light adaptation, it is difficult to assess the significance of the differences in I_0 obtained from various studies. However, I_0 for rods in *Bufo* eyecups using the same experimental set-up and protocol discussed in this report is approximately 1 *Rh** s^{-1} (D. Copenhagen & T. Reuter, unpublished observations). This suggests a real difference between the minimum adaptation levels of snapping turtle and *Bufo* rods. In considering the basis of the difference, it is interesting to note that the length of the snapping turtle outer segment rod (18 μm) is about $\frac{1}{4}$ that of the *Bufo* rod (70 μm). If the spatial spread of desensitization reported by Lamb, McNaughton & Yau (1981) for *Bufo* rods ($\lambda = 6\,\mu$m) is the same as in turtle rods, a single photo-isomerization might be expected to desensitize nearly the entire turtle rod. In contrast, the same spread of desensitization would adapt less than $\frac{1}{4}$ the much longer *Bufo* rod. Thus, four times as many photons would be needed to adapt the *Bufo* outer segment, consistent with the experimental findings.

Evidence against the spread of adaptation between rods

Several lines of evidence strongly mitigate against a pooling of adaptive signals between rods. Perhaps the most straightforward evidence is the difference between the summation fields for excitation and adaptation. The length constants for excitation, using a slit-shaped stimulus ranged from 50 to 70 μm. The excitatory signals are carried via the extensive electronic network, linking rods together (Owen & Copen-

hagen, 1977; Detwiler *et al.* 1980). The decline of adaptation with distance was more pronounced than that of an exponential decay having a space constant of 15 μm. Therefore, it appears that information on the state of desensitization in one rod is not carried in the electronic network to its neighbours. Furthermore, the similarity between the spatial profiles of adaptation and scattered light supports the hypothesis that a rod's sensitivity is governed almost entirely by photo-isomerization-induced desensitization in its own outer segment.

An adaptive mechanism whereby sensitivity is governed by membrane potential is ruled out by the lack of correlation between potential and sensitivity for displaced and superimposed background fields. Figs. 7, 8 and 11 showed that a displaced adapting stimulus could hyperpolarize the rod to a greater extent than a superimposed one and yet desensitize the rod less.

A comparison of the effects of spatially distinct backgrounds on flash sensitivity and response kinetics (Figs. 10*A* and 12*A*) could have ruled out the local computation of adaptation and supported the pooling hypothesis. Fig. 12*B* indicates that when the two disparate backgrounds were adjusted in intensity to elicit comparable desensitizations, the kinetics of the test responses were very similar. Furthermore, the similar dependence of flash sensitivity on background intensity for a slit and full field stimulus (Figs. 4 and 10*A*) is consistent with a single locus of adaptation. Therefore, these experiments provide additional supportive evidence for the local computation of adaptation within each rod outer segment and against a pooling of desensitization amongst neighbouring rods.

Our findings support the hypothesis that desensitization spreads longitudinally along the outer segment. They confirm previous studies indicating that adaptation cannot represent changes occurring at or just adjacent to the disk which absorbs a photon (Donner & Hemila, 1978; Bastian & Fain, 1979; Lamb, McNaughton & Yau, 1981). It now appears that backgrounds an order of magnitude dimmer than previously reported can halve sensitivity. This should place strong constraints on any photochemical hypothesis of how sensitivity is regulated (Lamb, 1981; Clark, Oakley & Pepperberg, 1982; Pepperberg, 1984).

Temporal decay of desensitization

The minimum intensity at which adaptation could be detected averaged 0·2 Rh^* s^{-1}. Since we concluded that there is little if any spread of adaptation between rods, it must be conjectured that a single photo-isomerization in a fully dark-adapted rod can desensitize that rod for at least several seconds. At the average minimum background intensity this period must last at least five seconds (1/0·2 Rh^* s^{-1}). In the case of the most adaptable rods, backgrounds which produced only one Rh^* every 10 s could more than halve the flash sensitivity. Thus, these rods must remain desensitized for periods longer than 10 s following a single photo-isomerization. In support of the relatively long-lived desensitization in rods, Lamb, McNaughton & Yau (1981) estimated that the decay of desensitization in outer segments of toad rods was about 3 s. Thus, it appears that in both turtle and toad, the desensitizing signals are influencing the state of adaptation of the rod for periods comparable to or larger than the response itself, which lasts for several seconds.

Comparison of the magnitude of desensitization for different background configurations

Experiments comparing the desensitization by full field and 3·5 μm slit-shaped stimuli (not included in Results) indicated that 0·7–1·4 (average = 1·0) log units more light was required in the slit to produce the same reduction in flash sensitivity. On the assumption that desensitization is computed locally, the difference in background intensity should be accounted for by optical factors.

Assuming that retinal scatter dispersed the profile of a narrow slit image in a manner described by the line spread function of eqn. (5), then the relation between the slit width and its peak intensity can be derived easily. This intensity can be compared to that of the full field background. It is possible to show by convolving a rectangular slit image with eqn. (5) that the peak intensity is

$$I(a) = 2(I_{FF}/\pi)(\tan^{-1} a/2b), \tag{6}$$

where a is the slit width and I_{FF} is the intensity of the full field background. Using $b = 9{\cdot}0$ μm, one calculates that a factor of 6·6 (0·82 $\log_{10}$) times as much light should have been required to elicit an equivalent desensitization by the slit as compared to the full field. This calculated difference is in reasonable agreement with the experimental findings.

Similarly, one ought to be able to account for the differences in intensity required for comparable desensitizations by spot and annular backgrounds (Fig. 7). Experimentally the annular intensity needed to be twenty times that of the spot for equal adaptation. Direct scans of the images impinging on the retina indicated that the illumination at the centre of the annulus was $\frac{1}{40}$ of that from the spot (Fig. 1*B*). Scatter in the retina must tend to increase the light falling on the rod at the centre of the annulus. To account for the measured reduction in sensitivity from the annulus on the basis of light scatter one must only postulate as much scatter in the retina as the light passes through it, as has already occurred in the stimulator prior to reaching the retina.

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