

## RESPONSES OF ROD BIPOLAR CELLS IN THE DARK-ADAPTED RETINA OF THE DOGFISH, *SCYLIORHINUS CANICULA*

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### SUMMARY

1. Responses to light were recorded from bipolar cells in the retina of the dogfish, *Scyliorhinus canicula*, under dark-adapted conditions. The identity of the cells was confirmed by Procion Yellow staining.

2. More than 95% of the bipolar cells sampled were of the type which depolarized to a spot of light. These are termed depolarizing bipolar cells. In most cells, illumination of the surround had little effect on the responses elicited from the central receptive field.

3. The mean flash sensitivity of the depolarizing bipolar cells was 270 mV/Rh\*\* (where Rh\*\* signifies rhodopsin photoisomerization per rod for full field illumination).

4. The mean flash sensitivity of horizontal cells under the same conditions was 8 mV/Rh\*\*. In a limited sample of hyperpolarizing bipolar cells the highest flash sensitivity was 42 mV/Rh\*\*.

5. The high flash sensitivity of the depolarizing bipolar cells indicates a large voltage gain at its synapse with rods. On the assumption of a rod flash sensitivity of 2 mV/Rh\*\* the mean gain at the synapse was 135, but for some cells the gain was in excess of 500.

6. Responses of depolarizing bipolar cells to dim flashes could be approximated by the impulse response of a 12–16 stage low-pass filter, whereas horizontal cell responses could be fitted by a low-pass filter of six sections. The implied filter at the rod-bipolar cell synapse is tuned to the higher frequency components of rod signals, thereby improving temporal resolution in the rod pathway.

7. Depolarizing bipolar cell responses to test flashes are reduced by weak background illumination (less than 0.1 Rh\*\*/sec). This desensitization, which would not be expected to affect rod responses, could be explained by a shift in the operating point to a less sensitive region of the intensity–response curve as a result of the large depolarization elicited by the background.

8. The results of current injection into the cell in darkness and during the response to light are consistent with the release by rod terminals of a transmitter which closes ionic channels in a conductance path having a reversal potential of –8 mV, transmitter release being suppressed by light.

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## INTRODUCTION

The rod visual pathway, when dark adapted, is capable of detecting a light stimulus from which a few quanta (of the order of 10) are absorbed within an area covered by some 500 rods (Hecht, Shlaer & Pirenne, 1942; Brumberg, Vavilov & Sverdlov, 1943; van der Velden, 1944). This high sensitivity is very likely established within the retina, for Barlow, Levick & Yoon (1971) have shown that retinal ganglion cells of the cat respond with extra impulses when only one or two photons are absorbed within their receptive field. Despite considerable progress in recent years in recording from the different cell layers of the vertebrate retina, it is still not understood how this great sensitivity is achieved. There is an emerging body of quantitative information on the size of rod signals and their spread to other rods by electrotonic coupling (Schwartz, 1973, 1976; Fain, 1975; Copenhagen & Owen, 1976; Detwiler, Hodgkin & McNaughton, 1978). Transmission of rod signals at the intermediate stages between the photoreceptors and ganglion cells is known only in a qualitative way.

The present study was undertaken in order to obtain quantitative information on the response characteristics of bipolar cells in the rod visual pathway under dark-adapted conditions and to gain some understanding of the way in which visual signals are processed at the rod-bipolar synapse. The retina of the dogfish, *Scyliorhinus canicula*, was chosen for study because its retina contains a high proportion of rods (with a rod-cone ratio which may be greater than 100:1) and its bipolar cells are relatively large permitting stable recording for periods of up to an hour. This paper gives a general description of the responses of rod bipolar cells to flashes and steps of light, their electrical properties, the gain characteristics at their synapse with rods and the way in which weak backgrounds modify the response. The implications of high gain and filtering at the rod-bipolar cell synapse are discussed in relation to detection of dim light.

The accompanying paper (Ashmore & Falk, 1980) is concerned with the single-photon signal in the bipolar cell and rod-bipolar cell convergence. A third paper (J. F. Ashmore & G. Falk, in preparation) analyses the power spectral density of voltage noise in bipolar cells and deals more fully with the problem of signal-to-noise.

## METHODS

Adult spotted dogfish, *Scyliorhinus canicula*, were obtained from the Marine Biological Association, Plymouth, and kept in a circulating sea-water aquarium at 10 °C for periods usually of up to a month. The animals were kept on a diurnal light-dark cycle but were shielded from bright light. Before an experiment the animal was dark adapted overnight. After decapitation and pithing, both eyes were enucleated under very dim red light, hemisected and cut into pieces about 1 cm<sup>2</sup>. Rapid, careful dissection appears to be critical. Room temperature was maintained in the range 14–18 °C, since at higher temperatures preparations tended to be less stable. Pieces of the eyecup could be kept on ice in darkness for up to 6 h without deterioration.

After removal of some of the vitreous, a piece of eyecup was mounted in a chamber through which 100% moist oxygen flowed and placed in a light-tight Faraday cage. The electroretinogram was monitored continuously and the preparation discarded when the light intensity required to elicit a *b*-wave of 10  $\mu$ V increased by more than about 1 log unit from its initial dark-adapted value. The e.r.g. of *Scyliorhinus* is very similar to that recorded in the skate (Dowling & Ripps, 1971) with a time to peak of the *b*-wave of 400–500 msec from the stimulus

flash for flashes in the linear range of response. Retinas which contained the most sensitive bipolar cells reported below had noticeably lower *b*-wave 'thresholds', with an initially smaller latency to peak, lengthening as the preparation deteriorated after more than about 2–3 h in the chamber. As far as could be ascertained, given the limitation that fewer than eight bipolar cells were sampled in any given retina, the intensity–response relation for the *b*-wave was the same as for the average bipolar cell relation when the peak amplitudes were normalized.

#### *Light stimulus and calibration*

The light stimulus for these experiments was provided by a dual-beam photostimulator of conventional design operating from a 6 V, 20 W quartz iodine bulb run on a stabilized DC supply. The beam was passed through a bandpass interference filter (Grubb Parsons) with a central wave-length of 498 nm and a band width of 22 nm at 50% of peak transmission. Pen motors were used to operate the beam shutters delivering highly reproducible flashes of 15 msec duration.

The optics were arranged to give a reduction of approximately unity to the circular apertures and annuli used as stimuli. The stimulus termed 'large field' was a circular field of light, 0.9 cm in diameter on the plane of the retina.

Gelatine neutral-density filters were individually calibrated against air on a spectrophotometer. No allowance was made for inter-reflexions, since at any one time no more than three filters were in the light path where inter-reflexions might occur. Using a formula given by Stokes (1862), we estimate that the light intensity would be underestimated by less than 4% assuming a reflectivity of glass of 10%.

The blue-green light falling on the preparation was calibrated with a Tektronix J16/J6502 irradiance probe, recently calibrated by the manufacturer and specified accurate to 7% over the visible spectrum. A photodiode, placed in the plane of retina, was calibrated frequently against the radiometer and used routinely to check the light source intensity through the bandpass filter before each experiment to allow for decline in the power output from the quartz iodine bulb during its lifetime. A decline of 11% in the light flux was detected over a sample period of approximately 60 hours running time, justifying the assumption of constancy during an experiment.

The irradiance of the source, after passing through the bandpass filter was converted to the equivalent photon flux density (photons  $\mu\text{m}^{-2} \text{sec}^{-1}$  at 498 nm). The calibration was further checked by a photomultiplier (EMI 9824 QA with bialkali cathode) in single-photon counting mode. In order to take into account the spectral variation of quantum efficiency of the photomultiplier, the output of the photomultiplier was corrected by the factor  $\int \lambda E(\lambda) T(\lambda) d\lambda / \int \lambda E(\lambda) T(\lambda) Q(\lambda) d\lambda$ , where  $E(\lambda)$  is the energy of the source ( $\text{erg cm}^{-2} \text{sec}^{-1} \text{nm}^{-1}$ ),  $T(\lambda)$  is the transmission of the bandpass filter and  $Q(\lambda)$  is the quantum efficiency of the photomultiplier at the wave-length  $\lambda$ .  $Q(\lambda)$  was taken from the manufacturer's specifications.  $E(\lambda)$  was determined by measuring the irradiance of the source through narrow-band interference filters (Baylor & Hodgkin, 1973) with the Tektronix radiometer and was found to agree with that expected from the Planck relation for a colour temperature of 3250 °K (close to the nominal colour temperature of 3200 °K, as stated by the manufacturer). These methods agreed on the absolute light calibration to within 15% (0.07 log units).

#### *Absorption of light by rods*

To convert light fluxes incident upon the retina to rate of photoisomerizations per rod, we take a specific axial density of rhodopsin of  $0.014 \mu\text{m}^{-1}$  at 500 nm (Liebman & Entine, 1968), a quantum efficiency for photoisomerization of 0.67 (Dartnall, 1968) and a reflectivity of the tapetum of 90% (Denton & Nicol, 1964). The geometric cross-section of *Scyliorhinus* rod outer segments suspended in vitreal fluid is  $7.1 \mu\text{m}^2$  and their mean length is 28  $\mu\text{m}$ . Thus the probability of absorption of light by the rod outer segments is 0.594 on one passage, and allowing for absorption after tapetal reflection, the photoisomerization cross-section would be  $7.1 \times 0.67 \times (0.594 + 0.594 \times 0.406 \times 0.9) = 3.8 \mu\text{m}^2$ . This figure will be an over-estimate since some of the light will pass between the photoreceptors as well as being scattered by other retinal layers. Denton & Nicol (1964) measured the optical density of the isolated dark-adapted retina of *Scyliorhinus* and obtained a mean value of 0.23 at 500 nm. This value for the optical

density together with the other assumptions given above would yield a photoisomerization cross section of  $3 \mu\text{m}^2$  which is the value we have adopted. For notational purposes a rhodopsin molecule bleached per rod will be denoted by  $\text{Rh}^{**}$ . Thus 1 photon  $\mu\text{m}^{-2}$  is equivalent to 3  $\text{Rh}^{**}$ .

#### *Electrical recording*

Recordings were made with electrodes pulled from commercially available borosilicate tubing containing a small capillary fused to the wall (Clark Electromedical, Pangbourne), and backfilled with 4 M-potassium acetate. Electrodes usually had resistances in the range 250–400 M $\Omega$  measured in the vitreous. Bevelled electrodes, which passed larger currents, were used in the experiments to inject Procion yellow to mark the cells or for cell resistance measurements.

In the experiments to be described, maintaining a state of dark adaptation in the preparation was considered of paramount importance. The electrode was positioned over the retina using only enough light barely to discern its position and was then advanced hydraulically from outside the light-tight box.

Impalements were made in the area of the reflecting tapetum but away from the bright tapetal streak, with the electrode making an angle of approximately  $45^\circ$  to the retinal plane. To locate cells responding to light while at the same time maintaining dark adaptation, dim, full-field flashes, bleaching about 0.03 rhodopsin molecules/rod were delivered at 4 sec intervals. The centring of spots and annuli on an impaled cell was performed by moving an edge over the field in orthogonal directions (Ashmore & Falk, 1976). Electrical signals were recorded via an ultra-low-input capacitance preamplifier (Kootsey & Johnson, 1972) and stored on magnetic tape for subsequent playback and analysis. The DC level was continuously monitored via a digital voltmeter. To extend the range of the FM tape recorder while recording at relatively high gain, the DC level was stored via a sample-and-hold circuit before each light stimulus and used to recentre the response around an arbitrary zero on the recorder. With full capacitative compensation the system band width was 0–2 kHz through a 500 M $\Omega$  pure resistance.

The neutral density filter in the light beam was coded and stored as an analog signal on one channel of the tape recorder. Dim light flashes of different intensities were presented in a pseudo-random order and the responses were subsequently sorted and averaged on a digital computer. This method compensates for any small instabilities in the recording over the period of time necessary to accumulate the data.

#### *Electrical properties of cells*

Current was applied through the recording electrode by means of a current pump circuit and the voltage drop across any resistance in series with the cell's capacitance was balanced by a bridge circuit. For such experiments electrodes with resistances of about 150–200 M $\Omega$  were used.

Current pulses in darkness and in light were applied as a train of pulses of fixed amplitude and polarity or as a sequence of pulses whose amplitude was incremented digitally in 32 steps from  $-1$  to  $+1$  nA. The latter method of applying a pulse ramp allows the determination of the current–voltage relation in a short space of time. However, the non-linearity of the electrode's electrical properties often precluded its use, since it was necessary to balance the bridge for the electrode resistance at a fixed current level. Corrections were made for electrode non-linearity observed after withdrawal of the electrode from the cell.

In the plot of voltage against current, the voltages were measured as displacements from the dark level. When a ramp of pulses was used, the pulses were applied in darkness and during steps of light lasting 1.5 sec. Otherwise pulses were applied in darkness and during brief flashes of light and the voltage displacement from the dark level measured near the peak of the flash response.

#### *Procion-yellow staining of cells*

Cells were impaled by electrodes filled with 6% (w/v) of Procion M4RS. Hyperpolarizing current pulses (about 0.5–2 nA, 400 msec pulses at 1.5 Hz) were passed for 6–10 min while the membrane potential and the response to flashes of light were monitored. Cells continued to respond after injection of dye. Histological preparation of the tissue followed standard techniques (Stretton & Kravitz, 1973; Kaneko, 1970).

## RESULTS

*Identification of cells*

The histology of the *Scyliorhinus* retina is similar to the retina of the smooth dogfish *Mustelus* both as to thickness of the different layers and the size of cells (Witkovsky & Stell, 1973*a*; Stell & Witkovsky, 1973*a, b*). Cells, presumed to be horizontal cells or bipolar cells, were impaled 70–100  $\mu\text{m}$  from the vitreal surface. It was not possible to record from the rods.

Cells in which the internal potential in the dark was  $-40$  to  $-85$  mV, giving hyperpolarizing responses of up to 90 mV and with a large receptive field (greater than 3 mm), were classified as horizontal cells (Kaneko, 1971*a*; Dowling & Ripps, 1971). Although there was little doubt that these were horizontal cells, four cells were injected with Procion yellow, of which two were later recovered. These were large cells immediately subjacent to the rod bases.

The responses to light of about 200 cells, presumed to be bipolar cells, were recorded. Some cells gave stable recordings for more than 1 hr. The cells were often located in close proximity to horizontal cells. In some cases the electrode jumped spontaneously from one cell type into the other during the recording.

Almost all of these cells were of the type which depolarized in response to a centred spot of light. This class is frequently referred to as on-centre, but in this paper we shall use the term depolarizing bipolar cell. Four out of thirteen depolarizing bipolar cells, injected with Procion yellow, were recovered and the best example is illustrated in Pls. 1 and 2. This cell has a tulip-shaped perikaryon, 27  $\mu\text{m}$  across. The dendrites emerge from the distal portion of the perikaryon and extend laterally in the outer plexiform layer, giving a dendritic spread of at least 105  $\mu\text{m}$ . The axon descends vertically through the inner nuclear layer and then turns in a horizontal direction to end in a large knob-like terminal in the proximal third of the inner synaptic layer. This cell appears to be identical to the b-type bipolar cell described from Golgi stained material in the retina of the smooth dogfish, *Mustelus* (Witkovsky & Stell, 1973*a*).

