J. Physiol. (1983), **340**, *pp.* 569–597
With 16 *text-figures*
Printed in Great Britain

AN ANALYSIS OF TRANSMISSION FROM CONES TO HYPERPOLARIZING BIPOLAR CELLS IN THE RETINA OF THE TURTLE

BY J. F. ASHMORE* AND D. R. COPENHAGEN

From the Department of Ophthalmology, University of California, San Francisco, CA 94143, U.S.A.

(*Received 16 August 1982*)

SUMMARY

1. Voltage noise was recorded from centre-hyperpolarizing bipolar cells in the retina of the snapping turtle. The identity of the cells was confirmed by intracellular staining.

2. The variance of the voltage fluctuations of the membrane potential present in the dark was suppressed by up to 30-fold by 100 μm diameter light spot stimuli centred on the cell's receptive field. Such noise reduction is expected when light hyperpolarizes the photoreceptors and reduces the rate of release of transmitter from the terminals.

3. The spectra of the fluctuations were analysed as the sum of two components: (a), a component with power band width limited to below approximately 10 Hz, and (b), a component $S_h(f)$ of the form

$$S_h(f) = S_h(0)/(1+(f/f_0)^2)^2,$$

with $f_0 = 27$ Hz. The two components were attributed (a) to the noise generated in the cones and transmitted through the synapse to the bipolar cells and (b) to the action of transmitter on the bipolar cell membrane.

4. The component $S_h(f)$ attributed to the action of transmitter on the bipolar cells corresponded to an event approximately 14 ms in duration. The event had a peak amplitude in the range 17·6–223 μV with a mean of 69·5 μV. It is estimated that, in the dark, the number of such events contributing to the noise is about 9200 s^{-1}.

5. It is estimated that each elementary noise event in the cones controls approximately thirty of the transmitter-related events at the synapse.

6. Responses to flashes of darkness applied on steady illumination were analysed by a method of matched filtering. The responses fluctuated in amplitude, and the analysis of this fluctuation suggested an elementary event of approximately the same amplitude as found from the noise analysis.

7. Enlarging the diameter of the stimulus spot to 1500 μm repolarized the bipolar cells with an associated increase in voltage noise. Implications for the synaptic mechanisms of the centre–surround organization are discussed.

* Present Address: Department of Physiology, The Medical School, University Walk, Bristol BS8 1TD.

INTRODUCTION

A small spot of light falling on the centre of the receptive field of a bipolar cell can produce either a depolarizing or hyperpolarizing response in the cell. Although the pharmacological basis for the two response types has not been fully clarified, the differences represent two distinct post-synaptic actions of the photoreceptor transmitter, (Toyoda, 1973; Saito, Kondo & Toyoda, 1979; Ashmore & Copenhagen, 1980), on cells which can be recognized as morphologically distinct, (Famiglietti, Kaneko & Tachibana, 1977; Weiler & Marchiafava, 1981). The continuous release of transmitter from the photoreceptor terminals in the dark leads to observable fluctuations in the bipolar cell membrane potential (Simon, Lamb & Hodgkin, 1975), and it is the analysis of such fluctuations which should characterize the nature of transmitter action and synaptic interactions at the outer plexiform layer. Noise analysis has been used to investigate transduction (Hagins, 1965; Schwartz, 1977; Lamb & Simon, 1977; Baylor, Matthews & Yau, 1980) as well as synaptic mechanisms in photoreceptors, (Ashmore & Copenhagen, 1980; Ashmore & Falk, 1982).

This paper describes the membrane potential fluctuations in hyperpolarizing bipolar cells of the turtle and presents an analysis of the noise spectrum which identifies components associated with the cones and with the mechanisms of synaptic transmission. A related analysis has been applied to the rod–bipolar system in the dogfish (Ashmore & Falk, 1982), but where the properties of the rods had to be inferred. In the turtle, both pre- and post-synaptic contributions to the noise can be recorded and can be used to separate synaptic mechanisms. Following a description of the electrical properties of the cells, we shall describe a component of the bipolar cell noise spectrum which arises from the action of the cone transmitter upon the post-synaptic channels. The duration of transmitter action is found to be 5–10 ms, approximately an order of magnitude shorter than the cell's response to a light flash. We estimate that the contribution of synaptic mechanisms to the noise present in the pathway is about 27 % of the total noise at all light levels. It will also be shown that an analysis of the noise in the bipolar cells during large and small field retinal illumination places restrictions on the synaptic connectivity of the horizontal cells in the turtle's outer plexiform layer.

METHODS

Experiments were performed on isolated eyecups of the common snapping turtle *Chelydra serpentina* having carapace lengths of 25–40 cm. The eyecup was placed in a chamber through which moistened 100 % oxygen was passed. The vitreous was drained down to a depth of 10–50 μm by Kleenex wicks. One snapping turtle retina provided recordings from several bipolar cells during a single experimental session, (cf. Schwartz, 1974).

The experimental chamber and light stimulation arrangement has been described in detail elsewhere (Copenhagen & Owen, 1976). The light stimulus was delivered down one port of a Zeiss dissecting microscope. The intensity of the unattenuated light was $5{\cdot}5 \times 10^5$ photons s^{-1} μm^{-2} at 650 nm delivered to the retinal surface. Conventional retinal recording techniques were used. Recording electrodes with 2–3 cm shanks were pulled from clean Omega-dot glass (o.d. 1 mm, i.d. 0·6 mm) on a Livingstone puller, and were back-filled with 2 M-potassium acetate. Most of the micro-electrodes had resistances of 500–800 MΩ when measured in the vitreous, with noise levels less than 0·1 mV r.m.s. when band-limited from 0–200 Hz. Electrodes were advanced using a high-speed stepper motor (Brown & Flaming, 1977) at nearly normal angles to the retinal surface.

Cells were recorded in the superior nasal sector of the retina about 3–5 mm opposite the optic nerve. This area corresponds to that studied by Baylor & Hodgkin (1973) and Copenhagen & Owen (1976). Data was recorded on an FM tape recorder for subsequent analysis. Experiments were performed at room temperatures, 20–24 °C.

Data analysis

The procedures for performing the power spectral analysis closely followed Bendat & Piersol (1971). The recorded voltage was passed through a four-pole low pass 100 Hz Butterworth filter and sampled at rates of 2·5 or 4 ms per sample into 1024 point frames. Noise variance was computed from those sampled frames. The data were corrected for slow drifts by removal of the linear trend

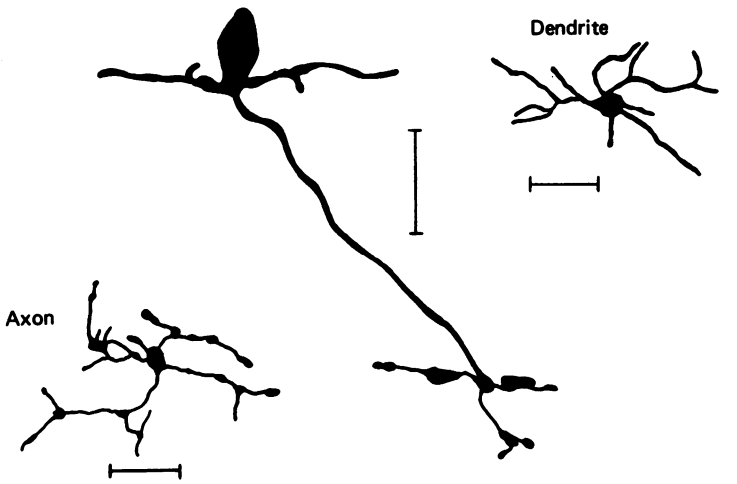

Fig. 1. Camera lucida drawing of a Lucifer Yellow stained hyperpolarizing bipolar cell. The dendritic and axonal arborizations were first viewed in flat-mount before embedding and serial sectioning. Scale bars, 20 μm.

(Bendat & Piersol, 1971) but were also directly coupled using a sample and hold circuit to offset the mean membrane potential. The effect of trend removal on the lowest frequency points in the spectrum was estimated to be less than a 10% correction for points below 2 Hz, (cf. Ashmore & Falk, 1982). These data blocks were multiplied by a Hanning window before processing by the Fast Fourier Transform algorithm. Between six and fifteen spectra were usually averaged and then frequency smoothed over five points to reduce the standard error. The effect of frequency smoothing upon the fitting of the spectra is negligible, since the spectral models described below depend upon fitting an inflexion in the experimental spectra between 10–30 Hz, where, at the chosen sampling rate, frequency smoothing reduces the resolution to 0·5 Hz.

Dye staining of cells

Both depolarizing and hyperpolarizing bipolar cells were marked with Lucifer Yellow. Cells were impaled with micro-electrodes containing 4% solution of the dye and filled by ionophoretic injection of 5–10 nA min. The retina was fixed in 4% paraformaldehyde, 0·5% glutaraldehyde at 4 °C overnight. Whole mounts were viewed following dehydration and clearing. Following identification of the cell, the tissue was embedded in Epon and sectioned at 10 μm.

A camera lucida drawing of a Lucifer-injected hyperpolarizing bipolar cell is shown in Fig. 1. Both the axonal and dendritic arborizations are illustrated as viewed from the vitreal and photoreceptor surfaces of the retina. The axonal arborization of the hyperpolarizing bipolar cells recovered lay exclusively in the distal two-thirds of the inner plexiform layer in contrast to the depolarizing bipolar cells which had axonal arborizations confined to the proximal half of the inner plexiform layer. Similar bipolar cell axon stratification has been described in the goldfish,

(Famiglietti *et al.* 1977) and in the turtle retina (Weiler & Marchiafava, 1981). It was often observed that the axon of a strained cell ran obliquely and arborized in a position peripheral to the cell body.

Theoretical section

The spectra to be analysed here were found to have a shape which could not be fitted by a simple Lorentzian or product of Lorentzian functions, (Neher & Stevens, 1977). They will be analysed instead on the basis of the following model.

Fig. 2 shows the block diagram for the cone–bipolar cell system. V_c and V_b are the cone and bipolar cell membrane potentials. Noise measured at V_c represents fluctuations of the intracellularly recorded membrane potential, while that measured at V_b arises both from the noise transmitted from the cone and that originating from the synapse with the bipolar cell. The noise spectrum at V_b will be derived below.

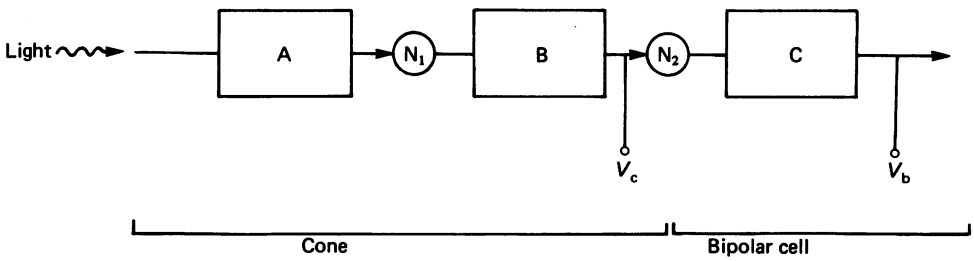

Fig. 2. Model for noise processes in cones and in synaptic transmission. A, B and C are low pass filters; A includes the initial transduction stage converting a light input into a membrane potential. Two filters in the cones are required because the cone noise spectra and the response to a light flash differ in temporal characteristics. V_c and V_b are the membrane potentials in the cone and the bipolar cell respectively. Noise sources N_1 and N_2 generate Poisson trains of delta functions where the rate parameters are instantaneously proportional to the input signals. See text.