The three other depolarizing bipolar cells which were recovered had their perikarya also in the distal part of the inner nuclear layer, but could not be characterized further as to subclass of bipolar cell. No cell with a Landolt club was stained, although such bipolar cells are to be found in the dogfish retina (Neumayer, 1896; Witkovsky & Stell, 1973*a*).

A rare group of cells comprising six out of about 200 cells suspected to be bipolar cells gave hyperpolarizing responses of the type illustrated in Fig. 16. The hyperpolarizing cells had receptive fields for their centre response of 200–300  $\mu\text{m}$ . One hyperpolarizing cell, located at a depth of 70  $\mu\text{m}$  was injected with Procion yellow and recovered. The cell is illustrated in Pl. 3. Numerous stout processes emerge from the distal half of the cell body and ramify in the outer plexiform layer. Two processes exit from the proximal end of the perikaryon, course obliquely through the inner nuclear layer to run laterally along the distal margin of the inner synaptic layer for more than 100  $\mu\text{m}$  in either direction. In many respects, the morphology of this cell resembles the a-type of bipolar cell in *Mustelus*, described by Witkovsky & Stell (1973*a*). The cell soma is somewhat larger than that of any other bipolar

cell which was Procion stained so that the paucity of recordings probably reflects a relatively small population in the retina. Since most of the observations reported here were made on depolarizing bipolar cells, the latter will be referred to simply as bipolar cells.

### Depolarizing bipolar cells

A total of fifty-eight cells were studied which gave depolarizing responses to a bright flash of light in excess of 10 mV and which were held over a sufficient time to characterize their response over a wide range of light intensities. These cells had internal potentials in the dark which ranged from  $-27$  to  $-65$  mV with a mean value of  $-46.8$  mV for the sample. The results for twenty representative cells are given in detail in Table 1, together with the mean values for all fifty-eight cells. The response characteristics are tabulated for full-field illumination. It will be shown in the section on the receptive field of bipolar cells that in a well dark-adapted retina, these response characteristics reflect that of the centre of the receptive field of the bipolar cell and are not significantly altered by the surround.

TABLE 1. Response characteristics of depolarizing bipolar cells

Cell	$E_D$ (mV)	$V_{max}$ (mV)	$S_F$ (mV/ Rh**)	$S_S$ (mV sec/ Rh**)	$t_i$ (msec)	$I_{\frac{1}{2}}^{-1}$ (flash/ Rh**)	$\beta$	$T$ (msec)
1	-56	26.8	118	44.4	370	2.6	1	510
2	-27	15.6	154	58.3	372	8.9	0	375
3	-42	35.4	128	65.5	510	1.5	2.5	580
4	-51	29	2790	—	—	12.7	2.5	418
5	-56	29.5	1860	645	346	14.5	3	456
6	-50	28.6	610	275	450	4.2	2	510
7	-35	24.6	26	10.2	392	1.2	0	504
8	-45	27.8	39	12.5	295	1.3	0	540
9	-50	31	1109	—	—	4.3	3.5	528
10	-44	25	265	93.5	355	3.4	3	516
11	-50	23.5	455	145	318	9.8	3	492
12	-29	27	324	120	370	4.5	2	460
13	-49	25.5	17	10.5	618	0.4	0	610
14	-47	29.5	11	3.4	312	0.4	0	520
15	-55	24.6	360	—	—	4.8	2	440
16	-40	18.2	93	—	—	5.1	0	620
17	-52	40	40	—	—	1.0	0	560
18	-50	11.0	87	—	—	7.3	0	510
19	-40	11.0	39	16.4	420	3.7	0	550
20	-40	21.8	22	—	—	0.6	1	500
Mean <sub>1</sub>	-45	25.3	405	115	394	4.6	1.3	514
Mean <sub>2</sub>	-47	22.2	269	—	—	3.8	1.0	—

$E_D$  is the internal potential in darkness;  $V_{max}$  is the maximum response to bright light;  $S_F$  and  $S_S$  are the flash and step sensitivities, respectively;  $t_i$  is the cell integration time, given by the quotient  $S_S/S_F$ ;  $I_{\frac{1}{2}}$  is the flash intensity giving a half-maximum response;  $\beta$  is the constant in eqn. (4) which determines the slope of the log intensity-response curve;  $T$  is the time to peak of the response within the linear range.

Mean<sub>1</sub> is the mean of the cells listed in the table; mean<sub>2</sub> is the mean for fifty-eight cells which had a maximum response amplitude in excess of 10 mV.

*Response to flashes*

*General features of the response.* The response of a depolarizing bipolar cell to 15 msec flashes of diffuse light is shown in Fig. 1 for a wide range of light intensities.

The response of a typical bipolar cell to a flash of light (less than 0.05 Rh\*\*/flash) consisted of a depolarization reaching a peak 400–600 msec after the flash. There was a latent period before the response could be observed to rise from the baseline noise. Responses to dim flashes showed pronounced amplitude fluctuations. These fluctuations in response have been analysed in the following paper (Ashmore & Falk, 1980).

At higher light intensities there was a shortening in the time to the peak of the response (Fig. 1). The maximum response amplitude for the cell shown in Fig. 1 was 31 mV, but in other cells responses up to 40 mV were observed (Table 1). Once the response amplitude approached a saturated level, the effect of still brighter light flashes was to shorten the time scale of the response during its phasic part. This shortening of time scale at higher light intensities is evident in the crossover of the curves during the falling phase of the response, seen in Fig. 1. At light intensities in excess of 1 Rh\*\*/flash (equivalent to log attenuation 3.0), the phasic part of the response was followed by a prolonged depolarizing after-potential whose duration and amplitude increased with light intensity (Fig. 1*B*). In some cells, including the cell illustrated in Fig. 1, the depolarizing after-potential for certain light intensities contained a small damped oscillatory component with a period of approximately 400 msec, but which damped out within a few cycles. Brighter lights exceeding 50 Rh\*\*/flash were usually sufficient to abolish these oscillations. In the majority of cells (but not the cell of Fig. 1), the depolarizing after-potential, produced by light intensities greater than about 10 Rh\*\*/flash, was followed by an after-hyperpolarization of a few millivolts which decayed along a time course of a few seconds. This after-hyperpolarization was similar to that which followed the turning off of a long step of light (e.g. bottom record of Fig. 4).

*Flash sensitivity.* One of the most prominent features of the response to flashes was that a measurable response could be elicited at extremely low light intensities. As shown in Fig. 1, there was a response exceeding 1 mV when the flash bleached one rhodopsin molecule per 676 rods (corresponding to log attenuation = 5.89) with saturation of the response evident at 12 Rh\*\*/flash (log attenuation = 1.97). The four lower records of Fig. 2 show signal averaged responses of the cell of Fig. 1 to dim flashes of light in the range from 1 rhodopsin molecule bleached per 3700 rods to 1 rhodopsin molecule bleached per 450 rods. Over at least this range, the response was graded linearly with light intensity so that the effect of doubling the light intensity was simply to double the amplitude.

Following Baylor & Hodgkin (1973), we define the flash sensitivity,  $S_F$ , by

$$S_F = dV/dI \quad (1)$$

as  $I \rightarrow 0$ , where  $I$  is measured as Rh\*\*/flash and  $V$  is measured at the peak of the flash response.  $S_F$  is thus the limiting slope of the intensity response curve for the cell at dim light intensities, with units mV-flash/Rh\*\*. For notational convenience, however, this will be abbreviated to mV/Rh\*\* without confusion. Although  $S_F$  was originally defined in the context of individual photoreceptor measurements, it

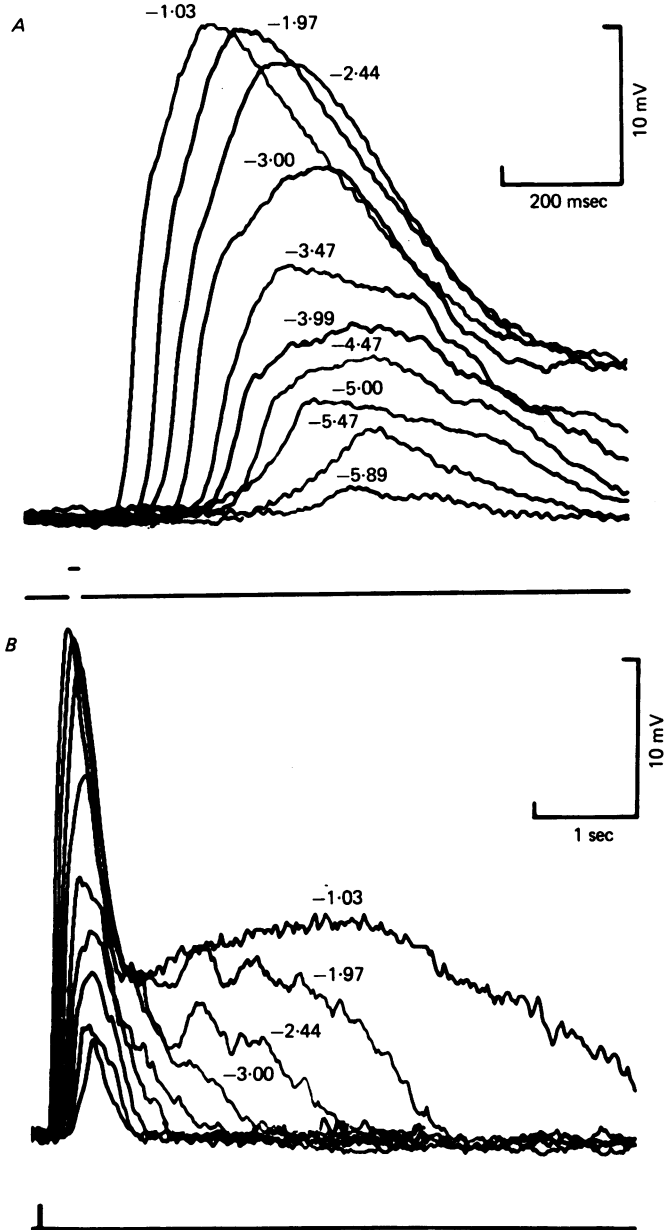


Fig. 1. Responses of a depolarizing bipolar cell to 15 msec flashes of blue-green light of varying intensity which illuminated the full retinal field. *A*, responses recorded at a fast sweep. *B*, responses of the same cell plotted on a slower time base. For clarity, the response at the lowest light intensity in *A* has been omitted in *B*. The timing of the flash is indicated by the trace below each set of records. The responses in this and most subsequent Figures have been digitized and superimposed on an *X-Y* plotter. The numbers by the records in this figure indicate the log of the flash intensity  $I$ , relative to the unattenuated light beam, so that  $\log I = 0$  corresponds to 1150 Rh\*\*/flash. Internal potential of the cell in darkness  $-50$  mV; maximum response amplitude 31 mV. Flash sensitivity 1109 mV/Rh\*\*. Temperature 16.5 °C. Cell 9 of Table 1.



is a quantity which also has meaning for post-synaptic retinal cells when the entire centre of their receptive field is illuminated.  $S_F$  is numerically equal to the response which would be observed in the post-synaptic cell if (a) the cell responded linearly to light and (b) a flash photoisomerized one rhodopsin molecule in every rod con-

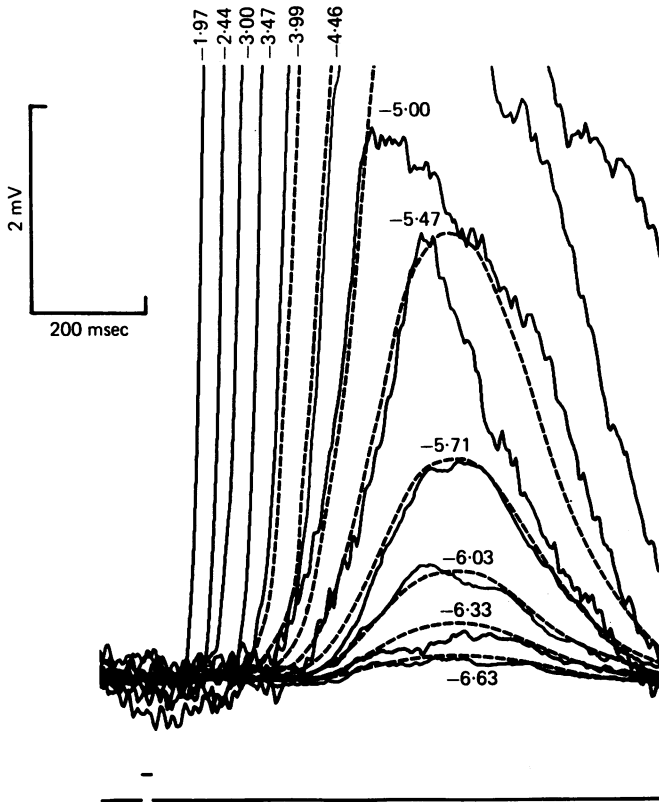


Fig. 2. Time course of the response of a depolarizing bipolar cell to a flash compared with a linear model of the response. Each dashed curve is the impulse response of a 17-stage low-pass filter with a time constant per stage of 33 msec, with response amplitude scaled by the factor by which the light intensity increases. The responses were obtained from the same cell illustrated in Fig. 1. The responses at the four lowest light intensities (attenuation 5.71–6.63 log units) are the result of signal averaging eight responses at each intensity, the flashes of different intensities being presented in pseudorandom fashion. Note that the lowest intensity corresponds to a mean of 1 rhodopsin molecule bleached per 3700 rods. The other responses illustrated (log relative intensity -5.47 to -1.97) are the same as shown in Fig. 1, but amplified.

verging onto the cell. In the case of the cell illustrated in Fig. 1 and 2, the flash sensitivity was 1109 mV/Rh\*\*. The mean value for fifty-eight cells was 269 mV/Rh\*\* (Table 1). In three cells  $S_F$  exceeded 1000 mV/Rh\*\* (including the Procion stained cell in Pls. 1 and 2), and in nine cells it exceeded 300 mV/Rh\*\*.

The response was half-saturated in some cells when 1 rod out of 13 had absorbed an effective photon (Table 1). The mean half-saturating flash intensity corresponded

to an absorbed photon in 1 rod out of 3.8, which would presumably be well within the linear response range of the rods.

*Time course of the response to dim flashes.* The responses,  $V(t)$ , in the linear range shown in Fig. 2 may be fitted by

$$V(t) = S_F I(t/T)^{n-1} \exp [-(n-1)(t/T-1)], \quad (2)$$

the time-dependent part being the normalized impulse response (having unity peak amplitude) of an  $n$ -stage, low-pass filter with a time constant for each stage,  $\tau = T/(n-1)$ .  $T$  is the time to the peak of the response. Such a function has been used to fit the responses of photoreceptors (Fuortes & Hodgkin, 1964; Penn & Hagins, 1972; Pasino & Marchiafava, 1976). For the cell illustrated,  $n = 17$ , although for such a large number of stages, the parameter  $n$  is not critical. In other cells, the number of stages required to fit the dim flash response ranged from 12 to 16, significantly higher than required to fit vertebrate photoreceptor responses where  $n$  ranges from 4 to 7. Equation (2) mimics the features of the response where there appears to be a finite delay before the potential increases from the base-line noise. In Fig. 2, the time to peak,  $T = 528$  msec, would give a time constant for each stage of 33 msec.

Fig. 2 shows the superposition of the curves obtained by fitting eqn. (2) to the response in the linear range and scaling up by the factor by which the light intensity increased. Reasonable agreement between the theoretical curves and the initial 4 mV of the rising phase of the response was obtained. The falling phase could not be fitted by the theoretical curve outside the linear range, the response reaching a peak sooner and then falling more rapidly than would be predicted. The time course of response outside the linear range cannot be described simply by a non-linear transformation of amplitude of the kind that would account for saturation of response. It appears that there are also time-dependent processes which must be considered to give a full account of the shape of the response over a wide range of light intensity.

*Intensity-response relationship.* In about one half of the cells studied, the relationship of peak amplitude of response,  $V$ , to flash intensity  $I$  could be fitted by a rectangular hyperbola of the form

$$V = V_{\max} I / (I + I_{\frac{1}{2}}), \quad (3)$$

where  $V_{\max}$  is the maximum response which could be elicited by a bright flash and  $I_{\frac{1}{2}}$  is the half-saturating light intensity. For the remaining cells the intensity-response curves were less steep than predicted by eqn. (3). The data from the bipolar cells were fitted by the empirical relationship,

$$V = V_{\max} I / [I + \sigma \exp (\beta V / V_{\max})], \quad (4)$$

where  $\sigma$  is a constant parameter having units of light intensity and  $\beta$  is a dimensionless constant for the cell. Equation (4) reduces to (3) in the case  $\beta = 0$  when  $\sigma = I_{\frac{1}{2}}$ . At low light intensities responses depend linearly on light intensity as

$$V = V_{\max} I / \sigma \quad (5)$$

and since

$$I_{\frac{1}{2}} = \sigma \exp (\beta / 2) \quad (6)$$

the relationship between flash sensitivity,  $S_F$ , and  $I_{\frac{1}{2}}$  is

$$S_F = [\exp(\beta/2)]V_{\max}/I_{\frac{1}{2}}. \quad (7)$$

Values for  $\beta$  for some representative cells are given in Table 1. Peak response amplitude normalized to the maximum response is plotted against log light intensity for 8 cells in Fig. 3. The curves are drawn according to eqn. (4) for different values of  $\beta$  which determines the shape of the curve whereas  $\sigma$  determines the position along the log  $I$  axis. It may be observed from Table 1 and Fig. 3 that the intensity-response curves for the cells with the largest flash sensitivities were less steep than a rectangular hyperbola and required  $\beta > 0$ .

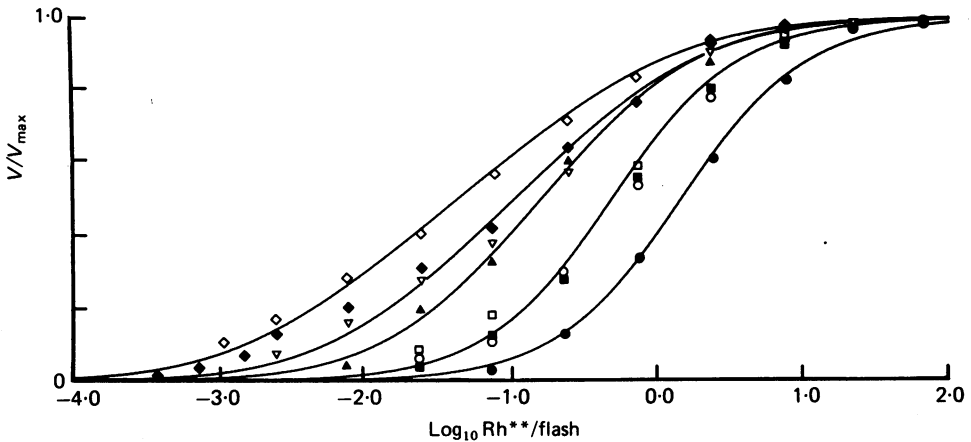


Fig. 3. Intensity-response relation for eight cells, with response normalized to the maximum response amplitude for each cell. Abscissa:  $\log Rh^{**}/\text{flash}$ . Each symbol represents a different cell. The curves were generated using eqn. (4) with value of  $\beta$ ,  $\sigma$  (from left to right): 3, 0.01; 2, 0.04; 1, 0.1; 0, 0.5; 0, 1.5, where  $\sigma$  is given in  $Rh^{**}/\text{flash}$ . No attempt has been made to provide the best fit to the data points. The maximum amplitude of the response for the different cells ranged between 17 and 35 mV. It will be seen from the displacement of the points at low light intensities that there was a significant trend such that those cells with the highest flash sensitivities (greater than 1000 mV/ $Rh^{**}$ ) were characterized a  $\beta \simeq 3$ , while cells with flash sensitivities 100 mV/ $Rh^{**}$  and less had an intensity response relation approximated by a rectangular hyperbola ( $\beta = 0$ ).

#### *The response to steps of light*

The response of a bipolar cell to steps of light is shown in Fig. 4. The light intensity ranged from one photon absorbed per 743 rods per sec ( $\log$  attenuation = 7.77) to 79.3  $Rh^{**}/\text{sec}$  ( $\log$  attenuation = 3.00). As with flash responses, there was a delay of several hundred milliseconds before the recorded potential increased above the baseline in response to dim steady lights. The delay decreased as the light increased in intensity. For the cell illustrated, at intensities less than about 1  $Rh^{**}/\text{sec}$ , the potential increased monotonically to reach a steady mean value which was maintained during the light. With brighter lights the response had an initial transient peak. With lights of near saturating intensities, the transient peak was followed by a slow

recovery of depolarization with the potential reaching the same internal potential as during the peak if the light was kept on sufficiently long (shown in the bottom record of Fig. 4 at slow sweep). Most of the cells studied with steps of light showed this slow increase in the potential after an initial peak. Some cells, however, maintained a steady depolarized level during bright steps of light (of intensity greater than about 10 Rh\*\*/sec) at about 40–60% of the response amplitude of the initial phasic component.

When the light was turned off, the cells repolarized with a delay of less than a second for light intensities lower than about 100 Rh\*\*/sec. There was frequently a hyperpolarizing undershoot of the dark potential whose magnitude depended on light intensity and the duration of the step. As noted by Schwartz (1974) in the turtle, the after-hyperpolarization increased as the duration of the step was increased. The after-hyperpolarization was more prominent in cells of low sensitivity. The characteristics of the response to a step of diffuse light, which has just been described, were also observed using centered spots of light, 150  $\mu$ m in diameter.

A prominent feature of the response to steps which is illustrated in Fig. 4 is the increase in noise in dim light and the reduction of noise with bright light which produces response saturation. Power spectral analysis of the noise shows an increase in the noise power over the band width 0–5 Hz, most of the noise being due to photon shot events (Ashmore & Falk, 1977). This aspect of the response to steps of light will be considered in a later paper (J. F. Ashmore & G. Falk, in preparation).

#### *Step sensitivity and integration time*

A characteristic property of a linear system is superposition such that the response to a step will be the integral of the response to an impulse, in this case a brief flash. This property may be assigned a parameter, the integration time, which is a measure of the time taken to change to the new steady potential when a long step of light is applied. The integration time is defined as the integral of a flash response when the peak amplitude is normalized to unity (Baylor & Hodgkin, 1973).

Fig. 5B shows the results obtained by numerical integration of the averaged response to dim light flashes (Fig. 5A), superimposed on the response to 1.1 sec steps of light. The light intensity was increased by a factor of approximately 2 between steps. There was agreement between the calculated and observed step responses for depolarizations less than about 1.5 mV, but at larger depolarizations the responses departed from linearity.

It will be noticed in Fig. 5 that the rising phase of the step response was reproduced on the assumption of linearity for larger depolarizations, up to about 4 mV. A similar observation also applied to the rising phase of the flash responses in the cell of Fig. 2, suggesting that there is a non-linear process which operates after a delay of 200 msec, possibly voltage linked. This process may account for the shortening of the time to the peak of response at higher light intensities and the departures of the intensity–response curves from a rectangular hyperbola observed in many cells.

The step sensitivity  $S_S$  (Baylor & Hodgkin, 1973) is defined by

$$S_S = (dV/dI)_{I \rightarrow 0}, \quad (8)$$

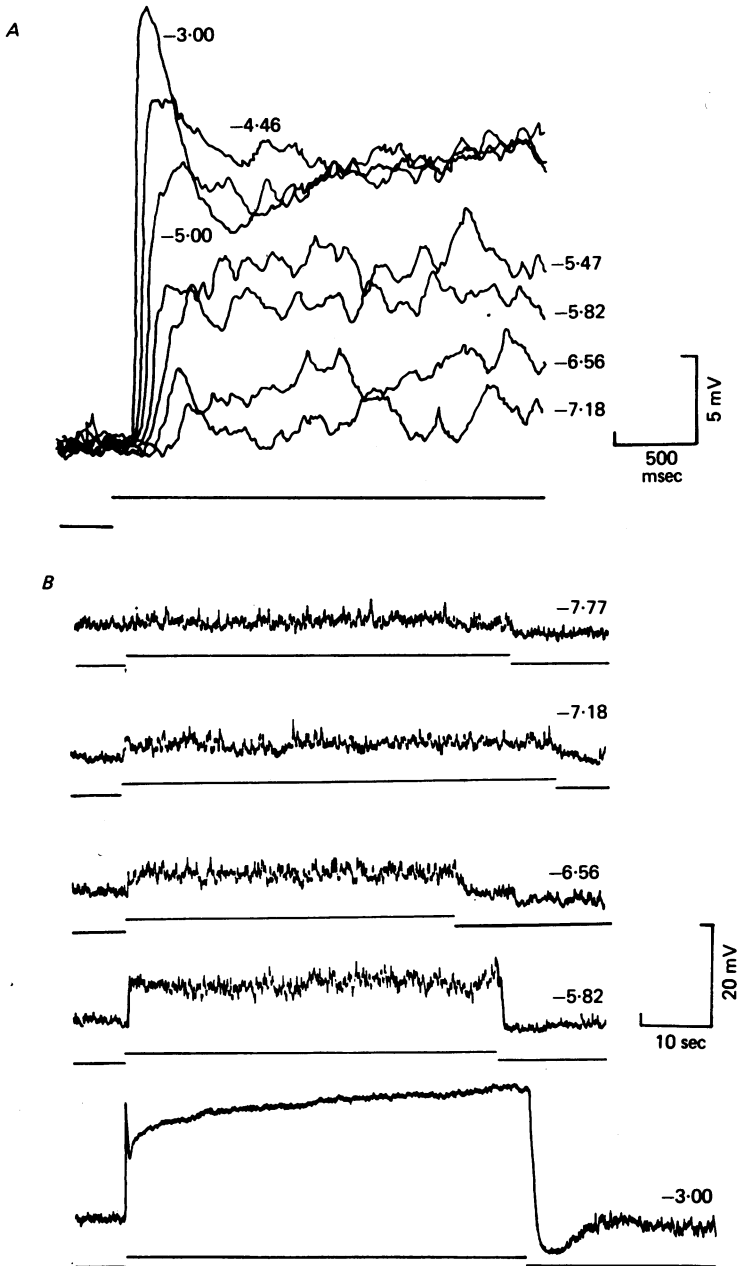


Fig. 4. Response of a depolarizing bipolar cell to steps of light. *A* and *B* show the responses on different time scales. Numbers by the records show the negative log attenuation of the light beam of unattenuated intensity  $7.93 \times 10^4$  Rh\*\*/sec diffusely illuminating the retina. The records in *A* are tracings from an XY plotter; the records in *B* are from a pen recorder and are displaced for clarity. The traces under the response records indicate the duration of the light. The dimmest light, (*B*, top) was equivalent to  $743^{-1}$  Rh\*\*/sec. Note the change in 'noise' during the period of illumination. Cell 6 of Table 1. Temperature 17.5 °C.

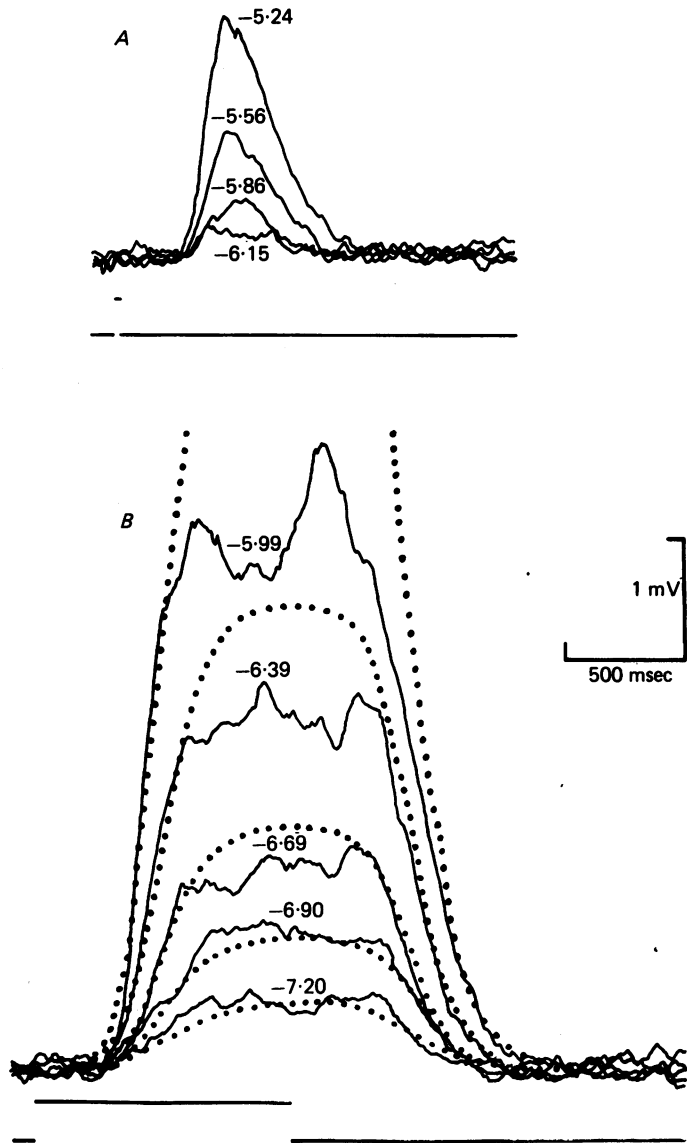


Fig. 5. Responses of a depolarizing bipolar cell to flashes and steps of light demonstrating linear characteristics of the cell. *A*, signal averaged responses to 15 msec flashes (16 sweeps at each intensity). *B*, signal averaged responses to a 1 sec pulse of light. 16 sweeps averaged for the three lowest intensities, 8 sweeps for the next highest and 4 sweeps for the brightest intensity. Numbers by each record give the negative log attenuation of the light beam; the unattenuated light corresponded to  $9.1 \times 10^4 \text{ Rh}^{**}/\text{sec}$ . The dotted lines were obtained by numerical integration of the signal averaged response to 15 msec flashes producing a response of 1.2 mV peak amplitude. The relative scale was adjusted by the linear factor appropriate to the light intensity. The integration time of the cell was 355 msec (cell 10 of Table 1). Temperature 13 °C.

where  $V$  is the mean depolarization produced by a steady light  $I$  ( $Rh^{**}/sec$ ).  $S_S$  is measured in  $mV \cdot sec/Rh^{**}$ . Values of  $S_S$  ranged from 3.4 to 645  $mV \cdot sec/Rh^{**}$  with a mean value of 115  $mV \cdot sec/Rh^{**}$  (Table 1).