Ashmore & Falk (1982) have argued that a component of the noise generated in rod bipolar cells may be considered as arising from shot events due to transmitter action, the elementary transmitter events ('shot events') summing to produce the observed noise. In this paper we consider the action of transmitter on the cone bipolar cell as a 'shot' process, (i.e. a process consisting of elementary events ocurring randomly in time, acting independently and summing linearly). The elementary event will be designated $a(t)$. If these events have Poisson arrival times $\{t_i\}$ with a mean frequency λ the potential due to their post-synaptic summation would be given by

$$V(t) = \sum_{t_i} a(t-t_i). \tag{1}$$

The spectrum of the noise would then be given by

$$S_a(f) = 2\lambda |\tilde{a}(f)|^2, \tag{2}$$

for $f > 0$ and where $\tilde{a}(f)$ is the Fourier transform of $a(t)$, (Rice, 1944). The factor 2 arises since single-sided spectra only will be considered. However, if λ is not a constant, but time varying, then the spectrum becomes the sum of two terms. Rice gives an expression in the case where $\lambda = \lambda(t)$ is a known function of time, (Rice, 1944, eqn. 2.6–11):

$$S_b(f) = 2\bar{\lambda}|\tilde{a}(f)|^2 + \left(\frac{2}{T}\right)|\tilde{\lambda}(f)|^2 |\tilde{a}(f)|^2, \tag{3}$$

where

$$\bar{\lambda} = \left(\frac{1}{T}\right)\int_0^T \lambda(t)\,dt, \tag{4}$$

when the measurements are made over a large time interval $(0, T)$. Thus $\bar{\lambda}$ is the mean rate of occurrence of the events. A similar expression is given formally by Sigworth (1981) who considers the effect of non-stationarity upon power spectra.

A simple generalization of eqn. (3) is the case where $\lambda(t)$ is itself a random variable described by a shot process, i.e. $\lambda(t)$ is a linear sum of terms:

$$\lambda(t) = k \sum_{t_i} m(t - t_i), \tag{5}$$

where k is a constant with the dimensions of s^{-1} and $m(t)$ (dimensionless) are the 'events' which are Poisson distributed in time with a mean rate of occurrence ν s^{-1}. Using the same methods of proof as Rice it may be shown that the spectrum of $V(t)$ becomes

$$S(f) = 2\bar{\lambda}|\tilde{a}(f)|^2 + S_\lambda(f)\,|\tilde{a}(f)|^2,$$

or

$$S(f) = S_h(f) + S_l(f), \tag{6}$$

for $f > 0$ where $S_\lambda(f)$ is the spectrum of the random variable $\lambda(t)$ given by eqn. (5).

$\bar{\lambda}$ is the time-averaged mean frequency of the events $a(t)$ and given by

$$\bar{\lambda} = \nu k \int_0^\infty m(t)\,dt = \nu k |\tilde{m}(0)|, \tag{7}$$

and arises from eqn. (5) by application of Campbell's theorem (Rice, 1944). The spectrum of this doubly stochastic process thus consists of two terms, S_h and S_l: S_h is the spectrum of the shot process with elementary event $a(t)$; S_l arises from the fluctuations in the instantaneous rate of arrival of the events $a(t)$ and is the product of the spectrum of $\lambda(t)$ and the modulus squared of the Fourier transform of $a(t)$. Physically, this term arises in the spectrum from noise events in the presynaptic terminal which have been filtered by the synapse. The two terms are seen to add since the two noise sources are independent. In this model, the ratio of the zero frequency asymptotes of the two spectral terms is

$$b = S_l(0)/S_h(0) = k \int_0^\infty m(t)\,dt, \tag{8}$$

and is thus the number of shot events $a(t)$ controlled by one unitary event, $m(t)$, causing a fluctuation in the rate $\lambda(t)$.

Eqn. (6) will be applied to noise in hyperpolarizing bipolar cells. $a(t)$ will be identified with the elementary transmitter event and $\lambda(t)$ as the membrane potential in the cones, itself a fluctuating quantity, (Lamb & Simon, 1977). It will be assumed that, to a first approximation, the operating range of the synapse is linear, so that the rate of transmitter arrival is linearly related to the presynaptic membrane potential. The basis of this assumption will be examined further below. This assumption implies that there the rate limiting stage is the post-synaptic action of transmitter and not the kinetics of release and/or diffusion in the synaptic cleft. $S_\lambda(f)$ in eqn. (6) will be identified with the voltage noise spectrum in the cones and thus will be set proportional to the voltage noise spectrum $S_c(f)$ in the cones:

$$S_\lambda(f) = AS_c(f), \tag{9}$$

where A is a constant. $S_c(f)$ has been studied in the red- and green-sensitive cones of the turtle by Lamb & Simon (1977). From their observations and our own (J. F. Ashmore & D. R. Copenhagen, unpublished) on red cones in *Chelydra*, $S_c(f)$ will be taken as the stereotyped form

$$S_c(f) = S_c(0)/((1 + 4\pi^2\tau_1^2 f^2)(1 + 4\pi^2\tau_2^2 f^2)), \tag{10}$$

a product of two Lorentzians with $\tau_1 = 54{\cdot}9$ ms and $\tau_2 = 9{\cdot}2$ ms. The time constant τ_2 is interpreted as the time constant due to the filtering action of the cone membrane. In the present experiments, the spectral decomposition (eqn. (6)) is sensitive only to the frequencies in this spectrum below about 10 Hz. Although τ_1 and τ_2 may be functions of the light intensity, (τ_1 decreasing at higher incident light levels corresponding to a speeding up of the light response), the range of light intensities used in these experiments was usually a factor over 300 times less intense than the brightest lights used by Lamb & Simon (1977). In addition, the low effective sensitivity of the sample of cones recorded

(see below) suggests that in the present experiments the range of light stimuli used would not have led to the adaptational effects described by Lamb & Simon in the cone spectra except possibly in a few experiments noted below. It should be noted that if the cone spectrum is itself a multi-component spectrum, as described here for the bipolar cells, an apparent shift in τ_1 could also have arisen from different light-dependence of the constituent spectral components. The invariance of the shape of bipolar cell spectra described below does suggest, however, that insufficient cone adaptation occurred in the present experiments to alter the form of $S_1(f)$ in the spectral decomposition.

RESULTS

A total of ninety-seven hyperpolarizing bipolar cells were recorded with stable resting potentials for periods up to 90 min long. Twenty-five of the cells showed responses to both rod and cone inputs. Such cells were characterized by a higher sensitivity to 500 nm light, a time-to-peak for the rod mediated responses of 400–500 ms with peak amplitudes up to 8 mV. The stimulus used in the experiments to be reported below was light of wave-length 650 nm, and thus primarily stimulated the red cones impinging on these cells. In most of the experiments on noise, 100 μm diameter spots were centred on the cell to minimize interaction from the surround mechanisms mediated by horizontal cells. Although depolarizing bipolar cells were also recorded, (see Ashmore & Copenhagen, 1980), the properties of the hyperpolarizing bipolar cells only will be described here. For brevity these cells will be referred to as bipolar cells.

Transfer characteristics of the synapse

Fig. 3 shows typical recordings from a red cone and a bipolar cell recorded in the same retinal area. In most experiments it was found that the maximum cone responses exceeded those of the bipolar cells. In this example, the bipolar cell had a maximum peak amplitude $V_{max} = 14$ mV. The maximal response of the cone using the unattenuated beam was 70 % greater ($V_{max} = 24$ mV). In some cases, however, bipolar cell peak hyperpolarizations in excess of 20 mV could be elicited (Table 1). In general, bipolar cells reached a maximal hyperpolarization at lower light intensities than the cones. The bipolar cell response showed a nearly constant time to peak over its operating range. This is consistent with a synapse which amplifies, (Ashmore & Falk, 1980*a*; Schwartz, 1974).

Fig. 3*B* shows the intensity–response curves for this pair of cells. The dotted line shows a fit to the data from both cells using a hyperbolic function of the form

$$V = V_{max} I/(I+\sigma), \tag{11}$$

where σ is the stimulus intensity at which the response is half maximal. A slightly better fit to the points near saturation can be obtained using a modified hyperbolic function described in Ashmore & Falk (1980*a*), eqn. (4). The slow approach to saturation in the bipolar cell was probably due to light scatter which elicited a surround antagonism. The difference in the σ, the half-saturating intensity of the stimulus, remains constant for a fixed choice of function fitting the responses, but the value of the half-saturation intensity differs. The effective synaptic voltage gain

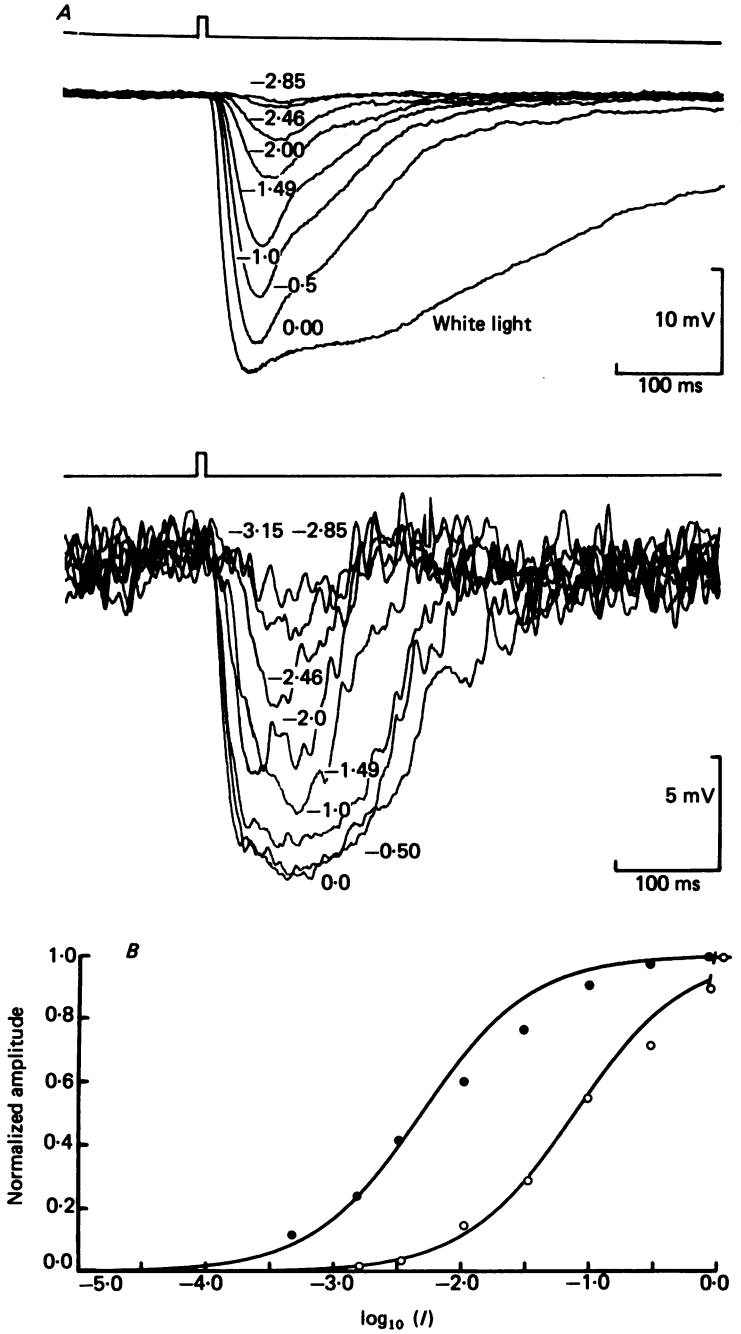

Fig. 3. *A*, response of a red-sensitive cone (top) and a hyperpolarizing bipolar cell (below) to light flashes. The cells were recorded from eyecups of the same animal within 1 h of each other, the bipolar cell being recorded earlier. The stimulus was delivered to both cells as a 90 μm diameter 650 nm light spot, except in the case where white light was used to saturate the cone response. Log attenuation of the incident beam is indicated by each record. *B*, intensity *vs.* normalized response curves for the cells shown in *A*, ●, bipolar cell, $V_{max} = 14$ mV; ○, cone, $V_{max} = 24$ mV. The continuous curves through the data points are drawn using eqn. (11) with log $\sigma = -1{\cdot}1$ and $-2{\cdot}3$ for the cone and bipolar cell respectively.

in the dark (Ashmore & Falk 1980*a*) for the red cone–bipolar cell synapse can be determined from the ratio of the flash sensitivities in the bipolar cell (S_{Fb}) and cone (S_{Fc}) by

$$A = \left(\frac{dV_{post}}{dV_{pre}}\right) = \left(\frac{dV_{post}}{dI}\right) \Big/ \left(\frac{dV_{pre}}{dI}\right) = S_{Fb}/S_{Fc}, \quad (12)$$

where S_{Fb} and S_{Fc} can be determined from the linear slope of intensity–response curves. The bipolar cell flash sensitivity was measured with 100 μm diameter spots of light, which corresponded approximately to the peak sensitivity of the cell (Richter & Simon, 1975). Larger diameter spots reduced the peak response due to surround effects.