The integration time,  $t_1$ , is related to the flash and step sensitivities by

$$S_S = S_F t_1. \tag{9}$$

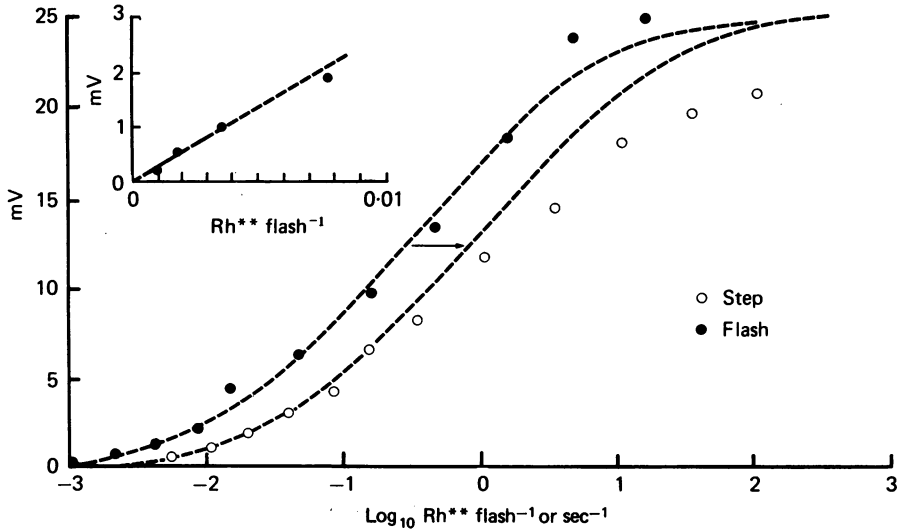


Fig. 6. Comparison of peak response amplitude versus light intensity for flashes (●) and steps (○) of full-field illumination. Data from cell shown in Fig. 5. Abscissa,  $\log I$  where  $I$  is measured as  $Rh^{**}/flash$  and  $Rh^{**}/sec$  for flashes and steps, respectively. The lowest four points in each curve are the result of signal averaging. Inset: the peak amplitude of the response to flashes, plotted on a linear scale. The dashed line drawn through the points for flashes (●) is given by eqn. (4) with  $\beta = 3$ . When displaced by 0.45 log units to the right, the same curve provides a fit to the step response data at low light levels extending up to about 30% of maximum response amplitude. The shift corresponds to an integration time of  $10^{-0.45} sec = 355 msec$ . For steps of intensity in excess of approximately 0.3  $Rh^{**}/sec$ , the deviation of the points from the translated curve indicates a progressive shortening of the effective  $t_1$ . At these light intensities the response to a step consisted of a phasic peak followed by a plateau of lower amplitude.

For a response fitted by eqn. (2), in terms of the parameters  $n, T$ ,

$$t_1 = (n - 2)! [e / (n - 1)]^{n-1} T. \tag{10}$$

An approximation which is within 2% of the exact expression, when  $n \geq 5$ , is

$$t_1 \simeq (2\pi)^{\frac{1}{2}} T (n - 1)^{\frac{1}{2}}. \tag{11}$$

Values for the integration time ranged from 250 to 618 msec with a mean of 394 msec (Table 1).

Fig. 6 shows intensity-response data for flashes and steps for the same cell illustrated in Fig. 5 over a wide range of light intensities. The curve fitted to the flash responses was based on eqn. (4) with  $\beta = 3$ . The same curve shifted to the right

TABLE 2. Some characteristics of rod horizontal cell responses

Cell	$-V_{\max}$ (mV)	$E_D$ (mV)	$S_F$ (mV/Rh <sup>**</sup> )	$T$ (msec)
1	74	-59	7.5	520
2	50	-67	8.0	410
3	60	-60	11.2	—
4	90	-47	8.2	490
5	45	-84	8.8	420
6	38	-59	4.5	440
7	67	-52	11.0	450
8	70	-48	7.8	510
9	45	-45	4.5	390
10	42	-47	5.0	460
11	55	-71	6.9	470
12	68	-65	—	—
Mean	58	-59	7.6	450

The symbols are the same as used in Table 1 and in the text; a negative  $V_{\max}$  indicates that the response is a hyperpolarization.

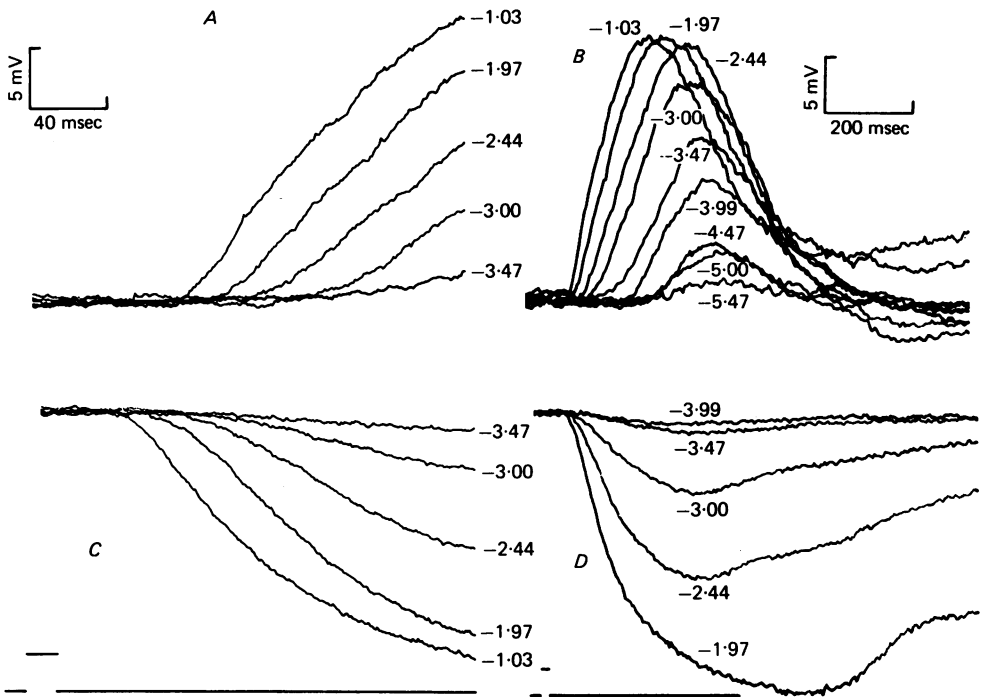


Fig. 7. Comparison between the flash responses of a depolarizing bipolar cell (*A* and *B* at fast and slower sweeps) and a neighbouring horizontal cell (*C* and *D*). The unattenuated light intensity was 1195 Rh<sup>\*\*</sup>/flash. The numbers by the records indicate the negative log attenuation of the photostimulator beam, delivering 15 msec flashes over a large field. The timing of the flash is shown by the lowest trace. Bipolar cell 15 of Table 1: flash sensitivity 360 mV/Rh<sup>\*\*</sup>. Horizontal cell 9 of Table 2: flash sensitivity 4.5 mV/Rh<sup>\*\*</sup>.



by 0.45 log units provided a fit to the peak step responses at light levels which extended beyond the linear range. At light levels greater than about 0.3 Rh\*\*/sec, when the step response exceeded about 5 mV, the step response data points fell below the curve.

*Comparison of bipolar with horizontal cell responses*

*Differences in flash sensitivity.* Characteristic features of horizontal cell recordings were the prolonged and large responses of up to 90 mV following bright flashes (Table 2). The potential of horizontal cells in the dark ranged from -45 mV to about -85 mV in retinas which were believed to be in good physiological condition (as judged from the e.r.g. and the high sensitivity of the bipolar cells). Deterioration of the retina was indicated not only by a *b*-wave of diminished amplitude in response to test flashes, but also a high internal negativity for horizontal cells in the dark, ranging to -120 mV in some cells and similar to the internal potential of the cell at the peak of the response to bright flashes.

The horizontal cell responses had a linear range from which the flash sensitivity,  $S_F$ , was determined analogously to the bipolar cells. The results from eleven cells are given in Table 2. There was much less variation in the determined flash sensitivity, which was in the range 4.5-11 mV/Rh\*\* with a mean value of 7.6 mV/Rh\*\*.

Fig. 7 shows the comparison between the response of a bipolar cell and an adjacent horizontal cell into which the electrode jumped spontaneously following the recording of the intensity-response series for the bipolar cell. Since these recordings were from regions of the retina which were probably separated by less than 20  $\mu$ m and in the same physiological state, the flash sensitivities can be compared directly. The flash sensitivity for the horizontal cell was 4.5 mv/Rh\*\*, that of the bipolar cell was 360 mV/Rh\*\*. This large difference in sensitivity is evident in the figure. It can be seen that, for small responses, about 80 times as much light was required to elicit a response in the horizontal cell of the same amplitude as the bipolar cell response. Cell 2 of Table 2 is also a member of a pair with a flash sensitivity of 8 mV/Rh\*\*. The adjacent bipolar cell had a flash sensitivity of 192 mV/Rh\*\*.

*Comparison of time course of responses.* Compared with horizontal cell flash responses, there was a longer delay for the bipolar cell to reach a criterion response amplitude. The longer delay in the bipolar cell response is readily apparent in Fig. 7A and C where the rising phase of the responses of the two cells are shown at fast sweep speed. The time course of response of the horizontal-bipolar cell pair in their linear range is illustrated in Fig. 8. The responses have been superimposed and normalized to the same peak amplitude. It is evident that, although the bipolar cell response began with a longer delay, following the delay, it had a more rapid time course than the horizontal cell response with a higher maximal rate of rise and a more rapid return of potential to the dark level. The curve b fitted to the bipolar cell response represents the function in eqn. (2) for a 16-stage low-pass filter with time constant  $\tau$  for each stage of 29.3 msec. The curve h fitted to the horizontal cell responses is based on an equation of the form

$$V = c(e^{-\alpha_1 t} - e^{-\alpha_2 t})^5, \quad (12)$$

where  $c$  is a constant proportional to light intensity and  $\alpha_1 = 0.41 \text{ sec}^{-1}$  and

$\alpha_2 = 8.26 \text{ sec}^{-1}$ . Equation (12) is the impulse response of a low-pass filter with six stages of delay (the stages having unequal time constants) and is similar in form to that used by Schwartz (1976) and Detwiler *et al.* (1978) to fit rod responses in the turtle retina.

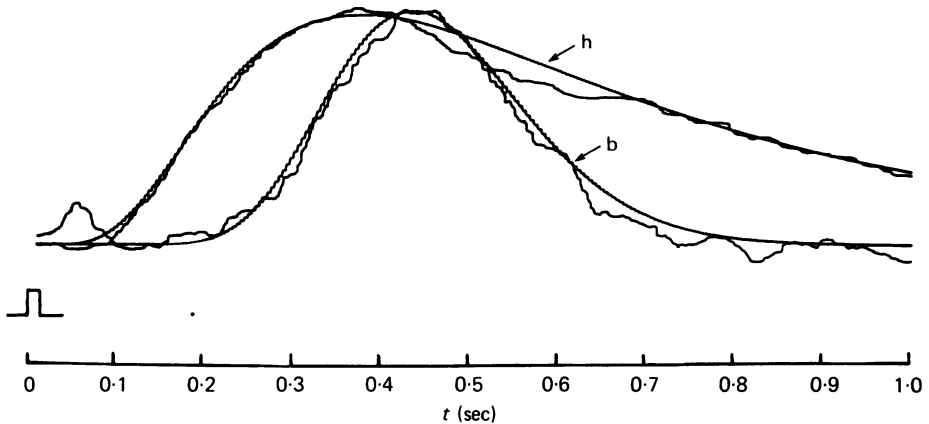


Fig. 8. Time course of the small signal flash responses of the bipolar cell and neighbouring horizontal cell shown in Fig. 7. The responses have been superimposed and normalized to the same peak amplitude. The responses were filtered through a low-pass filter (10 Hz cut-off frequency). The curve b fitted to the bipolar cell response was generated from eqn. (2) with  $n = 16$  and  $T = 439$  msec. The curve h fitted to the horizontal cell response was generated from eqn. (12) with  $\alpha_1 = 0.41 \text{ sec}^{-1}$  and  $\alpha_2 = 8.26 \text{ sec}^{-1}$ .

#### *The rod-bipolar cell synaptic transfer function*

The transfer function for the rod-bipolar cell synapse can be determined if we assume the form of the rod response at the synaptic terminals. For a linear system the bipolar cell response,  $V_b(t)$ , can be related to the rod response,  $V_r(t)$ , by the convolution

$$V_b(t) = \int_0^t g(t-t') V_r(t') dt', \quad (13)$$

where  $g(t)$  is the impulse response of the synaptic filter. Taking the Laplace transform of eqn. (13), one obtains

$$\tilde{V}_b(s) = \tilde{g}(s) \tilde{V}_r(s), \quad (14)$$

where the tilde signifies Laplace transformation and  $g(s)$  is the synaptic transfer function completely specifying the input-output relations of the synapse. The functional form of  $g(s)$  can be derived by using the fit to the flash response of rods and bipolar cells. The bipolar cell response is well fitted by eqn. (2) with  $n = 16$ . Although eqn. (12) may be a better model for the rod response, the use of eqn. (2) for the rod input (with  $n = 6$ ) leads to a particularly simple transfer function whose properties are qualitatively similar to those implied by the use of eqn. (12).

The synaptic transfer function in the frequency domain will be given by the Fourier transform  $T(f)$  of the impulse response. It can be shown that

$$T(f) = g(i2\pi f) = -\frac{S_{sb} (1 + i2\pi f\tau_r)^6}{S_{sr} (1 + i2\pi f\tau_b)^{16}}, \quad (15)$$

where  $i = \sqrt{-1}$ , subscripts refer to rod and bipolar cells and the negative sign inversion of polarity at the synapse. The synaptic gain is given by the modulus of eqn. (15). The rod-bipolar cell transfer function has some of the characteristics of a lead-lag filter. As can be seen from eqn. (15), the DC gain at the synapse is given by the ratio of the light step sensitivities of bipolar cells and rods (seen at the synaptic terminals). Synaptic gain increases with frequency over the passband so that certain of the higher frequency components of rod transient signals will be enhanced in passing through the synaptic filter (Fig. 3 of Ashmore & Falk, 1979).

The power spectral density of voltage noise in bipolar cells due to noise at the rod terminals, whether of origin more distally in the rod or due to fluctuations in transmitter release (Falk & Fatt, 1972, 1974a), would be given by the modulus squared of the transfer function if that noise source had wider bandwidth than rod signals. A peak in the noise spectrum of bipolar cells at 3–5 Hz, as well as the steep roll-off at frequencies greater than about 5 Hz predicted from eqn. (15), have been observed in darkness and during dim steady illumination (J. F. Ashmore & G. Falk, in preparation; see also Fig. 2 of Ashmore & Falk (1977)).

#### *Receptive field organization*

In most previous studies, retinal bipolar cell responses have been characterized by a centre-antagonistic surround organization (Werblin & Dowling, 1969; Kaneko, 1973; Toyoda, 1973; Matsumoto & Naka, 1972; Schwartz, 1974). In the present study concerned with the dark-adapted state, such an organization for depolarizing bipolar cells was difficult to demonstrate. Fig. 9 compares the flash responses to a 200  $\mu\text{m}$  diameter spot and to diffuse illumination when the intensity of illumination was adjusted by neutral density filters so as to match the peak amplitudes of response to the two patterns of illumination. The results are shown for two cells of differing flash sensitivities. Fig. 9A illustrates the responses of a cell with a flash sensitivity of 550 mV/Rh\*\* (half-saturation at 1/7 Rh\*\*) and shows that diffuse and spot illumination could be matched over the response range of the cell at least up to 90% of saturation with no demonstrable effect of the surround. The intensity-response curves could be superimposed with a relative shift of 0.75 log units. On the assumption of a linear model for summation over area and a sharply defined, circular receptive field, this shift would imply a receptive field size for this cell of  $200 \times 10^{0.375} = 470 \mu\text{m}$  in diameter.

The cell shown in Fig. 9B had a lower flash sensitivity, 70 mV/Rh\*\* (half-saturation at 1 Rh\*\*). In this cell, enlarging the area of the stimulus increased the peak response amplitude for all but the highest intensities. The responses to the spot of light and to diffuse illumination could be matched by a relative shift of 0.5 log units to give an apparent receptive field size corresponding to  $200 \times 10^{0.25} = 356 \mu\text{m}$ . There did, however, appear to be some interaction with the surround evident at the higher light intensities such that the response to diffuse illumination was faster than the response to a spot, reaching a peak earlier and falling more rapidly.

It has been supposed that the antagonistic surround of bipolar cells is mediated via the horizontal cells (Werblin & Dowling, 1969; Naka & Nye, 1971; Naka & Witkovsky, 1972). The difficulty in demonstrating surround antagonism of depolarizing bipolar cells in the dark-adapted retina may arise simply as a consequence of the very much higher sensitivity of bipolar cells compared with horizontal cells. Consistent with this idea is the observation that annular illumination not only

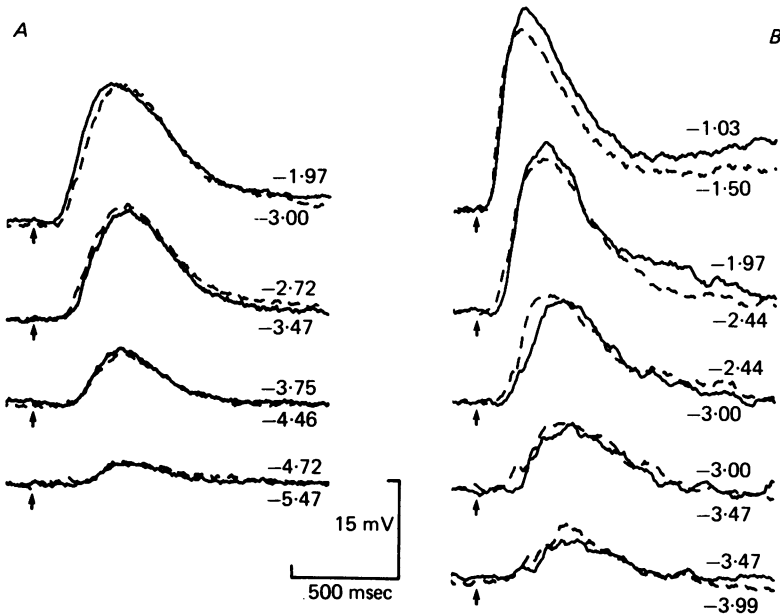


Fig. 9. Flash responses of depolarizing bipolar cells to a centred 200  $\mu\text{m}$  spot (continuous trace) and to large field illumination (dashed trace). The numbers above and below each pair of records represent the relative intensity of the light in log units for the 200  $\mu\text{m}$  spot and diffuse illumination, respectively. Light intensity of log  $I = 0$  was equivalent to 1275  $\text{Rh}^{**}/\text{flash}$ . A 15 msec flash was delivered at the time indicated by an arrow. *A*, cell with a flash sensitivity of 550  $\text{mV}/\text{Rh}^{**}$ . Internal potential in the dark  $-44$  mV; maximum depolarization produced by light 25 mV. Temperature 14  $^{\circ}\text{C}$ . *B*, cell with a flash sensitivity of 70  $\text{mV}/\text{Rh}^{**}$ . Internal potential in the dark  $-40$  mV; maximum depolarization with light 31 mV. Temperature 15  $^{\circ}\text{C}$ .

failed to suppress the depolarizing responses of sensitive bipolar cells to a centred spot of light, but added to the depolarization as a result of scattering into the central field. The relative decrease in the effect of a surround on cat ganglion cell responses which occurs with dark adaptation (Barlow, Fitzhugh & Kuffler, 1957; Enroth-Cugell & Pinto, 1972) may be explained likewise by the differentially higher gain of the pathway generating the centre response.

The determination of the size of the receptive field centre by the method described, giving a value of 300–500  $\mu\text{m}$  in diameter is likely to be an over-estimate as a result of light scattering and imperfections of centring and focusing of the spot. In the following paper (Ashmore & Falk, 1980), a mean receptive field size, 160  $\mu\text{m}$  in diameter, was estimated by an entirely different method, less dependent on optical quality.

*The effects of background light*

To test the effects of background light on bipolar cells, brief test flashes, 15 msec in duration, were applied 940 msec after the start of a light pulse lasting 2 sec, i.e. at a time which is about 3 times the integration time of the bipolar cell. The results of one such experiment are shown in Fig. 10. Fig. 10A illustrates the responses to test flashes of different intensities ranging from 0.04 to 4.1 Rh\*\*/flash superimposed

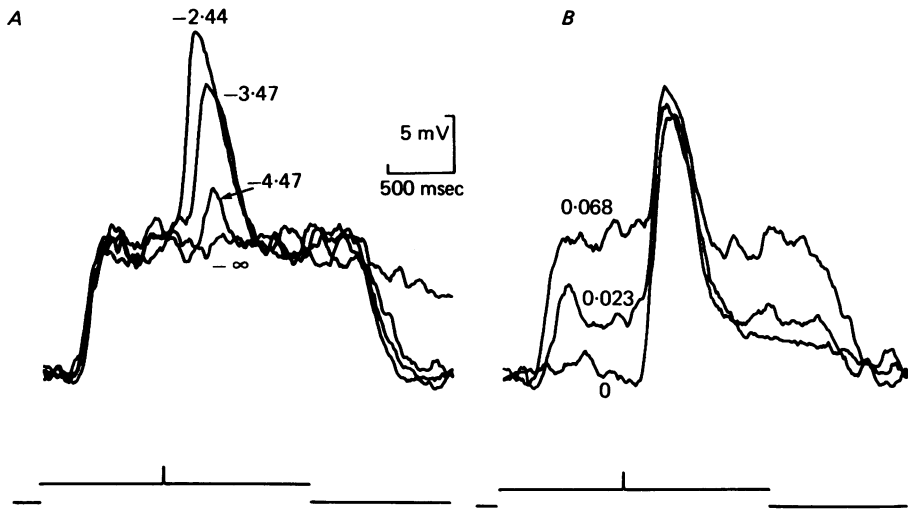


Fig. 10. The effect of background light on the responses of a bipolar cell to 15 msec test flashes of diffuse light. In *A*, a background of fixed intensity (0.068 Rh\*\*/sec) was applied for 2 sec and the response to a test flash of varying intensity recorded. The timing of the light stimuli is monitored on the bottom record. The unattenuated flash intensity corresponded to 1220 Rh\*\*/flash and the numbers by each response indicate the negative log attenuation of the light channel which provided the test flashes. *B*, the responses of the same cell to a test flash of fixed intensity as the background was varied. The intensity of the test flash was equivalent to 0.405 Rh\*\*/flash which, in the absence of a background, produced a response of peak amplitude 75% of the maximum depolarization,  $V_{max}$ . The numbers by each record indicate the intensity of the background as Rh\*\*/sec. Cell 5 of Table 1. Flash sensitivity 1860 mV/Rh\*\* and step sensitivity 645 mV.sec/Rh\*\*. Temperature 17.5 °C.

on a background of 0.068 Rh\*\*/sec, which produced a mean depolarization of 11 mV, nearly 40% of the maximum depolarization which could be produced by light. The peak depolarization (measured from the dark level) in response to a bright test flash was not reduced by the background. The effects of different backgrounds on the response to a test flash which, in the absence of a background, resulted in a peak depolarization which was about 75% of the maximum, is illustrated in Fig. 10B. It can be seen that the peak depolarization, when measured from the dark level, increased with background intensity and that the time course of response to the test flash was not significantly altered by the background.

In the linear range of the bipolar cell response, the effect of a weak background light and the response to a flash should summate, giving a net peak depolarization

from the dark,

$$V = S_F I + S_B I_B = S_F (I + t_1 I_B), \quad (16)$$

where  $I_B$  is the background light intensity (Rh\*\*/sec),  $I$  is the intensity of the test flash (Rh\*\*/flash), and  $S_F$  and  $t_1$  are defined through eqns. (1) and (9). Moreover, the data from the cell shown in Fig. 10 can be fitted beyond the linear range by replacing the light intensity  $I$  for a flash in eqn. (4) by  $I + I_B t_1$ . The results of such a fit are shown in Fig. 11. The integration time was determined by the measurement of step and flash sensitivities, and the curves were obtained from

$$V = V_{\max} (I + t_1 I_B) / [I + t_1 I_B + \sigma \exp(\beta V / V_{\max})]. \quad (17)$$

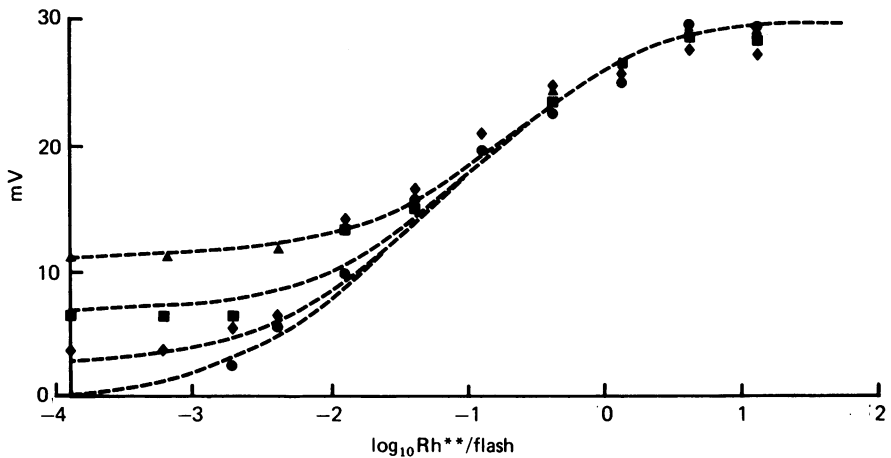


Fig. 11. Effect of background light on the potential changes produced by test flashes. The peak amplitude of response is measured as the displacement from the dark level. The data were obtained from the cell illustrated in Fig. 10. Data points correspond to backgrounds of: (●) no background, (◆)  $5.14 \times 10^{-3}$  Rh\*\*/sec, (■)  $2.30 \times 10^{-2}$  Rh\*\*/sec, (▲)  $6.8 \times 10^{-2}$  Rh\*\*/sec. Abscissa,  $\log_{10}$  Rh\*\* delivered as a flash while the background light was on. The dashed lines are drawn according to eqn. (17) with  $V_{\max} = 29$  mV,  $\beta = 3$ ,  $\sigma = 1.58 \times 10^{-2}$  Rh\*\*/flash,  $t_1 = 0.346$  sec.

This equation is formally similar to that used by Naka & Rushton (1966), but modified to include the integration time over which summation of backgrounds takes place and the empirical parameter  $\beta$  which describes the deviation of the intensity-response relation from a rectangular hyperbola. The fit to the data implies that the effects of weak backgrounds and test flashes add and that the only form of desensitization which occurs is due to non-linear summation evident in the intensity-response relation for flashes in the absence of backgrounds. If there were some additional form of desensitization, as occurs in rods, the curves in the presence of backgrounds would cross the curve for flash responses in the absence of background (see e.g. Kleinschmidt & Dowling, 1975).

The potential change produced in the rods by the weak backgrounds used in the experiment illustrated in Figs. 10 and 11 may be estimated if the rod flash sensitivity

is assumed to be 2 mV/Rh\*\* and the rod integration time is taken to be 1 sec. In this case, background intensities of 0.0051 Rh\*\*/sec and 0.068 Rh\*\*/sec would produce mean hyperpolarizations of 0.01 and 0.14 mV, respectively. Any desensitization in the rods produced by these backgrounds would be negligible (Baylor &

TABLE 3. Electrical properties of bipolar cells

Cell	$E_D$ (mV)	$V_{max}$ (mV)	$\tau_D$ (msec)	$R_D$ (M $\Omega$ )	$R_L$ (M $\Omega$ )	$E_r$ (mV)	$G_r/G_0$	$R_{mL}$ ( $\Omega$ cm <sup>2</sup> )
1	-37	15	2.8	10.3	4.3	-10	5.9	1170
2	-50	24	3.0	19.2	—	—	—	—
3	-60	14	1.7	16.0	—	—	—	—
4*	-27	15	2.0	28.8	(6.6)	-6.7	16.4	460
5*	-58	21	2.9	28.9	(19.2)	+6.0	1.3	1900
6*	-37	25	2.0	20.3	(8.1)	+4.1	4.9	790
7	-40	24	2.6	44.4	14.1	-6.0	7.0	830
8	-58	23	4.1	39.1	13.3	-23.0	4.6	1390
9	-48	14	2.5	15.7	(7.4)	-21.0	4.3	1180
Mean	-46	19	2.6	24.8	10.4	-8.0	6.3	1100
S.E.	$\pm$ 3.8	$\pm$ 1.6	$\pm$ 0.24	$\pm$ 3.8	$\pm$ 2.0	$\pm$ 4.2	$\pm$ 1.8	$\pm$ 177

\* Indicates that input resistance was determined by means of a ramp of current pulses applied during steps of light.