A sample of eleven red-sensitive cones was recorded in the same retinal area using large field (spots greater than 400 μm in diameter) illumination conditions to reduce the effect of cone–cone coupling. The mean flash sensitivity, S_{Fc}, for this sample of eleven cones was 16·5 μV photon^{-1} μm^2 with a range of 4·9–34 μV photon^{-1} μm^2. These values fall within the range of sensitivities previously reported for turtle cones (Baylor & Hodgkin, 1973; Baylor & Fettiplace, 1975). A possible reason for the low sensitivities would have been screening by the melanophores of the pigment epithelium. However, since the eyes were kept in a dark adapted condition, when such pigment migration does not occur (Copenhagen & Owen, 1976), a more likely cause is non-coaxial alignment of the stimulus beam with the cones. Baylor & Fettiplace (1975) showed that cones exhibit a directional sensitivity such that the sensitivity falls off when the light enters more than about 5–10° off axis. In a few experiments analysis of the cone light responses to orthogonally oriented, plane-polarized light suggested that the light entered these less sensitive cones at angles greater than 10° off axis.

Column 3 of Table 1 shows the flash sensitivities of a sample of bipolar cells determined from the linear range responses to 100 μm diameter spots. S_{Fb} ranged from 30 to 860 μV photon^{-1} μm^2 with a mean of 221 μV photon^{-1} μm^2, when measured with a 100 μm diameter spot. The mean gain at this synapse was thus 221/16·5 = 13·4.

Electrical properties of the bipolar cell synapse

Although the electrical properties of the recording electrodes were in general unfavourable to passing current, in six cells the electrical characteristics of the cell membrane were studied. Fig. 4 shows the results from the best experiment. When current was passed using an active bridge circuit, the input resistance showed an increase during the light-induced membrane hyperpolarization. In this experiment, the exponential time constant increased from 9 to 13 ms. The slope resistance increased from 113 MΩ in the dark to 158 MΩ at a light-induced hyperpolarization of 12·5 mV. The straight lines through the data points intersect at 67 mV positive with respect to the dark resting potential, (−45 mV), suggesting a reversal potential of around +22 mV for the transmitter action.

These results suggest that the cone transmitter acts upon the bipolar cell by opening post-synaptic channels to ions with a relatively positive reversal potential. The effect of light is to reduce the number of open post-synaptic channels by reducing transmitter release from the cone terminals.

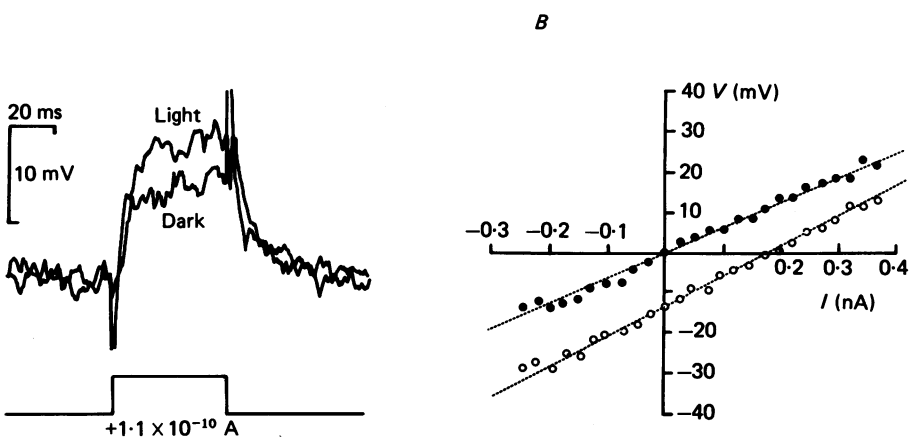

Fig. 4. Current–voltage properties of bipolar cells. *A*, response of a bipolar cell to depolarizing current (0·11 nA) passed in the dark and in light. Tracing from an *XY* plotter; the artifacts at onset and offset of the current step have been truncated. Scale, ordinate 10 mV, abscissa 20 ms. *B*, current–voltage curve for the same cell in dark (●) and in light (○). The current stimulus was a digitally produced sequence of pulses of increasing and alternating polarity injected using an active bridge circuit. The voltage responses have been corrected for the electrode nonlinearity by subtracting the *I–V* curve obtained when the electrode was withdrawn from the cell. Steady hyperpolarization of the cell in light, 12·5 mV. See text.

Voltage noise in hyperpolarizing bipolar cells

Fig. 5 shows the response of a hyperpolarizing bipolar cell to steps of light. In the dark this cell showed pronounced membrane potential fluctuations which were suppressed by steady light, (Simon *et al.* 1975; Ashmore & Copenhagen, 1980). At the brightest stimulus intensity, $5{\cdot}5 \times 10^5$ photons μm^{-2} s^{-1}, (650 nm), the residual noise appeared to be dominated by the noise of the recording system. In some experiments, the noise from the recording electrode could even have increased following withdrawal from the cell due to changing recording conditions, (e.g. Fig. 5). With this light stimulus, the cell initially hyperpolarized and then returned to a steady level of 12 mV below the dark resting potential with a time constant of about 3 s. There was a pronounced transient depolarization to 32 mV beyond the dark level when the light was turned off, following which the fluctuations were also reduced, possibly due to activation of a membrane rectification. The noise observed in the dark subsequently recovered as the cell repolarized to its dark resting potential. This cell was one of the noisiest encountered with a total noise variance in the dark of $3{\cdot}75$ mV^2.

Although we cannot rule out a small contribution to the noise generated by synapses with amacrine cells of the sustained response type, the voltage fluctuations were assumed to arise mainly from the cone–bipolar cell synapse and from signals generated in the cones for the following reasons:

(1) Bipolar cells are post-synaptic elements to photoreceptors, making direct synaptic contact with the cone pedicles, (Lasansky, 1978).

(2) The noise decreased during bright light steps, when transmitter release from the cone terminals is reduced.

(3) Cobalt suppresses the noise. Fig. 6 shows an experiment in which 2 mM-cobalt

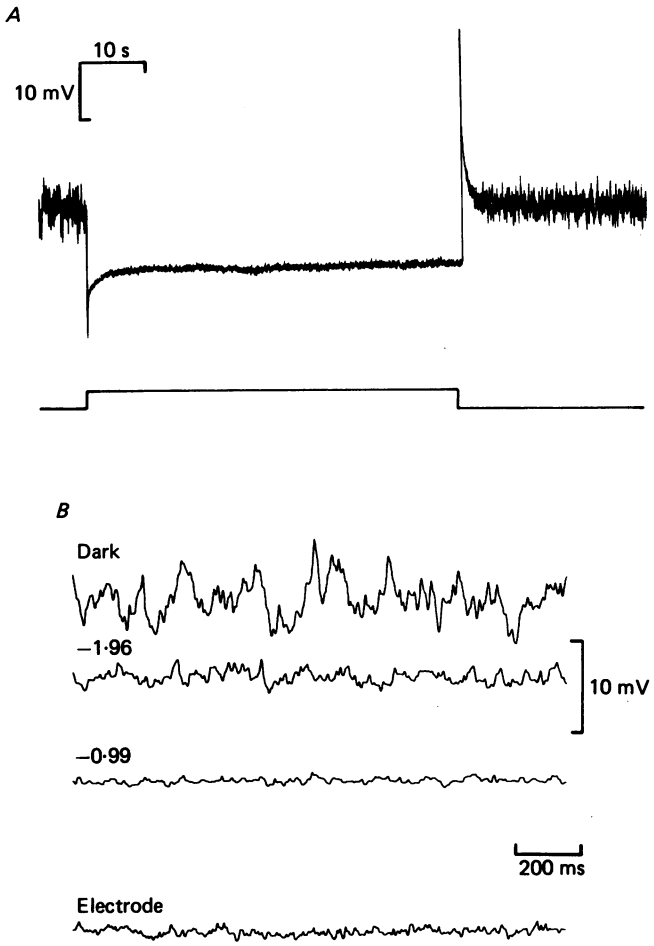

Fig. 5. Response of a bipolar cell to steps of light. *A*, response to a step lasting 58 s delivering $5{\cdot}89 \times 10^4$ photons μm^{-2} s^{-1}. Maximum hyperpolarization of the cell, 22 mV; $S_F = 665$ μV photons μm^{-2} s^{-1}. *B*, voltage fluctuations observed at a faster sweep speed. Voltage scales, 10 mV. Figures by each record indicate log attenuation of the stimulus. The bottom trace is the electrode placed extracellularly after spontaneously coming out of the cell, with variance 0·228 mV^2. The total variance of the record in bright light was 0·248 mV^2.

was allowed to flow onto the retinal surface around the recording electrode. In this case the noise was suppressed and the light response reduced by over 70% within 2 min. A noise reduction was obtained in six further experiments.

(4) Extrinsic current applied through the recording electrode did not produce a noise suppression.

Fig. 7 shows the result of an experiment using an active bridge circuit to polarize the cell with long current pulses. When the cell was hyperpolarized to approximately the same membrane potential by either light or current, the noise was not suppressed equally. Instead, during the hyperpolarizing current step the noise increased. This noise increase (0·304 mV^2) from the dark level was ascribed to the unfavourable

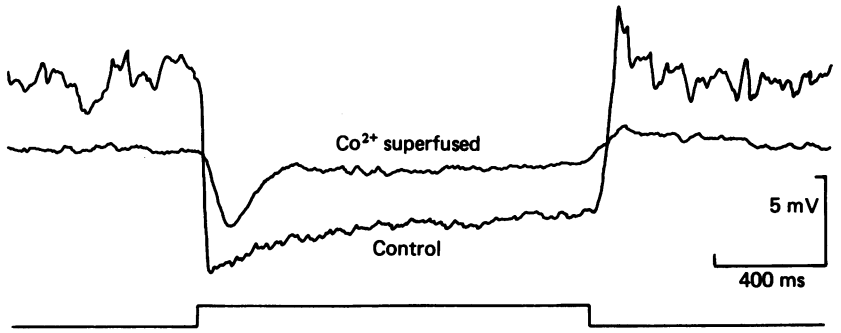

Fig. 6. Noise suppression by cobalt. 10 mM-Co^{2+} in turtle Ringer was superfused onto the retina via the tissue wicks. Superimposed are the control response to a 100 μm diameter light stimulus and the response 2 min following Co^{2+} application. Co^{2+} hyperpolarized the cell and reduced the noise variance from 0·64 to 0·013 mV^2 in the dark. In two other experiments, cells hyperpolarized by up to 10 mV with a suppression of the noise and in four other cases the noise was suppressed without any observable dark resting potential change. In all other attempts the cell was lost following cobalt superfusion.