The values of  $R_L$  in parentheses were obtained by extrapolation on the basis of eqn. (22). The measurements of input resistance used for the extrapolation were made when the light-induced potential change was approximately one-half the peak value,  $V_{max}$ , attained after a bright flash. The flash sensitivities of the cells ranged between 35 and 110 mV/Rh\*\*.

Hodgkin, 1974) compared with the desensitization of the bipolar cell, whose flash sensitivity was decreased approximately tenfold by the background of 0.068 Rh\*\*/sec (or 1 rhodopsin molecule bleached/(15 rod. sec)). We conclude that, because of the high gain at the rod-bipolar cell synapse, weak backgrounds which produce insignificant changes in rod sensitivity drive the bipolar cell potential to levels at which responses no longer sum linearly.

Eqn. (17) cannot be expected to hold over all background intensity levels. An indication of this may be seen in Fig. 4 where steady light intensities in excess of about 0.5 Rh\*\*/sec gave rise to an initial transient peak. Fig. 6 shows that the intensity-response curve for steps does not superimpose well on the intensity-response curve for flashes at higher intensities, whereas eqn. (17) predicts that the curves should be identical (when shifted along the log  $I$  axis). We have not made any quantitative measurements at high background levels indicating the extent to which rods might contribute to the desensitization, although rod desensitization may enter at background levels in excess of 0.5 Rh\*\*/sec where the sag in the bipolar cell potential to a plateau becomes evident.

#### *Electrical properties of bipolar cells*

*Resistance in darkness.* The input resistance in darkness,  $R_D$ , measured in nine cells, varied from 10 to 44 M $\Omega$  with a mean value of 25 M $\Omega$  (Table 3). Time constants in darkness,  $\tau_D$ , determined from the nearly exponential rise of the voltage response

to the applied current, were in the range 1.7–4.1 msec, with a mean value of 2.6 msec. If one assumes a membrane capacitance  $C_m$  of  $1 \mu\text{F}/\text{cm}^2$ , the observed time constants would be compatible with a membrane resistance  $R_m$  of 2000–4000  $\Omega \text{ cm}^2$ .

The input resistance of a spherical cell is

$$R = R_m/(\pi d^2), \quad (18)$$

where  $d$  is the cell diameter. A cell  $25 \mu\text{m}$  in diameter with a membrane resistance of  $3000 \Omega \text{ cm}^2$  would have an input resistance of  $153 \text{ M}\Omega$ , which is 6 times the mean input resistance in the dark. It is likely that loading by the dendritic tree of the cell lowers the input resistance substantially, since the Golgi studies of Witkovsky & Stell (1973*a*) indicate that the surface area of the dendrites greatly exceeds that of the cell body. The contribution of the dendrites to the measured electrical properties can be estimated in the following way. If tapering and branching of the dendrites are ignored, as a first approximation, the dendrite can be represented as a cable with open-circuit termination at the distance  $l$  from the soma, since the end of the dendrite is sealed by a high resistance membrane. The input resistance of a dendrite, measured by current injection into the soma, will be

$$R = \left[ \frac{\pi}{2} \frac{d^{\frac{3}{2}}}{\sqrt{R_m R_1}} \tanh \frac{l}{\lambda} \right]^{-1}, \quad (19)$$

where  $R_m$  and  $l$  are defined above,  $d$  is the diameter of the dendrite and  $R_1$  is the resistivity of the dendrite axoplasm, and the space constant is defined by

$$\lambda = \frac{1}{2} \sqrt{(d R_m / R_1)}. \quad (20)$$

If we take  $d = 2 \mu\text{m}$ ,  $R_m = 3000 \Omega \text{ cm}^2$ ,  $R_1 = 100 \Omega \text{ cm}$ ,  $l = 100 \mu\text{m}$ , then the input resistance of a dendrite would be  $490 \text{ M}\Omega$ ; if there were twelve to fifteen such dendrites, the input resistance of the cell would be about  $30 \text{ M}\Omega$ . It would thus appear that the input resistance measured in the dark represents largely the properties of the dendrites.

Moreover, with a specific membrane resistance of  $3000 \Omega \text{ cm}^2$ , the space constant would be 3.9 times the assumed length of a dendrite so that the resistive properties of the dendrites can be replaced by an equivalent lumped circuit, thereby neglecting their cable properties. The error which is introduced by treating the dendrites as isopotential can be obtained from the ratio of  $l/\lambda$  to  $\tanh(l/\lambda)$  (as may be seen by replacing  $\tanh(l/\lambda)$  of eqn. (19) by  $l/\lambda$ ). For values of  $R_m$  of 2000–4000  $\Omega \text{ cm}^2$ , the input resistance of a uniformly polarized cylinder is within 4% of that for a cable of the same dimensions.

*Membrane conductance increase produced by light.* Fig. 12 shows the voltage displacement produced by current pulses in darkness (*a* and *d*) and during the response of a bipolar cell to a saturating brief flash of light (delivered at the arrow). Following the phasic peak response to light of  $23 \text{ mV}$ , there was a prolonged depolarization lasting approximately 10 sec before the cell repolarized to its dark potential of  $-58 \text{ mV}$ . At the peak of the light response (at *b*) there was a pronounced decrease in resistance as also occurred during the plateau (at *c*). When the cell repolarized, the input resistance returned to the dark level of  $39 \text{ M}\Omega$ , determined from the slope of the current–voltage relation (Fig. 13*A*).



In this cell, there was a delayed sag in the voltage displacement produced by current pulses during the response to light, which may be indicative of delayed rectification. However, tested with short current pulses of 20–40 msec duration producing voltage displacements of up to  $\pm 5$  mV from the potential in light, all the other cells studied had electrical characteristics which appeared linear (e.g. Fig. 13*B*). Toyoda, Fujimoto & Saito (1977) were able to demonstrate a modest degree of rectification in a depolarizing bipolar cell of the carp, the input conductance increasing by a factor of about 1.4 as the internal potential was displaced from  $-100$  to  $+60$  mV by applied current.

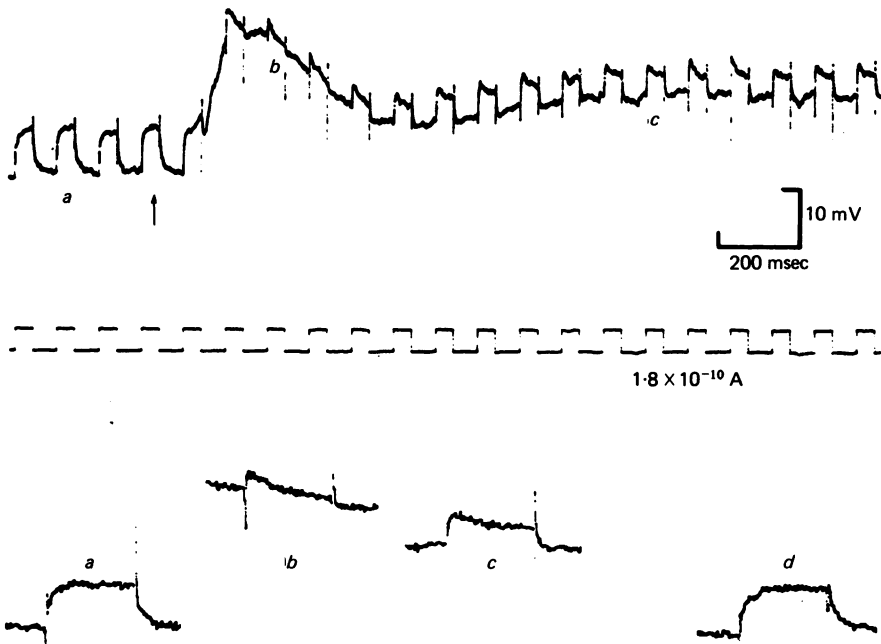


Fig. 12. Resistance changes in a depolarizing bipolar cell during the response to light. The top record shows the response to a saturating (large field) flash of light of intensity  $227 \text{ Rh}^{**}/\text{flash}$ . Superimposed upon the light response are the voltage displacements produced by depolarizing current pulses of  $1.8 \times 10^{-10}$  A (middle record) applied via a bridge circuit used to balance out the resistance in series with the cell. Following the peak of the response (near *b*) the potential of the cell remained at a more positive value than the level in the dark for about 10 sec. The lowest set of records *a*–*c* were taken at the times shown in the top record but are displayed at 5 times the sweep speed; *d* was obtained 15 sec after the flash when the potential had returned to its dark level. Cell 8 of Table 3. Flash sensitivity  $102 \text{ mV}/\text{Rh}^{**}$ . Temperature  $16^\circ \text{C}$ .

Fig. 13*A* shows a plot of the current–voltage relation in darkness and during the peak of the response to flashes of light for the cell illustrated in Fig. 12. In order to obviate the influence of membrane nonlinearity, in this cell, voltage displacements were measured 4 msec after the onset of the current pulse before there was any noticeable sag in the voltage displacement. Measured in this way, the resistance,  $R$ , fell from  $39.1$  to  $28.9 \text{ M}\Omega$  during a flash which produced a peak depolarization of  $9.1$  mV and to  $13.3 \text{ M}\Omega$  during the peak of a saturating flash depolarizing the cell

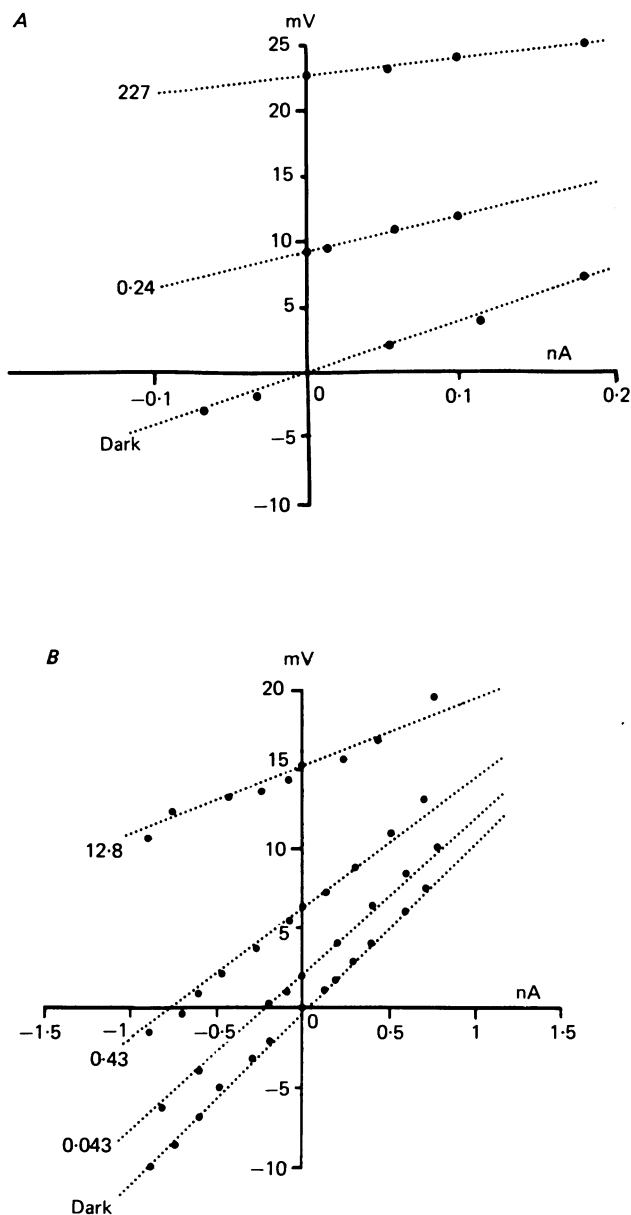


Fig. 13. Current-voltage relations in darkness and during the response to a light flash. Current pulses were timed so that a pulse occurred at the peak of the flash response. The ordinate is the voltage displacement from the dark level. Numbers to the left of each line indicate the light intensity as  $Rh^{**}/\text{flash}$ . *A*, the cell illustrated in Fig. 12 (cell 8). Potential in the dark  $-58$  mV. The lines intersect at a point  $35$  mV positive with respect to the potential in darkness to give  $E_r$  of  $-23$  mV. *B*, cell 1 of Table 3. Potential in the dark  $-37$  mV;  $E_r$   $-10.1$  mV. Flash sensitivity  $63$  mV/ $Rh^{**}$ . Temperature  $17^\circ\text{C}$ .

by 23 mV. The cell, whose current-voltage relations in light and in darkness are illustrated in Fig. 13*B*, had an input resistance of 10.3 M $\Omega$  in darkness and a resistance of 4.3 M $\Omega$  at the peak of the response to a flash which gave a saturated response. The resistance,  $R_L$ , during the maximal light-evoked depolarization is given in Table 3. The ratio of the resistance in darkness  $R_D$  to the resistance in bright light  $R_L$  at the peak of the response, measured directly or obtained by extrapolation, averaged 2.71.

It was evident that the time constant of the membrane shortened during the response to light but accurate measurements could not be made. Estimates of the specific membrane resistance of the cell in bright light,  $R_{mL}$ , based on the assumption that the time constant shortened in proportion to the decrease in input resistance, ranged from 460 to 1900  $\Omega$  cm<sup>2</sup> with a mean value of 1100  $\Omega$  cm<sup>2</sup>.

*Equivalent circuit of the bipolar cell.* A simple equivalent circuit model of the bipolar cell is illustrated in Fig. 14. The conductance of sites controlled by transmitter released from rod terminals is represented by the variable conductance  $g_r$  which ranges between zero and its maximum value  $G_r$ .  $E_r$  is the voltage source (or reversal potential) for this conductance path.  $E_0$  and  $G_0$  represent the voltage source and conductance of non-synaptic sites and  $C$  is the capacitance of the cell membrane. The circuit in Fig. 14 will apply irrespective of whether the transmitter released by the presynaptic cell opens or closes ionic channels.

The internal potential,  $E$ , of the bipolar cell is given by

$$E = E_r + \frac{(E_0 - E_r)}{1 + g_r/G_0} \quad (21)$$

$E_r$  may be evaluated from the current-voltage curves obtained in darkness and at different light intensities (Rushton, 1959) such as those illustrated in Fig. 13. When the voltage-current curves are linear, they will intersect at a common point at which the potential equals  $E_r - E_D$ , if the voltage is measured as the displacement from the potential in the dark,  $E_D$ . Estimates of  $E_r$  in different cells (Table 3) ranged from -23 to +6 mV with a mean of -8.0 mV.

Eqn. (21) indicates that the resistance change with light should be proportional to the voltage change, since eqn. (21) may be rewritten as

$$E - E_D = (E_r - E_0)(R_D - R)/R_0 = \frac{(E_r - E_0)}{R_0} \cdot \Delta R, \quad (22)$$

where  $E_D$  and  $R_D$  are, respectively, the potential and input resistance in the dark and  $R_0 = 1/G_0$  is the input resistance of the cell when all the transmitter modulated channels are closed. The results for three cells are plotted in Fig. 15. In some other cells a proportional relationship between resistance change and response amplitude had to be assumed in order to extrapolate to the value of the input resistance at the maximum of the response in bright light, when the resistance change and response were known for other light intensities. This was necessary in those cells (cells 4-6) in which resistance was measured by means of a ramp of current pulses during steps of light (as described in Methods), since it was not possible to complete the measurement during the brief phasic peak of the response to bright light.

The ratio  $G_r/G_0$  could be computed from the data using the equivalent circuit model and eqn. (22), if it were assumed that at the peak of the light response the

subsynaptic conductance reached its maximum value  $G_r$ . Estimates for  $G_r/G_0$ , when  $E_0$  was taken equal to  $-90$  mV, are given in Table 3. The mean value was 6.3. A lower bound would be 2.4, the mean value if  $E_0$  were taken as the potential in the dark.

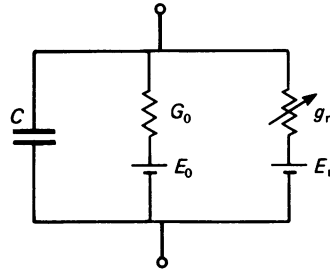


Fig. 14. Equivalent circuit model for a bipolar cell. See text.

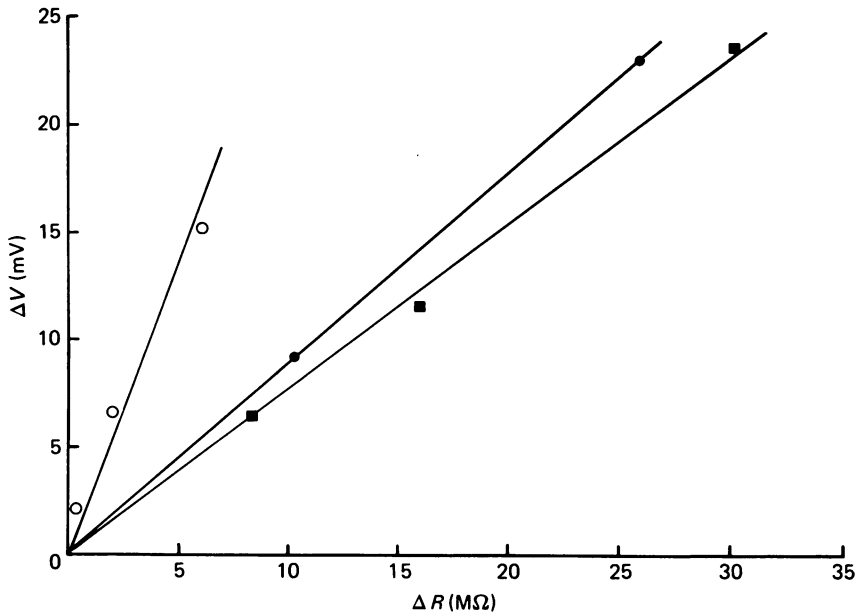


Fig. 15. The proportional relationship between depolarization ( $\Delta V$ ) and resistance decrease ( $\Delta R$ ) with light. The results for three bipolar cells are shown. In order of decreasing slope, they are cells 1, 8 and 7 whose properties are summarized in Table 3.

### Hyperpolarizing bipolar cells

Less than 5% of the cells studied and thought to be bipolar cells gave hyperpolarizing responses to spots of light in the centre of their receptive field, as well as to diffuse illumination. Large field illumination resulted in responses which were oscillatory, the oscillations becoming particularly prominent for a range of higher light intensities.

One such cell is shown in Fig. 16. The responses from the cell stained with Procion yellow and illustrated in Pl. 3 were very similar. Flashes presented as a  $210\ \mu\text{m}$  spot of light produced a graded hyperpolarization, which at high light intensities peaked earlier, followed by a more gradual return to the dark level. Flashes presented as a spot  $500\ \mu\text{m}$  or larger resulted in a more complex waveform with numerous

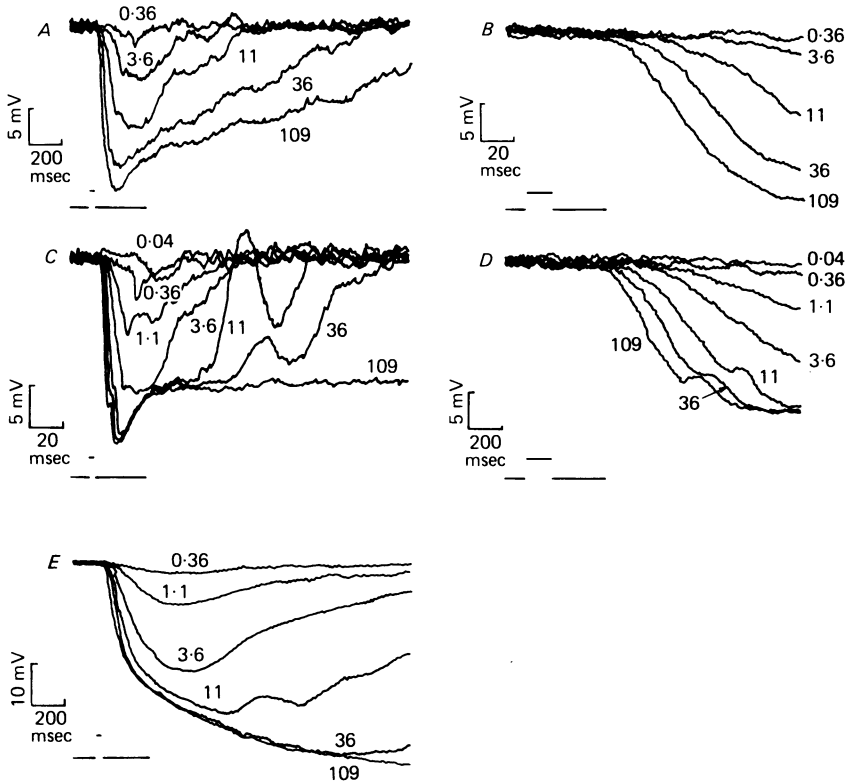


Fig. 16. Responses of a hyperpolarizing bipolar cell and adjacent horizontal cell to flashes of light. *A* and *B*, (fast sweep), responses of a hyperpolarizing bipolar cell to a spot  $210\ \mu\text{m}$  in diameter. *C* and *D*, (fast sweep), responses of the same cell to flashes of diffuse light, showing depolarizing notches on the initial hyperpolarizing phase. The numbers by each of the records indicates the light intensity in  $\text{Rh}^{**}/\text{flash}$ . Internal potential in the dark,  $-52\ \text{mV}$ . Maximum hyperpolarization  $23\ \text{mV}$ . Flash sensitivity  $41.8\ \text{mV}/\text{Rh}^{**}$ . *E*, responses of horizontal cell penetrated shortly afterwards. Diffuse light stimulus; cell 5, Table 2. Flash sensitivity  $8.8\ \text{mV}/\text{Rh}^{**}$ .

depolarizing inflexions superimposed on the hyperpolarization and the appearance of notches on the rising phase of the response to higher light intensities. There were prominent damped oscillations at approximately  $4\ \text{Hz}$  occurring during the repolarization phase, which disappeared at flash intensities greater than about  $50\ \text{Rh}^{**}/\text{flash}$  when the plateau phase of the response was prolonged and lasted more than a second. The maximum hyperpolarization observed in these cells was  $27\ \text{mV}$  (cell of Pl. 3).

Not shown in Fig. 16 were the responses to a flash of light presented as an annulus. In that case the response also showed oscillatory components but which were even less well damped than when the centre was also illuminated. The results indicate that the oscillatory part of the response is contributed by a surround mechanism. Similar features have been described in hyperpolarizing cone bipolar cells in the turtle retina (Richter & Simon, 1975; Marchiafava & Torre, 1978). However, in the cells of the dogfish retina, no purely depolarizing response to surround illumination was observed. The size of the central receptive field was estimated to be  $300\ \mu\text{m}$  in diameter.

Fig. 16*E* shows the response from a horizontal cell which was penetrated immediately after recording from the cell illustrated in Fig. 16*A-D*. There were no prominent oscillations in the horizontal cell response to large field illumination, but a small oscillation appears at the same light intensity which produced the large oscillatory response in the bipolar cell (Fig. 16*C*). The oscillation in the horizontal cell lags behind that in the bipolar cell. It thus seems unlikely that the oscillatory response in the hyperpolarizing bipolar cell originates from the surround mediated by horizontal cells. An alternative possibility is that the surround effects in hyperpolarizing bipolar cells are contributed by wide-field amacrine cells making reciprocal or other synapses with bipolar cells (Dowling & Boycott, 1966; Witkovsky & Stell, 1973*b*) or by interplexiform cells (Hedden & Dowling, 1978.)

In the small sample studied, the flash sensitivity of the bipolar cells was significantly lower than for the depolarizing bipolar cells. The cell illustrated in Pl. 3 had a  $S_F = 28\ \text{mV/Rh}^{**}$  and the cell in Fig. 16 had the highest flash sensitivity with  $S_F = 42\ \text{mV/Rh}^{**}$ . In the linear response range of these cells, the delay in the response was shorter than for the depolarizing bipolar cells, as has been described in other preparations (Nelson, 1973; Marchiafava & Torre, 1978).

#### DISCUSSION

Bipolar cells form the signal pathway from the rods to the ganglion cells, so that bipolar cell characteristics will determine many of the properties of ganglion cell responses. Because of the higher flash sensitivity of the depolarizing compared with the hyperpolarizing bipolar cell, it would be tempting to hypothesize that the depolarizing bipolar cells form the principal pathway for transmission of dim light signals. A serious objection to such a generalization derives from the recent observations of Nelson, Kolb, Famiglietti & Gouras (1976) on the rod bipolar cells of the cat retina. In a small sample of cells they reported that the rod bipolar cells, whose identity was confirmed by Procion dye staining, were of the hyperpolarizing type. However, they described only responses to light pulses delivering more than 1000 photons per  $\mu\text{m}^2$  per sec.

#### *Synaptic voltage gain*

The results indicate that there was a large signal gain at the synapse between the rods and the depolarizing bipolar cells. Assuming a mean value for the flash sensitivity of dogfish rods of  $2\ \text{mV/Rh}^{**}$  the synaptic gain, given by the ratio of bipolar to rod flash sensitivities, would have been greater than 200. Individual rod flash

sensitivities in turtle have been reported as high as 5 mV/Rh\*\* (Detwiler *et al.* 1978). However, the gain at the synapse made by the rods with the horizontal cells was very much lower, by more than an order of magnitude.

It is worth noting that synaptic gain determined from the ratio of flash sensitivities is independent of the number of rods converging onto the bipolar cell: increasing the area of the bipolar cell dendritic tree will reduce the single-photon signal in proportion, but will also increase the total number of photons absorbed by the same fraction.

Falk & Fatt (1972, 1974*a, b*) have argued that the maximum gain,  $A_{\max}$ , that can be obtained at a synapse where ionic channels are opened by transmitter action is given by

$$A_{\max} = \frac{b}{4}(E_r - E_0), \quad (23)$$

where  $E_r$  and  $E_0$  are the voltage sources in the equivalent circuit of the post-synaptic cell in Fig. 14. The parameter  $b$  is a constant, the reciprocal of which is the potential change at the presynaptic terminals required to produce an  $e$ -fold change in transmitter release, which derives from the empirical finding that, at a number of synapses, transmitter release increases exponentially with presynaptic membrane depolarization (Liley, 1956; Kusano, Livengood & Werman, 1965; Katz & Miledi, 1967; Martin & Ringham, 1975).

Synaptic gain would be higher if there were a steeper dependence of transmitter release on membrane potential of photoreceptors than is found at conventional synapses. We can examine this proposition by estimating  $b$  in eqn. (23) for the rod-horizontal cell synapse. It is likely that at this synapse the transmitter increases the conductance of ionic channels with a reversal potential for this path  $E_r = 0$ , as shown by the work of Trifonov, Byzov & Chailahian (1974) for other horizontal cells. We take the small-signal synaptic gain  $A_{\max} = 11.1/2 = 5.6$  and  $E_0 = -120$  mV, the internal potential observed in dogfish horizontal cells in bright light. This leads to an estimate for  $b$  of 0.17 mV<sup>-1</sup> or a 6 mV change required to produce an  $e$ -fold change in transmitter release which is comparable to values of 5–10 mV for an  $e$ -fold change, reported at a number of conventional synapses (Liley, 1956; Katz & Miledi, 1967; Martin & Ringham, 1975).

The resistance changes produced by light in depolarizing bipolar cells are consistent with a continual release in darkness by the rods of a transmitter which *closes* ionic channels forming a conductance path across the bipolar cell membrane with a reversal potential of about  $-8$  mV. Similar conclusions have been reached by Kaneko (1971*b*) and Toyoda (1973), although recent observations on depolarizing bipolar cells (contacted by rods and cones) in the carp suggest that the transmitter released by cones acts by *opening* ionic channels in a path with a reversal potential more *negative* than the dark potential (Saito, Kondo & Toyoda, 1978).

Falk & Fatt (1974*a, b*) have suggested that a higher synaptic gain is possible at a synapse at which the transmitter acts by *closing* ionic channels, than at one at which transmitter opens ionic channels, provided that the conductance changes are proportional to transmitter release even when a large fraction of available post-synaptic sites are combined with transmitter.

Some rather more elaborate mechanism of transmitter action than found at

many chemical synapses seems to be required to account not only for the higher gain but also the temporal properties of the bipolar cell response.

### *Synaptic filtering*

The synapse between the rods and depolarizing bipolar cells can be characterized as operating at high gain for signals containing frequency components below about 5 Hz, steeply attenuating frequency components above 5 Hz and being tuned for frequency components in the range 2–4 Hz. It appears that the rod network may have some of the characteristics of a high pass filter, such that the high frequency components of the rod signal are attenuated less than the low frequency components as a signal spreads through the network (Detwiler *et al.* 1978). The tuned filter characteristics of the rod–bipolar cell synapse would be a specialization allowing the bipolar cell to sum dim light signals over a large area. A significant improvement in temporal resolution would be a further consequence of tuning at the synapse.