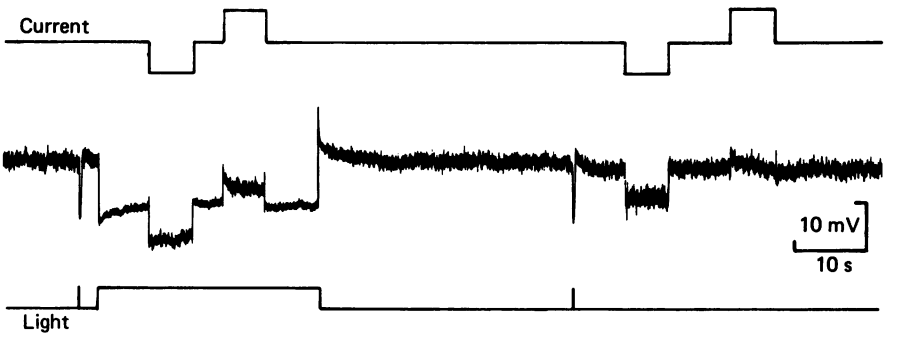

Fig. 7. Noise and extrinsic current. Current was injected with a bridge circuit into the cell during light and in the dark. Before each run, a flash of light was given, (bottom trace). Injected current, 0·09 nA, (current monitor, top trace). The total noise variance, computed from 5·12 s digitized frames, was 0·620 mV^2 (in dark) and 0·130 mV^2 (in light). Total noise variance with +(−) current: 0·915 (0·924) mV^2 (in dark) and 0·435 (0·416) mV^2 (in light).

properties of the fine electrodes, since the same current applied during the light step produced the same increase in noise variance (0·305 mV^2). Similarly, depolarizing current steps increased the noise variance by 0·286 mV^2 in the light and 0·294 mV^2 in the dark. If the membrane potential fluctuations were due to a voltage-dependent noise source, then significant differences in total noise variance would be expected for the same polarity current applied during light and in the dark. Since we found no difference, it seems that an intrinsic voltage-dependent noise source could have made only a small contribution to the voltage fluctuations observed.

The results which follow are analysed on the assumption that the noise is composed of elementary events due to transmitter action. It is suggested that the events tend to cluster in time as a result of fluctuations in the cone membrane potential which modulates their release.

The spectrum of the voltage noise

Fig. 8 shows the typical spectrum of voltage noise at two different levels of steady illumination with a 100 μm diameter spot of 650 nm light. The difference spectra are shown in Fig. 8*B* where the spectrum in bright light ($5{\cdot}5 \times 10^5$ photons μm^{-2} s^{-1}) has been subtracted to remove system noise. It is likely that light does not suppress the noise in the bipolar cells completely and thus the noise variance estimated in the dark might be slightly greater than indicated by the difference spectra.

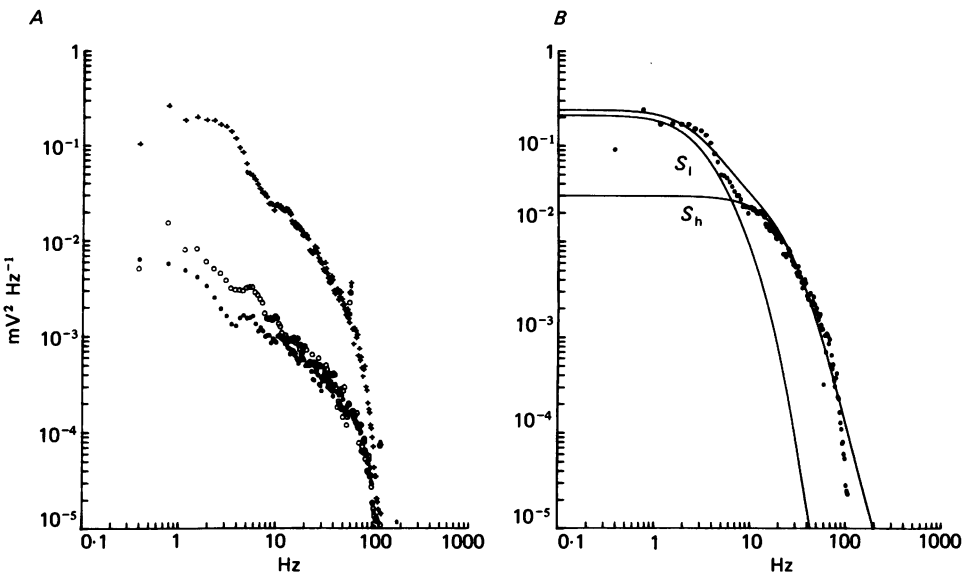

Fig. 8. Noise spectra of a hyperpolarizing bipolar cell. *A*, raw spectra of cell in dark (+) and with retinal illumination $4{\cdot}4 \times 10^4$ (○) and $5{\cdot}5 \times 10^5$ (●) photons μm^{-2} s^{-1}. Spot diameter, 100 μm. The data was filtered at 100 Hz and sampled at 400 Hz. Variance in dark, 0·965 mV^2; in bright light, 0·0365 mV^2. Maximum steady hyperpolarization, 6·8 mV. *B*, difference spectra (+ minus ●) for the same cell. The continuous lines are drawn using eqn. (13) with $S_l(0)/S_h(0) = 5$, $f_0 = 27$ Hz. Cell 8, Table 1.

Above about 50 Hz, the spectra approached an asymptotic behaviour which was close to f^{-4}. (Frequencies above 100 Hz were considered to be distorted by the recording band width and were removed by filtering.) Between 3–30 Hz the spectra behaved like f^{-a} where $a < 1$. In some cases, a pronounced inflexion was seen in the spectra, (see Fig. 9*A*). This behaviour precludes a fit by a simple product of Lorentzian functions, and suggests that the spectrum is composed of a sum of components. The power spectrum of the noise had wider band width than the power contained in the linear range response, which exhibited a half power point at about 2·5 Hz, (Fig. 14). We shall argue that this implies that the noise is composed of smaller, faster events than is present in the signal generated in the cone.

Fig. 9 shows sample difference spectra from two other cells. There was a pronounced inflexion in the spectra at around 10 Hz. These spectra also suggest the presence of two components. One component had power rolling off at about 7 Hz. The other

component had power extending to higher frequencies with a roll-off at about 25 Hz. All but two of the spectra measured could be analysed in terms of such a two component decomposition. The exceptions were cells which also had low flash sensitivities (less than about 50 μV photon^{-1} μm^{-2} s^{-1}), and whose spectra appeared to be composed of only one component with roll-off frequency at about 25 Hz. A possible cause might have been a reduced efficiency of transmitter release from the presynaptic terminal.

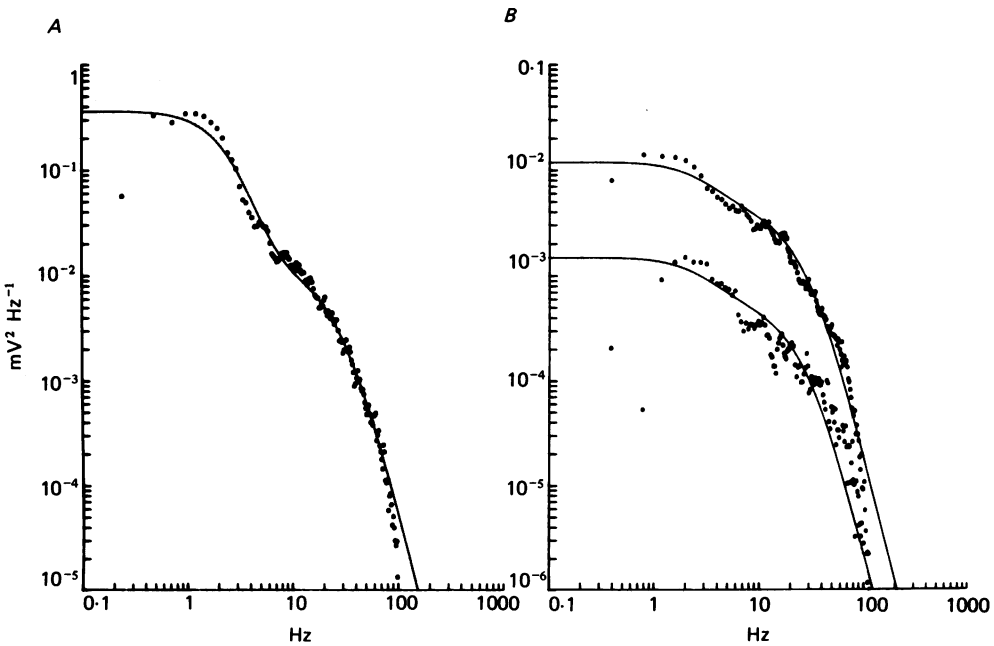

Fig. 9. Noise spectra from two bipolar cells showing extreme forms of spectra encountered. *A*, difference spectrum of cell 2, Table 1. Variance in the dark, 1·18 mV^2. Variance in bright light ($1{\cdot}52 \times 10^5$ photons μm^{-2} s^{-1}), 0·195 mV^2. *B*, difference spectrum of cell 16, Table 1. Variance in dark, 0·338 mV^2. Top spectrum, difference between dark and bright light ($5{\cdot}7 \times 10^4$ photons μm^{-2} s^{-1}). Lower spectrum, difference between noise spectrum at illumination $1{\cdot}75 \times 10^4$ photons μm^{-2} s^{-1} and bright light spectrum. Maximum hyperpolarization of cell, 12·7 mV.

Spectra were fitted with the two component model developed in the Theoretical Section. This model also provides a decomposition of the total noise variance into the sum of two terms.

$$\begin{aligned} S(f) &= S_l(f) + S_h(f) \\ &= bS_c(f)\,S_h(f) + S_h(f) \end{aligned} \tag{13}$$

where $S_h(f) = S_h(0)/(1+(f/f_0)^2)^2$ is chosen to fit the asymptotic behaviour of the spectra, (Ashmore & Copenhagen, 1980), and the cone noise spectrum is the normalized expression,

$$S_c(f) = 1/((1+(f/f_1)^2)\,(1+(f/f_2)^2)), \tag{14}$$

consistent with the data from cones in eqn. (10) if $f_1 = 2{\cdot}89$ Hz and $f_2 = 17{\cdot}3$ Hz.

The fit to the data points is shown on Figs. 8 and 9.

The simplest elementary event compatible with the form of S_h is

$$a(t) = a_{peak}(t/T)\exp(-t/T+1), \tag{15}$$

which formally corresponds to the impulse response of a buffered, two stage low-pass filter, each stage having time constant T and where a_{peak} is the peak amplitude of the event. One of the time constants T in eqn. (15) is presumably the membrane time constant (Ashmore & Copenhagen, 1980). For most of the cells, a fit could be obtained with $T = 5{\cdot}9$ ms ($f_0 = 25$ Hz). The half width of the event was thus 14·3 ms.

Although eqn. (15) represents a simple, monophasic event $a(t)$, a small biphasic component in $a(t)$ would still be compatible with the present data. Detwiler, Hodgkin & Lamb (1983) describe an experiment in which synaptic events were seen in a hyperpolarizing bipolar cell in *Pseudemys*. These events show an initial depolarizing phase lasting about 30 ms and a subsequent small hyperpolarizing phase of about the same duration. The modulus squared of their Fourier transform exhibited a slight maximum at about 13 Hz. If $S_h(f)$ were taken to be a peaked function rather than the double Lorentzian adopted, the inflexion point in some spectra could be fitted better. Similar considerations apply to the analysis of the noise spectra in rod bipolar cells, where the associated elementary event was inferred to contain both overshoots and undershoots, (Ashmore & Falk, 1982). It is thus unlikely that $S_h(f)$ is sharply peaked, and the adoption of the Lorentzian expression would not substantially affect the numerical conclusions below.

Eqn. 13 decomposes the total noise variance into a sum

$$\begin{aligned}\sigma^2 &= \int_0^\infty S(f)\,df \\ &= \int_0^\infty S_l(f)\,df + \int_0^\infty S_h(f)\,df \\ &= \sigma_l^2 + \sigma_h^2.\end{aligned} \tag{16}$$

Using eqn. (13) to fit the spectra, the ratio of the variances σ_l^2/σ_h^2 was obtained by numerically integrating the area under the curves $S_l(f)$ and $S_h(f)$. For the cells studied, the ratio was in the range 0·34–8·56 (Table 1, column 7), with a mean value of 2·73. The source of the variation remains unclear. There was no apparent correlation between S_{Fb}, the bipolar cell flash sensitivity, and σ_l^2/σ_h^2 although a large ratio may imply a larger event in the cone, and may possibly be associated with a smaller cone field.

Table 1 shows the results from eighteen cells for which complete spectra were obtained. Column 6 shows the value of b in eqn. (13) used to fit the spectra in the dark. b ranged from 2 to 50. According to eqn. (8) this is interpreted to mean that each elementary event in the cone controls 2–50 synaptic shot events in the bipolar cell.

Amplitude of the transmitter event. The peak amplitude of the elementary event, a_{peak}, was obtained from the slope of the variance σ_h^2 as a function of the mean depolarization V

$$a_{peak} = \phi\frac{d\sigma_h^2}{dV}, \tag{17}$$

where ϕ is a shape factor required to give the peak amplitude, (Ashmore & Falk, 1982). For an event given by eqn. (15) $\phi = 4/e = 1{\cdot}47$.

TABLE 1. Noise properties of hyperpolarizing bipolar cells

Cell	V_{max} (mV)	V_S (mV)	S_{Fb} (μV photon^{-1} μm^2)	σ_D^2 (mV^2)	σ_L^2 (mV^2)	$S_l(0)/S_h(0)$	σ_l^2/σ_h^2	a_{peak} (μV)	c_{peak} (μV)	N
1	12	9	50	13·3	2·48	—	6·47	233	1740	14
2	15	12·8	297	1·18	0·195	20	3·45	25·7	102	177
3	9	9	53	0·689	0·0402	10	1·84	37·3	79·1	57
4	24·6	12·7	665	3·22	0·248	50	8·56	35·5	350	130
5	12	10	130	1·15	0·0299	3	0·512	108	63·7	34
6	7·5	7	—	0·802	0·110	20	3·45	32·2	127	79
7	10	10	—	0·965	0·0349	4	0·684	80·1	63·1	45
8	15·4	9·5	860	1·10	0·043	5	0·860	86·7	85·9	40
9	17·5	12	227	2·13	0·279	4	0·684	136	107	32
10	11·6	10	30	1·28	0·215	25	4·42	28·5	145	127
11	12	9·1	72	8·18	3·35	30	5·15	126	742	26
12	15	5·4	172	0·591	0·140	4	0·684	71·9	56·7	27
13	13	7	423	0·821	0·169	5	0·860	72·6	71·9	35
14	16	11·2	59·9	0·895	0·278	2	0·340	59·6	23·3	68
15	9	7·5	87	0·356	0·0412	20	3·45	18·2	49·1	149
16	15·6	12·7	368	0·338	0·0854	2	0·340	21·5	84·3	214
17	14	13·0	189	0·299	0·0651	20	3·45	58·6	23·3	804
18	14	14	80·9	1·26	0·085	30	5·15	19·8	117	257
Mean	—	—	235	—	—	19·7	2·73	69·5	224	129

Cells 8, 9, 14 and 15 had positively identified rod inputs by the criteria described in the text. V_{max} refers to the peak amplitude of the response to a flash of light, V_S to the steady hyperpolarization in bright light; the difference between the two indicates the degree of relaxation of the membrane hyperpolarization following the light step. The flash sensitivity, S_{Fb} (column 3) is measured for all cells using a 100 μm diameter spot of light as stimulus. σ_D^2, σ_L^2 (columns 4, 5) are the total noise variance in the dark and in light respectively; the spectral decompositions, columns 6, 7, are explained in the text. The peak amplitude of the shot event $a(t)$, a_{peak} is given in column 8, corrected by a shape factor $\phi = 1{\cdot}47$. The peak amplitude of the event corresponding to the cone event is given in column 9, using a shape factor 1·63 obtained from numerical integration of eqn. (19); the ratio of the total power in the two components used to fit the spectra in the dark is given in column 7. N (column 10) is the number of simultaneous transmitter events occurring in the bipolar cell in the dark.

In practice, a_{peak} was determined either from the difference in the variance in the dark and in the light divided by the difference in membrane potential, or using incremental changes of variance and membrane potential produced by inserting calibrated 0·3, 0·6 or 0·9 neutral density filters in the optical stimulator on bright backgrounds. For reasons related to non-linearities to be discussed below, the former method underestimates a_{peak} by a factor of about 2. The peak size of the event $a(t)$ is given in Table 1, column 8. a_{peak} was found to lie in the range 18·2–233 μV with a mean of 69·5 μV.

The number of simultaneous events in the dark. The rate of occurrence of transmitter events may be calculated from the coefficient of variation of the noise. The number of simultaneous events in the dark is given by:

$$N = \sigma_h^2 \Big/ \int_0^\infty a^2(t)\,dt. \tag{18}$$

No correction for nonlinear summation has been included in the values given in Table 1. The values for N were in the range 14–804 with a mean of 129. Since the integration time of the bipolar cell elementary event was 14 ms, the number of events per second would have been about 9200.

The cone signals in the bipolar cell. The amplitude of the event recorded in the bipolar cell which would produce the observed low frequency component can be obtained similarly by analysis of σ_l using eqn. (17). If the noise event in the cones is $m(t)$, the transmitted signal $c(t)$ seen in the bipolar cell is the convolution of $m(t)$ with the impulse response of the synapse, $a(t)$, corresponding to a filtering by the synapse. Thus $c(t)$ = constant × $(a*m)(t)$ where * denotes convolution. The simplest form of $m(t)$ which gives rise to the cone voltage noise spectrum would be given by

$$m(t) = \text{constant} \times (\exp(-t/\tau_1) - \exp(-t/\tau_2)), \tag{19}$$

where τ_1 = 54·9 and τ_2 = 9·2 ms, (eqn. (14)). Because of the wide passband of the synaptic filter, little distortion of the cone noise signals is introduced by the synapse and $c(t)$ has approximately the same time course as the cone noise event, $m(t)$. Hence the shape factor corresponding to $c(t)$ is approximately equal to that deduced for $m(t)$ and equals 1·63, by numerical integration. The peak amplitude, c_{peak}, of the event is given in Table 1. c_{peak} was found to have a range from 23 μV to 1·74 mV with a mean peak amplitude of 224 μV. The mean peak amplitude of the voltage event estimated for isolated cones in *Pseudemys* was approximately 100 μV, (Lamb & Simon, 1977). Since the average post-synaptic signal is found to be larger by a factor of about 2, it may be suggested that the bipolar cells are in synaptic contact with relatively few cones in the electrically coupled network of these photoreceptors. The size of the dendritic field, 40–60 μm in diameter, supports this conclusion, and studies of the receptive field structure of the bipolar cells using slits of light as stimuli also indicate that the functional receptive field centre may be no more than 70 μm in diameter. Perfect resummation from all the cones in a cone's functional receptive field would produce cone signals up to thirteen times greater and those cells with a larger value for c_{peak} were presumably summing over larger numbers of cones. The smaller values for c_{peak} could have arisen in experiments in which the isolated cone event was less than 100 μV.

The number of simultaneous cone events in the dark. The number of events that would produce the observed variance σ_I^2 in the dark can be computed in the same manner as in eqn. (18), or more readily using eqn. (8), whence the number of simultaneous events is N/b. From Table 1, it is found that the mean number of cone events observed in the bipolar cell was 1370 s^{-1} with a range of 71 to 7640 s^{-1}.

Invariance of spectrum with membrane hyperpolarization

Fig. 10 shows spectra from a cell which were obtained at several different light intensities. The shape of the difference spectra was independent of the light intensity, differing only in an over-all scale factor. Thus the noise spectra could be fitted by

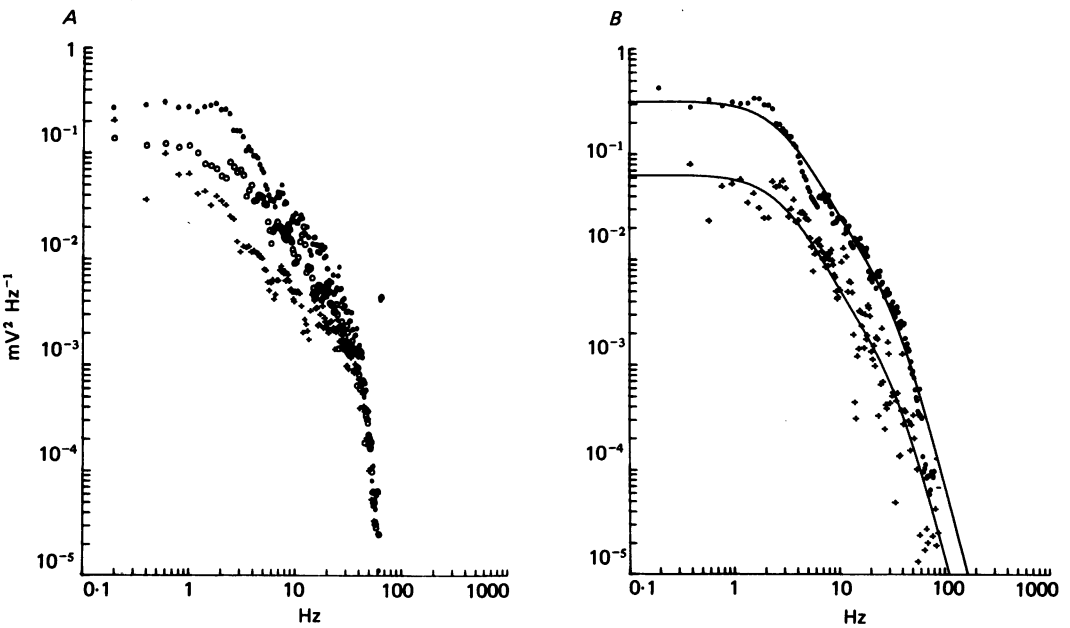

Fig. 10. Shape invariance of the noise spectra with light. *A*, raw spectra showing spectra (●) dark, 0·90; (○) $2{\cdot}76 \times 10^3$, 0·38; (+) $5{\cdot}5 \times 10^3$, 0·170, (photons $\mu m^{-2}\ s^{-1}$, mV^2). *B*, difference spectra obtained by subtracting the spectrum in bright light (+) from the spectra (●) and (○) in *A*. The continuous lines are both given by eqn. (13) with $S_l(0)/S_h(0) = 5$, $f_0 = 27$ Hz, but are scaled in the power axis only. Cell 8, Table 1.

eqn. (13) using b = constant at all light intensities. The difference spectra of the cell in Fig. 9*B* also showed this scaling behaviour. In contrast, the shape of the noise spectra in cones is a function of the stimulus intensity, with the corner frequency increasing at higher light levels, corresponding to a speeding up of the cone elementary events with increasing light intensities, (Lamb & Simon, 1977; J. F. Ashmore & D. R. Copenhagen, unpublished observations). The independence of the frequency parameters of the bipolar cell spectra strongly suggest that the synaptic transfer does not alter in time course during light adaptation. The fit to the spectral points is not sufficiently sensitive to determine whether the roll-off frequency of S_l increased, as would be suggested by the spectral data from cones, (Lamb & Simon, 1977). The relatively dim lights used in these experiments probably did not

sufficiently light adapt the cell. Invariance of the spectra is also consistent with the hypothesis that only one source of noise was present in bipolar cells, namely shot events produced by transmitter acting on the cells.

Variance vs. membrane potential

Linearity between variance and membrane potential allows the event size to be determined when the noise process is a shot process described by Campbell's theorem, (Rice, 1944). We examined this linear dependence for noise changes during repolarization of the membrane potential and for small decrements from maximum hyperpolarization.