*Signal-to-noise.* It has been suggested that filtering in the retinal signal pathway may provide an improved signal-to-noise ratio (Hagins, Penn & Yoshikami, 1970; Falk & Fatt, 1972, 1974*b*; Baylor & Fettiplace, 1977). For the maximal improvement in the signal-to-noise ratio in the presence of a presynaptic white noise source, the synapse should act as a filter matched to the rod signal. The rod–bipolar cell synaptic filter is not an optimum filter by the criterion of maximization of signal-to-noise amplitude, but may represent a compromise between signal ‘conditioning’, so as to present an adequate signal for triggering action potentials in amacrine and ganglion cells in very dim light and noise rejection to eliminate false responses.

*Equivalent circuit of the synaptic filter.* A filter, with a transfer function of the form of eqn. (15) can be constructed from a buffered cascade of six lead and ten lag networks with appropriately chosen time constants. It is difficult to see how the large number of delay stages in the filter, each with a time constant of about 30 msec, could arise from the electrical properties of the bipolar cell, since tested with rectangular current pulses, the bipolar cell behaved in the dark as if there were only a single stage of exponential delay with a time constant of about 3 msec. Behaviour equivalent to an inductance, giving phase lead, would occur if the internal potential of the bipolar cell were more positive than the potassium equilibrium potential and there were delayed rectification of potassium channels in the bipolar cell membrane similar to that found in the squid giant axon (Cole & Baker, 1941; Hodgkin & Huxley, 1952). However, there was no evidence for a large equivalent inductance associated with ionic channels in darkness.

### *Effects of backgrounds*

The results indicate that the bipolar cells became desensitized in the presence of backgrounds too weak to affect rod sensitivity. The effect of weak backgrounds on bipolar cell responses to test flashes could be explained satisfactorily by supposing that the effects of backgrounds and test flashes summate. The decrease in sensitivity then arises from the instantaneous non-linearity in the intensity–response relations. The high gain at the rod–bipolar cell synapse rendered weak backgrounds very effective, so that a background which was estimated to hyperpolarize the rods by less than 0.05 mV could drive a bipolar cell beyond the linear range of its response.



In view of the sensitivity of the mammalian visual system near absolute threshold, known psychophysically (Hecht *et al.* 1942) and from studies at the ganglion cell level in the cat (Barlow *et al.* 1971), it is likely that rod signals in the mammalian retina are also transmitted at high gain as in the dogfish. It is possible then to account for the desensitization of cat ganglion cells (Enroth-Cugell & Shapley, 1973) and the rise in threshold by a factor of 3 for detection of a flash by a human observer when the flash is superimposed on a background light from which one photon is absorbed per 100 rods per sec (Aguilar & Stiles, 1954; Rushton, 1963). If the gain at the rod-bipolar cell synapse were comparable to that inferred in the dogfish retina and if there were a gain of 5–10 at the bipolar-ganglion cell synapse, as found in the dogfish (J. F. Ashmore & G. Falk, unpublished), there would be no difficulty in explaining the rise in threshold on the basis of the shift in operating point.

It must be presumed that the effect of bright backgrounds, of about 1 photon/rod.sec or more, involves some form of adaptation. Without such adaptation to account for the wide operating range of the rod visual system the bipolar cells would saturate at about 20 Rh\*\*/sec and even moonlight would be dazzling. The mechanism for this adaptation need not necessarily involve changes in the cell post-synaptic to rods, since there is evidence that adaptation of rod responses to these higher background lights could play an important role in extending the operating range of the rod visual pathway (Kleinschmidt, 1973; Coles & Yamane, 1975; Fain, 1976).

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#### EXPLANATION OF PLATES

Abbreviations: r.o.s., rod outer segments; i.s., inner segments; o.n.l., outer nuclear layer (containing the nuclei of receptor cells); o.s.l., outer synaptic layer; i.n.l., inner nuclear layer (containing the cell bodies of horizontal, bipolar and amacrine cells); i.s.l., inner synaptic layer.

#### PLATE 1

Depolarizing bipolar cell injected with Procion yellow.

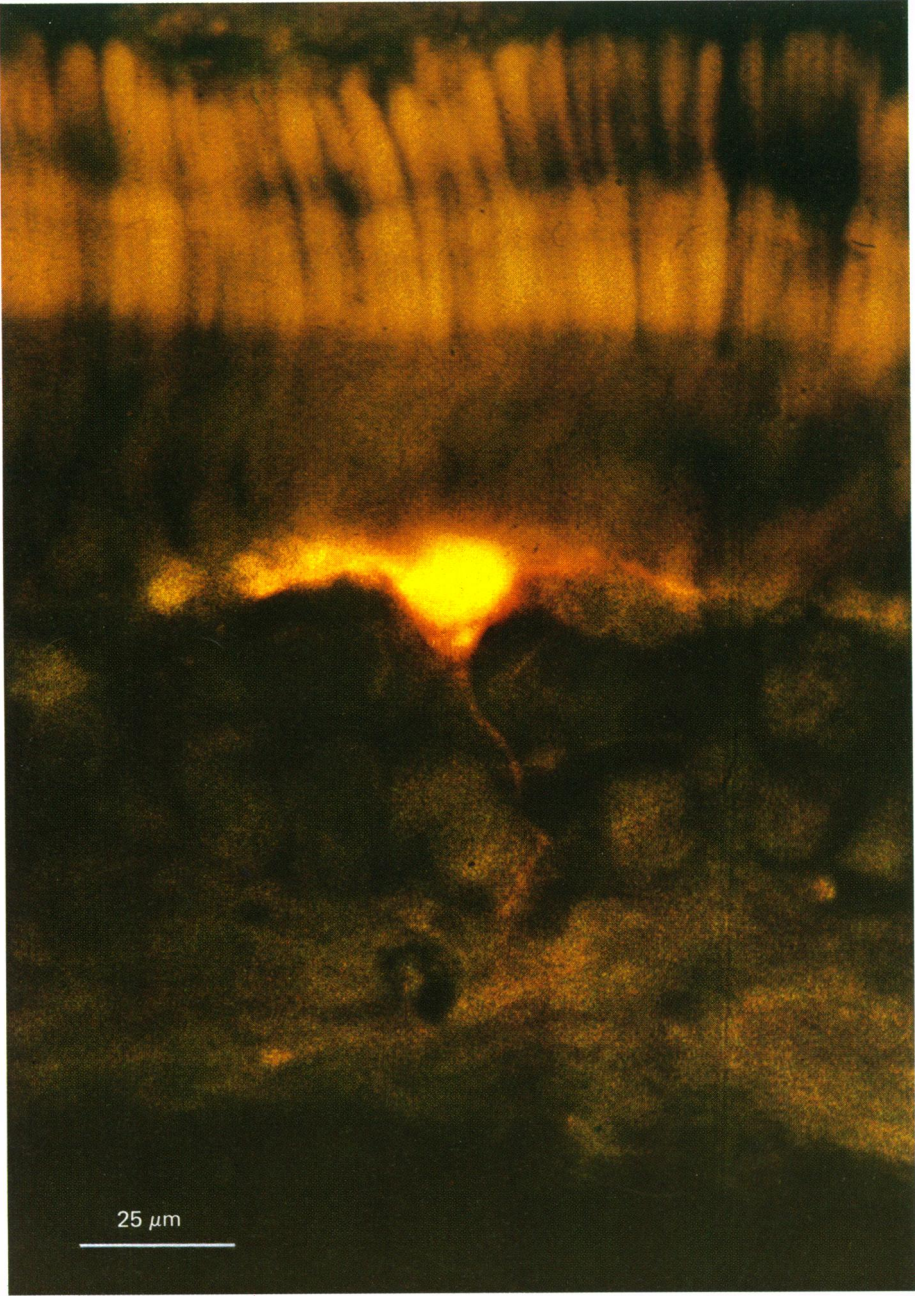
#### PLATE 2

Diagram of the Procion-injected, depolarizing bipolar cell of Pl. 1. Profiles of other cells in the inner nuclear layer were traced from a photograph of the section. Rod outlines are schematic and not to scale.

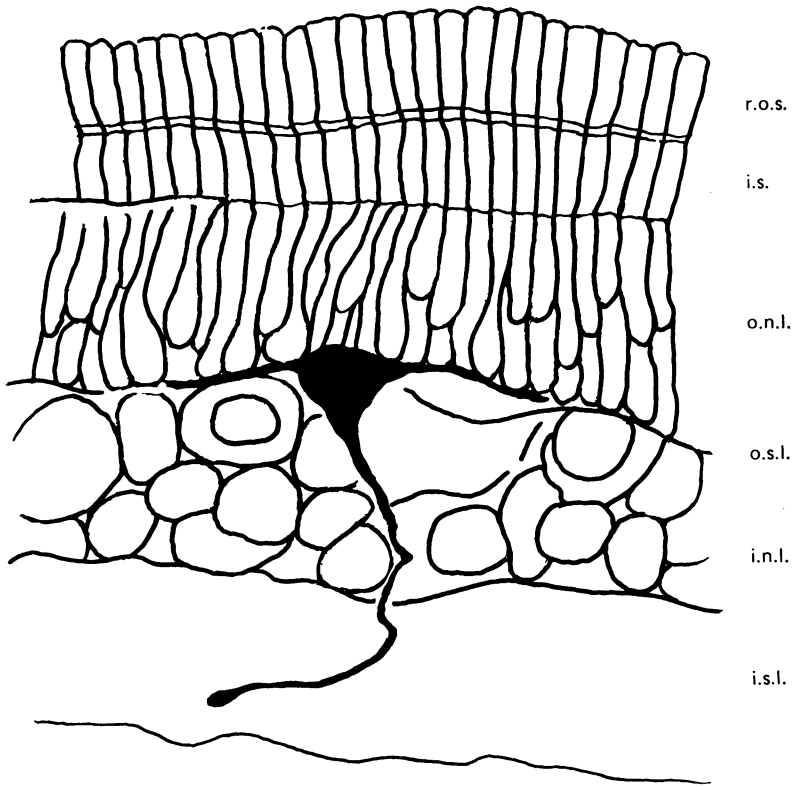
#### PLATE 3

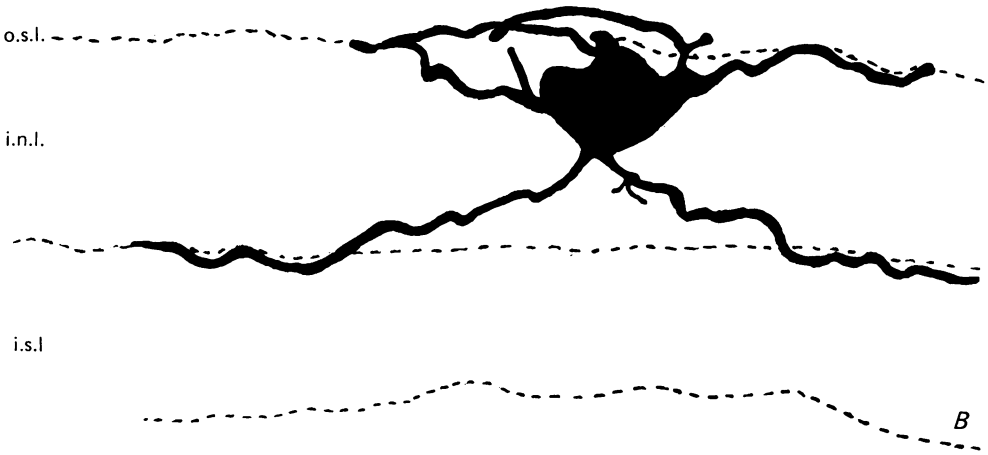
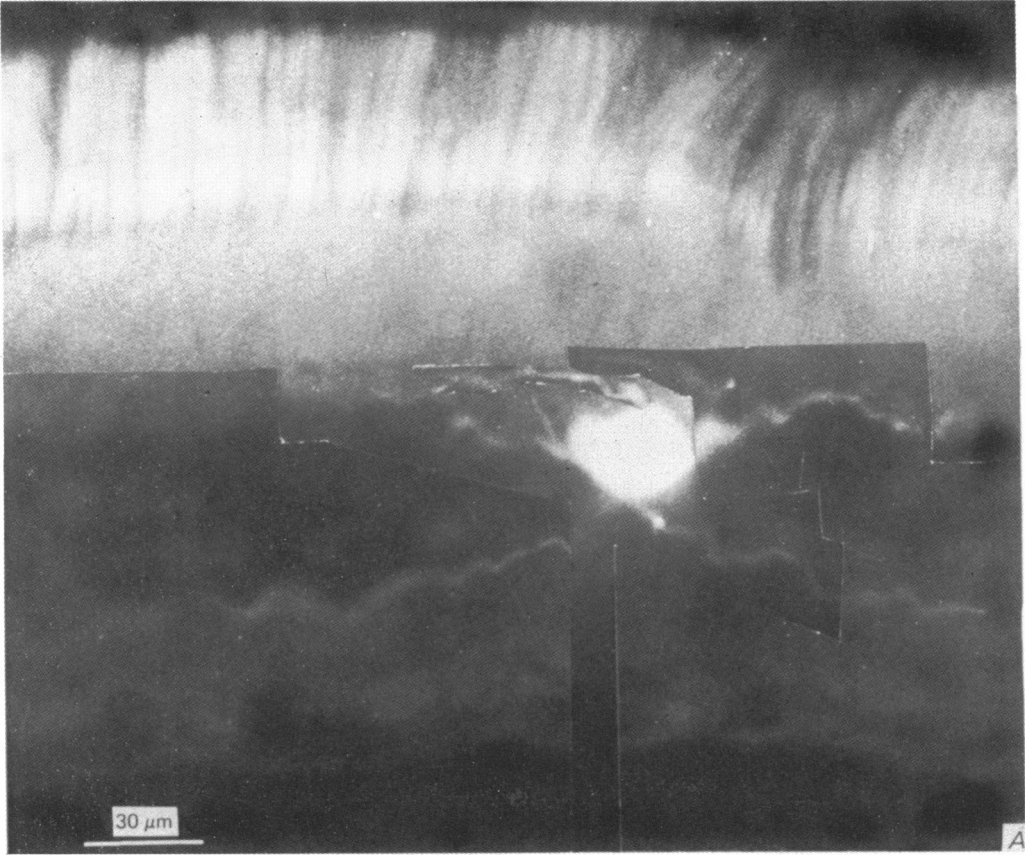
*A*, photomontage of a Procion-yellow injected cell in the inner nuclear layer which hyperpolarized in response to a spot of light. The cell was reconstructed from three serial sections. The fortuitous dip in the rod layer, seen in the upper right hand part of the photograph, served as a useful landmark in the reconstruction.

*B*, tracing made from photographs of the cell.



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