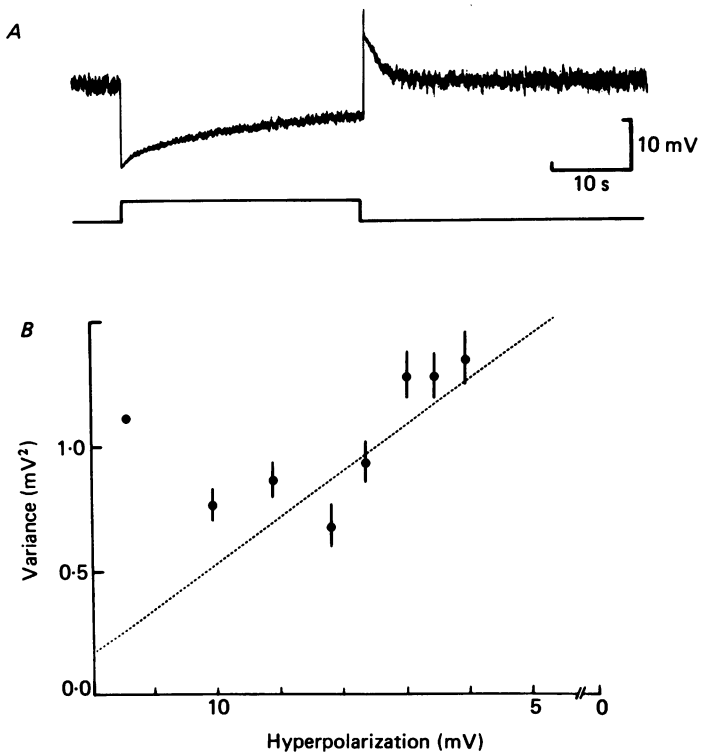

Fig. 11. Recovery of the total noise variance during a prolonged step. *A*, cell showing initial suppression of noise followed by an increase as the cell repolarized. *B*, variance of the record sampled in $T = 4{\cdot}096$ s blocks. Noise variance in the dark, 0·97 mV². The standard error (S.E.) of the variance estimate is given by the vertical bars using the expression S.E. $= (1/B_S T)^{\frac{1}{2}}$ where $B_S = 25$ Hz is the statistical band width of the signal (Bendat & Piersol, 1971, p. 278). The leftmost point is above the linear fit to the points due to an initially high base line drift. The dotted line is a least-squares fit to the remaining points. Cell 6, Table 1.

Fig. 11 shows an experiment in which the noise variance increased as the cell repolarized during a prolonged, bright light step. This behaviour was seen in twelve out of eighteen cells; the remaining cells showed maintained hyperpolarization after the initial peak, (e.g. as in Fig. 5). The dashed line in Fig. 11*B* is a least-squares fit to the points. Thus, at hyperpolarized membrane potentials, the total membrane

noise variance was roughly linear with the membrane potential. A linear dependence of variance on membrane potential is anticipated in the linear shot model, and, in this case, the analysis of the elementary shot events follows eqns. (13)–(17).

In some cells, however, the variance of the membrane potential fluctuations in the steady state showed a non-linear dependence on membrane potential during prolonged steps of light. Fig. 12 shows an experiment where a steady light was applied and then

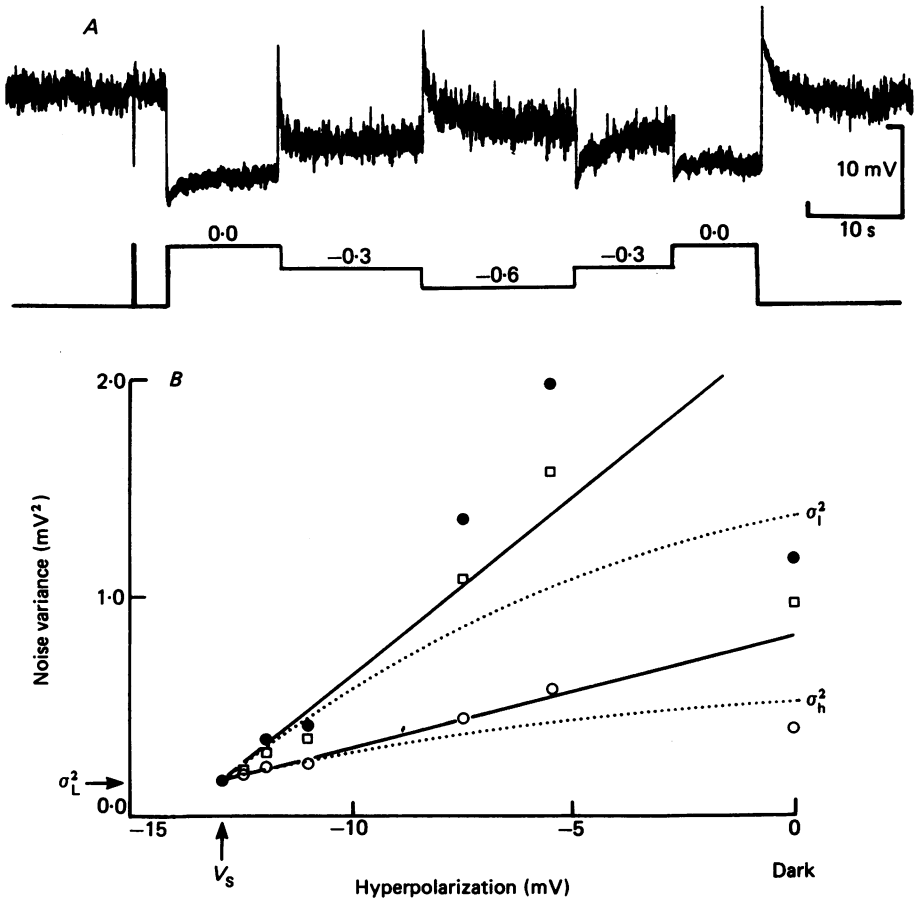

Fig. 12. Variation of the noise variance with membrane potential. *A*, step response of a cell to light at an intensity 381 photons $\mu m^{-2} s^{-1}$. Attenuation of the light by 0·3 and 0·6 neutral density filters, indicated on the light monitor trace, produced a prominant depolarizing transient at the beginning of each intensity change. The light step was preceded by a test flash. *B*, dependence of total noise variance on membrane potential (●), potential measured from the dark resting potential (−45 mV). Minimum noise variance in bright light, $\sigma_l^2 = 0{\cdot}19$ mV², (arrowed). The decomposition of difference spectra using $b = 20$ in eqn. (13) is shown for σ_l^2 (□) and σ_h^2 (○). The continuous lines are drawn by eye through these data and have slopes $k_l = 156\ \mu$V (□) and $k_h = 45\ \mu$V (○). The dotted curves are drawn according to eqn. (20) with the same initial slopes as the straight lines:

$$\sigma_i^2 = k_i U(1 - U/U_r)^3 + 0{\cdot}19,$$

for i = l or h where U is measured from the maximum light-evoked potential $V_S = 12{\cdot}8$ mV and where $U_r = 80$ mV corresponds to a reversal potential of +22 mV, (Fig. 4). U_r taken closer to 0 mV would not alter the curves significantly. Cell 2, Table 1.

reduced by two or four times by neutral density filters interposed in the beam. The noise increased as cell reached more depolarized potentials, (i.e. as the light intensity was reduced). However, when the membrane hyperpolarized by 4·5 mV from the dark, (corresponding to 95 photons μm^{-2} s^{-1}), the total noise variance was 1·96 mV^2 compared with 1·18 mV^2 in the dark. The total noise variance thus passed through a maximum.

Fig. 12*B* shows the data plotted from the same cell at a number of different light intensities. At the most hyperpolarized level, 12·8 mV, the noise was maximally reduced. The open symbols show the decomposition of the total variance into the sum $S_l + S_h$ obtained from fitting eqn. (13) to the difference spectra using a constant value $b = 20$. The dependence of the variance on membrane potential was roughly linear for hyperpolarizations $0{\cdot}5\,V_{max} < V < V_{max}$, i.e. in the low transmitter limit. However, the variance near the dark fell below the extrapolated line by a factor of 2. The values of a_{peak} and c_{peak} obtained from the linear slopes k at the most hyperpolarized levels were 66 μV (σ_h^2) and 254 μV (σ_l^2) compared with 26 and 102 μV, respectively, obtained using the difference between light and dark variance (Table 1).

A possible source of the observed non-linearity arises from the addition of post-synaptic membrane conductance which predicts a relation between total noise variance $\sigma^2(V)$ and membrane potential of the form

$$\sigma^2(V) = kU(1 - U/U_r)^3, \tag{20}$$

where k is a constant, $U = U - V_{max}$ is the potential measured from the point of maximal hyperpolarization (where all transmitter activated channels are closed) and $U_r = E_r - V_{max}$ is the reversal potential for transmitter action, (Katz & Miledi, 1972; Ashmore & Falk, 1982). The dotted curve on Fig. 12*B* shows a fit of eqn. (20) with $U_r = 80$ mV (corresponding to a reversal potential for transmitter action at about +20 mV). The poor fit suggests that other sources of non-linearity may be significant.

Voltage sensitivity of the membrane conductance could also have reduced the voltage noise in the dark. No rectification was seen using 50 ms current pulses, (Fig. 4). although a slowly activating rectifier could have accounted for the sag noted when longer current steps were applied (for example, in Fig. 7 for depolarizing current pulses). Another possibility is that the voltage noise recorded in cones passed through a maximum, before being suppressed by bright light (Lamb & Simon, 1977; Fig. 9). Such a noise increase in the cones would be transmitted to the bipolar cells, although accompanied by a mean reduction of transmitter release and hyperpolarization of the bipolar cell. It might be expected that in such a case, the bipolar cell noise spectrum would also change shape, b increasing to reflect a larger fluctuations of the cone transmitter release. Our failure to observe such a change in the spectra, (Fig. 10), may indicate the low sensitivity of the spectral fit to changes in the relative contribution of S_l and S_h, although we estimate that a 30% change in b could have been detected.

Depletion of transmitter from vesicles released from the cone terminals at high rates in the dark could also contribute to the observed noise maximum, by reducing the peak size of the unitary event in the dark. Such transmitter depletion would be less than about 50% to account for the data, and certainly less than transmitter pool depletion invoked to account for adaption at the sensory synapse of hair cells, (Furukawa, Hayashick & Matsuura, 1978).

Filtering properties of the synapse

A consequence of the model is that the bipolar cell responses can be predicted from the cone wave forms and from the form of $a(t)$ (eqn. (15), since $a(t)$ is also the synaptic transfer function (Ashmore & Copenhagen, 1980). Fig. 13 shows the results from a cone bipolar cell pair recorded within a few minutes from each other in the same retinal area. Both responses were in their linear range. The cone response was fitted

using an equal time constant filter model consisting of a cascade of seven buffered low-pass filters and time constant 20 ms per stage (Baylor & Hodgkin, 1974; Ashmore & Falk, 1980*a*). Passing this response through the synaptic filter with impulse response $a(t)$ gives the bipolar cell response to a flash of light with a time to peak delayed by 14 ms. The delay found experimentally was 20 ms. In five other cone bipolar cell pairs the mean delay between the peaks of the responses was 17 ms.

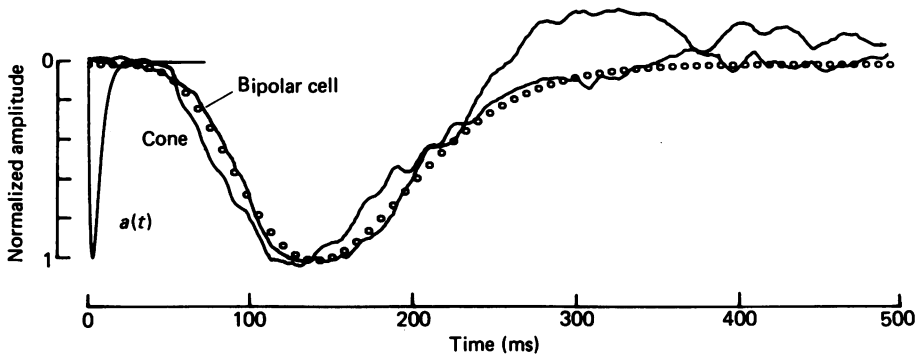

Fig. 13. Filtering properties of the cone-hyperpolarizing bipolar cell synapse. Normalized linear range responses of a cone and a bipolar cell both recorded within a few minutes of each other. The dim flash was delivered at $t = 0$. $a(t)$ shows the impulse response of the synaptic filter, eqn. (19), on the same time scale. O, the computed bipolar cell response obtained by numerically passing a fit to the cone response through a filter with impulse response $a(t)$. See text.

In some bipolar cells, a small after-depolarization was evident following the response even in the linear range. This effect was not predicted from $a(t)$ since this biphasic response is equivalent to a series high-pass stage with cut-off frequency below 0·2 Hz. It was not possible to model accurately any high-pass filtering characteristics since the noise spectral data is unreliable at such low frequencies. Differentiation equivalent to high-pass filtering has been described for the synaptic transfer from cones and rods to horizontal cells in turtle (Schnapf & Copenhagen, 1982) and from cones to ganglion cells in the same retina (Baylor & Fettiplace, 1977).

Responses to flashes of darkness

Depolarizing responses in the bipolar cells could be produced by transiently depolarizing the cone terminal with a 'dark flash' causing a synchronous release of transmitter. The fluctuations in the amplitude of the depolarizing response in the bipolar cell should reflect fluctuations in the number of transmitter-like events contributing to this response.

Flashes of darkness were given on bright light steps by interrupting the beam with an electrically driven shutter for 7 ms. Fig. 14 shows a comparison between the response produced and a linear range responses elicited from the dark using dim light flashes. The time to peak for the dark flash response is greater (155 ms) than the light-elicited response (140 ms), although the response had a shorter duration, and appeared to rise from the base line after a greater delay. These differences are evident in Fig. 14 which shows the spectrum of the noise in the dark, and the modulus squared

of the two responses (inset). The noise in the dark had considerably wider band width than either response. This is consistent with the suggestion that the response to dark flashes is composed of the multiple action of many events of the type $a(t)$.

The technique of matched filtering was used to extract fluctuations of low level responses (Ashmore & Falk, 1980*b*; Baylor, Lamb & Yau, 1979). The result from one

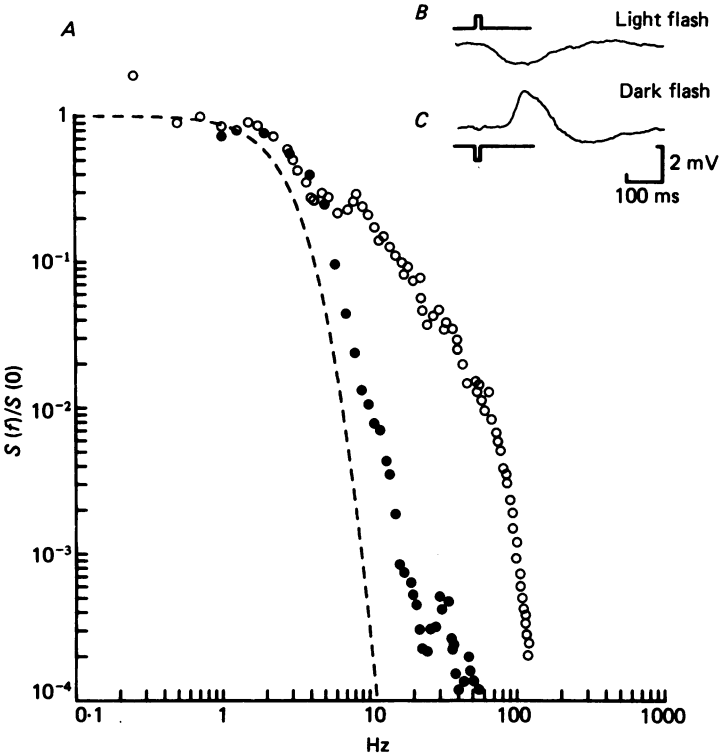

Fig. 14. Comparison of the voltage noise spectra in the dark with the spectrum of responses to short duration stimuli. *A*, ○, difference spectra of noise in bipolar cell between dark and during steady illumination at $1{\cdot}73 \times 10^5$ photons μm^{-2} s^{-1} (100 μm diameter spot). The total variance of the cell in the dark (light) was 0·591 mV^2 (0·140 mV^2). In this experiment, records were filtered using a 50 Hz low-pass filter. ●, modulus squared of the Fourier transform of the response of the cell to 7 ms flashes of darkness delivered during the bright light illumination. Twenty responses were digitized at 1 kHz and averaged before performing the Fourier transform. The scattered points between 20–60 Hz are due to noise remaining after signal averaging. Dotted line, the modulus squared of the Fourier transform of a model fit to the linear range response of the cell to dim light flashes:

$$S(f) = S(0)/(1 + (f/f_b)^2)^8,$$

with $f_b = 7{\cdot}95$ Hz. The model is an equal time constant, 8-stage filter model fit to the linear range response and has been used in preference to the response where noise in the dark obscures the low level signal. The chosen parameters correspond to 140 ms peak latency for the bipolar response. The spectra have been normalized by eye. *B*, response of the cell to 14 ms flashes of light. Average of four sweeps. Time to peak, 140 ms. *C*, response of the cell to 7 ms interruption of steady illumination of the same intensity as in *A*. Average of eight sweeps. The modulus squared of the Fourier transform is shown (●). Cell 12, Table 1.

such experiment is shown in Fig. 15. The fluctuations were small and records containing responses to a dark flash were compared with records containing the base line fluctuations alone. Fig. 15*B* shows the histogram obtained from seventy-four responses to a dark flash. The variance of the ensemble was 0·0967 mV^2. Using the record during bright line alone, matched filtering of thirty-five 2 s frames gave the ensemble variance 0·0795 mV^2.

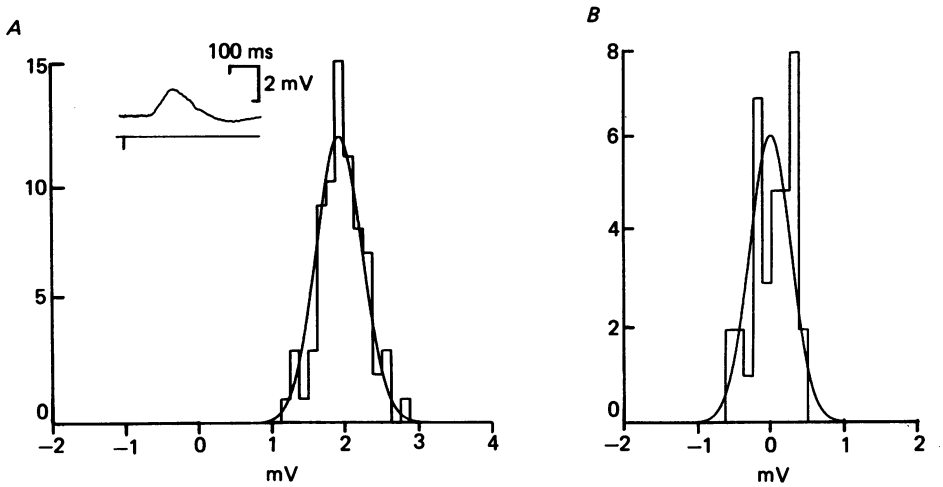

Fig. 15. Matched filtering of responses to a flash of darkness. *A*, histogram obtained by matched filtering of seventy-four responses of a cell to a 7 ms interruption of steady illumination at an intensity of $1·86 \times 10^5$ photons $\mu m^2 s^{-1}$. The cell was hyperpolarized by 13 mV. The template for the filter was the signal average of all the responses, (inset). The continuous curve is a Gaussian with variance $\sigma^2 = 0·097$ mV^2. Abscissa, amplitude of the filter output. Ordinate, number of responses. *B*, histogram obtained by matched filtering thirty-five frames of the record during steady illumination. The same template as in *A* was used to construct the filter. The continuous curve is a Gaussian with variance $\sigma^2 = 0·080$ mV^2. See text.

The ratio of these two variance estimates is 1·22. It may be asked whether the difference is above the chance level. Thus, if the null hypothesis is that the ratio is unity, the ratio of the two variances should be distributed as an F distribution with degrees of freedom 73 and 34. Since $F_{73,34;0·25}$ is about 1·21, there is a probability of about 25% that the observed ratio arises by chance. Thus, although the confidence limits for the event amplitude derived below are low, the estimate derived is to be considered an upper bound for the amplitude derived by this method.

Since the amplitude of the mean response was 2·1 mV, the amplitude of the event underlying these fluctuations would have been

$$(0·0967 - 0·0795)/2·1 = 8·2\ \mu V.$$

This is considerably smaller than found from the spectral analysis. However, the matched filter assumes that the event extracted is of the same duration as the signal average, (since an integral of the response is performed). The discrepency arises if the response is a composite of transmitter events of about 15 ms. Under these

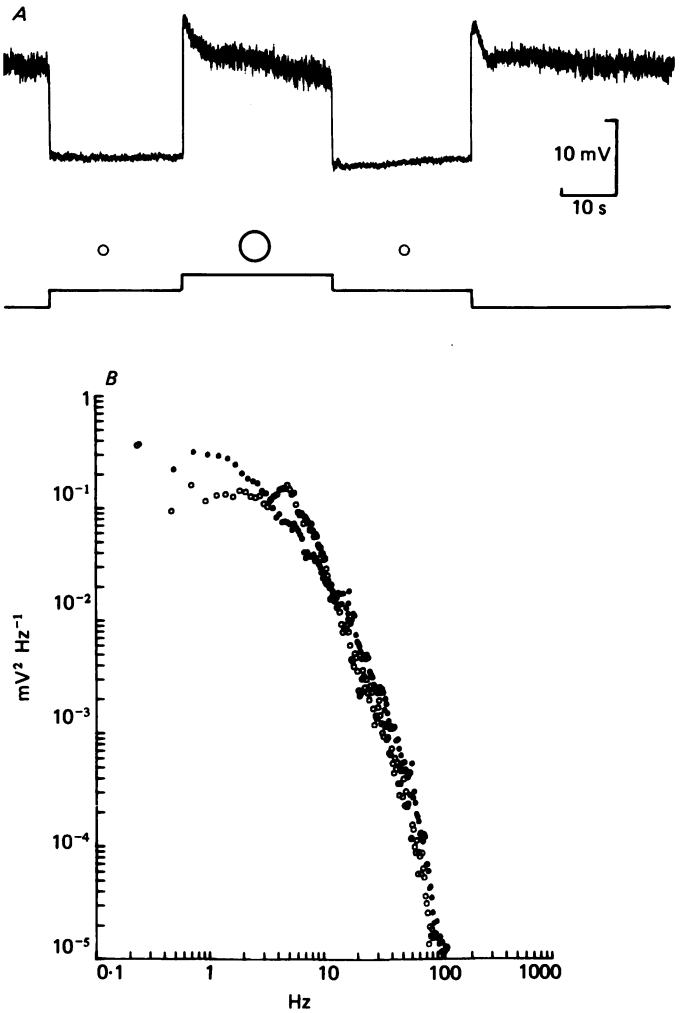

Fig. 16. Noise during surround illumination. *A*, response of a cell to 200 μm diameter spot of light at an intensity of 6×10^5 photons $\mu m^{-2}\ s^{-1}$. At the point indicated the spot diameter was increased to 1500 μm. *B*, noise spectra of the membrane potential with 200 μm *vs.* 1500 μm diameter spot illumination. ●, difference spectra between dark and 200 μm spot illumination; ○, difference spectrum between 1500 and 200 μm diameter spot spectra.

circumstances, the amplitude of the event can be obtained by equating the time integrals, and the peak amplitude would be

$$8{\cdot}2 \times 110\ \text{ms}/15\ \text{ms} = 60\ \mu\text{V},$$

which is within the range found for the transmitter event $a(t)$, (Table 1).

Noise from the surround

Fig. 16 shows the result of an experiment where a small diameter (200 μm) spot centred on a cell was suddenly increased to 1·5 mm and then reduced again. The large

spot repolarized the cell to a potential close to the dark level and increased the noise from 0·144 mV^2 to 1·39 mV^2. The total noise variance, uncorrected for the electrode contribution, was 1·48 mV^2 in the dark.

Fig. 16*B* shows the difference noise spectra. While the spectrum for the noise with small spot illumination had the same shape as that in the dark but scaled down the power axis, the spectra for the large spot and the dark coincided between 15 Hz and 100 Hz. This would be expected in a model for the noise where the unitary events $a(t)$, which contribute to $S_h(f)$, determine the membrane potential. The low frequency portions of the two spectra differed, the spectrum during large spot illumination showing an additional small peak at around 5 Hz and more power near 10 Hz than in the dark. An explanation may be that cone events speed up with light (Lamb & Simon, 1977) and this would be reflected in the voltage noise spectrum in the bipolar cell as the corner frequency of the component S_1 increases. Alternatively, activation of the surround could have altered the characteristics of the reciprocal feed-back onto cones which may be responsible for a slight oscillatory 'ringing' following a short annular flash (Piccolino & Gershenfeld, 1980), as well as oscillations seen in some red cones during sustained annular illumination (M. Piccolino, personal communication), and could have resulted in the small peak observed in the spectrum.

Although a sign-inverting pathway is interposed between the horizontal cells and the bipolar cell (Richter & Simon, 1975; Marchiafava, 1978), it is difficult to reconcile the present result with a direct synaptic input from the horizontal cell onto the bipolar cell unless special conditions are met. Since a large spot would hyperpolarize a horizontal cell further than a small spot, it would be expected that the horizontal cell transmitter output would be reduced by a large spot, and thus produce less noise in the bipolar cell. The opposite was observed. The results are consistent with a surround antagonism mediated by presynaptic modulation of the cone synaptic terminal. Piccolino & Gerschenfeld have provided evidence for a calcium current in cone terminals involved in feed-back from the horizontal cells and have suggested that this may be controlling the surround mechanism in the bipolar cell receptive field organization (Piccolino & Gerschenfeld, 1980). Another possibility is that transmitter released by the horizontal cells competes with the photoreceptor transmitter for post-synaptic sites on the bipolar cell membrane, so that a large spot would have the effect of making available more sites for photoreceptor transmitter on the bipolar cell (Werblin, 1977). In this case, the cell would repolarize while the membrane potential fluctuations also increase. This latter (feed-forward) scheme requires a delicate control mechanism to ensure that the large field response produces no net hyperpolarization of the cell.

DISCUSSION

The methods of this paper allow an analysis of the synaptic events involved in signal transmission at the outer plexiform layer. Five distinct types of events may be distinguished: (a) the response of the cone and (b) the response of the bipolar cell to a flash of light; (c) the noise event in the cone in darkness; (d) event (c) filtered through the synapse and contributing to S_1 in the bipolar cell; and (e) the elementary transmitter event in the bipolar cell noise. A summary of the measurable properties

of these events is given in Table 2. In Table 2, column 1, the single photon events in the cone and in the bipolar cell both depend on the assumed cone collecting area. Since the maximum cone flash sensitivity measured was 34 μV photon^{-1} μm^2, (page 576), it is likely that S_{Fb} was measured when the cone collecting area was below 10 μm^2, the value used by Baylor & Hodgkin (1973). The maximum single photon event in a bipolar cell may thus have been three to four times greater which is

TABLE 2. Summary of the events in signal transmission to bipolar cells

Event	Amplitude (μV)	T_{max} (ms)	Half-width (ms)	Frequency
(a) Cone photon event*	25	120	100	1 per photon
(b) Bipolar photon event	86	140	100	1 per photon
(c) Cone noise event†	100	25	40	1500 s^{-1}
(d) Cone noise event in bipolar cell	190	14	50	1370 s^{-1}
(e) Transmitter event in bipolar cell	70	8	14	11600 s^{-1}

* Data from Baylor & Hodgkin (1973).

† From Lamb & Simon (1977).

The cone signal (a) is the peak response obtained when the network coupling effects have been removed by large field illumination; the single photon signal in the bipolar cell is obtained from the maximal S_{Fb} (Cell 8, Table 1) assuming a cone collecting area 10 μm^2. Events (c), (d) and (e) are inferred from the noise measurements.

indicated by the synaptic gain of 8–13. This event would have been produced if exactly 1 photon were absorbed by every cone in the bipolar cell's receptive field. The elementary transmitter event in the hyperpolarizing bipolar cells had a mean peak amplitude of 70 μV. This result should be contrasted with a similar analysis performed in the depolarizing bipolar cells of the dogfish, where the amplitude of the elementary event associated with transmitter action was 12 μV (Ashmore & Falk, 1982). Allowing for the difference in input resistance between the two cell types, the elementary conductance events activated by transmitter would be comparable. If it is assumed that the input resistance of the bipolar cells recorded here was close to 100 MΩ and the driving force was approximately 40 mV, then the elementary transmitter event would correspond to a conductance change of 17 pS. This figure is close to that for a single transmitter activated channel in many other systems, (Neher & Stevens, 1977). Although release of transmitter from the cone is likely to occur by vesicular release, (Shaffer, Raviola & Heuser, 1982), low concentration of transmitter in the vesicles could contribute to an apparently molecular action of transmitter on the bipolar cells. Dispersion of transmitter by diffusion in the synaptic cleft appears to be unlikely since the light responses can be fitted using the simple filter model without the requirement for additional delays.

The number of transmitter events controlling the membrane potential in bipolar cells was found to be 9200 s^{-1}. Thus if twenty-five cones make synaptic contact with a single bipolar cell, corresponding to a dendritic field diameter of about 50–60 μm, the number of events per cone–bipolar contact would be about 370 s^{-1} and the number of simultaneous events would be five per cone contact. Each cone–bipolar contact may consist of between five to twenty synapses of ribbon or invaginating type, (Dacheux, 1982) so that at each synaptic release site the number of events per second may be

as low as 20 s^{-1}. If each of these events represents a vesicle of transmitter, it may be concluded that, in the dark, the rate of release and recycling of vesicular membrane is not high. If the events represent molecular action of transmitter on the bipolar cell membrane, then the metabolic requirements at the cone synaptic pedicle would be still lower since vesicular release could be below 1 s^{-1}. The low transmitter concentrations are also likely to indicate low post-synaptic receptor densities.

Events attributed to noise in the cones and transmitted through the synapse had a frequency of 1370 s^{-1}. For comparison, the equivalent dark light in the cone was estimated to be 2800 photons/s, (Lamb & Simon, 1977). Using the same methods to estimate the number of cone noise events when the noise in an isolated cone is 0·4 mV^2, an event 100 μV in amplitude, 40 ms integration time and shape factor 0·68 would have a frequency of about 1500 s^{-1}. This figure may be fortuitously close to the value obtained in the bipolar cells. If the bipolar cell dendrite resummed signals from 25 cones, the frequency of unitary events in bipolar cells would be expected to be 25 times greater than in the isolated cone, or approximately 25 times that found here. The source of the discrepency is not clear; however, if the fluctuations of the coupled cone membrane potentials were correlated, then a reduced number of cone events would be counted in the bipolar cell. It is to be noted that any non-linearity in the coupling between presynaptic membrane potential and transmitter release could have the same effect, although still giving rise to a linear transfer of signals between the cone population and the bipolar cell at low light intensities. Alternatively, if the noise in the cones consisted of more than one component, the number of low frequency events transmitted to the bipolar cell would be lower and possibly closer to the present estimate of 55 s^{-1} per cone.

As found in cones, the time integral of the cone noise event in the bipolar cells (190 μV × 50 ms) is close to the event induced by light, (86 μV × 100 ms), suggesting a close connexion between this component of the noise in the visual pathway and transduction mechanisms in the cone.

The synaptic filter

The presence of synaptic noise in the dark limits the effectiveness of the cone hyperpolarizing bipolar cell pathway as the signal path for dim light signals, (Baylor & Fettiplace, 1977; Ashmore & Falk, 1980). The pathway is better suited to conveying information about contrast in the photopic range where the synaptic noise contributes little to the signal. It is seen from Table 1, column 9 that the synaptic noise contributes $1/(1+2{\cdot}73)$ or 27 % of the total noise power present in the bipolar cell in the dark, most of the noise arising as noise events in the cones themselves. The degradation of signal-to-noise produced by synaptic transmission to the bipolar cell would thus be about 17 %. In rod depolarizing bipolar cells, it was estimated that the synaptic component degraded signal-to-noise in the visual pathway by 1·6 at 17 °C, (Ashmore & Falk, 1982), but it was suggested that this figure would be close to 1 at 37 °C because of the high Q_{10} the light transduction step in the rods. Comparable figures for the Q_{10} for transduction noise in cones is not available. However, using psychophysical measures, it has been suggested that the efficiency of human visual signal discrimination may be set by mechanisms in the retina, (Burgess, Wagner, Jennings & Barlow, 1981). The present results support the notion

that it is likely to be the photoreceptors, not the synaptic mechanisms, which limit such discrimination.

As for noise in the visual pathway due to synaptic activity, the constancy of the shape of the spectra at different light intensities also suggests that the synaptic noise contributes a constant fraction of the total noise present at the level of the bipolar cells. Such a noise source is thus multiplicative, rather than additive, to the noise generated in the cone photoreceptors. Multiplicative noise has been implicated in visual detection tasks, (van Meeteren, 1979) although it has been inferred to be a property of the photoreceptoral mechanisms.

The present noise measurements suggest that the synaptic filter between the cones and the hyperpolarizing bipolar cells cuts off cone signals with frequencies above about 20 Hz. At high background levels of illumination, the cone signals speed up, so that the time to peak of the cone signal shortens to about 60 ms, (Baylor & Hodgkin, 1974). The same reduction in time constant is also evident in the noise in the cones themselves, (Lamb & Simon, 1977). The independence of the spectral roll-off frequency and the membrane potential that was observed above suggests that there is no parallel timescale reduction at the synapse, and that the mechanisms of synaptic transfer provide a sufficiently wide passband to response to all the visual signals that are generated in the cone.

We thank Drs J. Belgum, I. J. Russell and J. L. Schnapf for helpful comments on the manuscript and W. Stewart for a gift of Lucifer Yellow. This work was supported by NIH grant EY01869.

REFERENCES

ASHMORE, J. F. & COPENHAGEN, D. R. (1980). Differing postsynaptic events in two types of retinal bipolar cell. *Nature, Lond.* **287**, 84–86.

ASHMORE, J. F. & FALK, G. (1980*a*). Responses of rod bipolar cells in the dark adapted retina of the dogfish, *Scyliorhinus canicula*. *J. Physiol.* **300**, 115–150.

ASHMORE, J. F. & FALK, G. (1980*b*). The single photon signal in rod bipolar cells of the dogfish retina. *J. Physiol.* **300**, 151–166.

ASHMORE, J. F. & FALK, G. (1982). An analysis of voltage noise in rod bipolar cells of the dogfish retina. *J. Physiol.* **332**, 273–297.

BAYLOR, D. A. & FETTIPLACE, R. (1975). Light path and photon capture in turtle photoreceptors. *J. Physiol.* **248**, 433–464.

BAYLOR, D. A. & FETTIPLACE, R. (1977). Kinetics of synaptic transfer from receptors to ganglion cells in turtle retina. *J. Physiol.* **271**, 425–448.

BAYLOR, D. A. & HODGKIN, A. L. (1973). Detection and resolution of visual stimuli by turtle photoreceptors. *J. Physiol.* **234**, 163–198.

BAYLOR, D. A. & HODGKIN, A. L. (1974). Changes in time scale and sensitivity in turtle photoreceptors. *J. Physiol.* **242**, 729–758.

BAYLOR, D. A., LAMB, T. D. & YAU, K-W. (1979). Responses of retinal rods to single photons. *J. Physiol.* **288**, 613–634.

BAYLOR, D. A., MATTHEWS, G. & YAU, K-W. (1980). Two components of electrical dark noise in toad rod outer segments. *J. Physiol.* **309**, 591–621.

BENDAT, J. & PIERSOL, A. G. (1971). *Random Data: Analysis and Measurement Procedures*. New York: Wiley.

BROWN, K. T. & FLAMING, D. G. (1977). New microelectrode techniques for intracellular work in small cells. *Neuroscience* **2**, 813–827.

BURGESS, A. E., WAGNER, R. F., JENNINGS, R. J. & BARLOW, H. B. (1981). Efficiency of human visual signal discrimination. *Science, N.Y.* **214**, 93–94.

COPENHAGEN, D. R. & OWEN, W. G. (1976). Functional characteristics of lateral interactions between rods in the retina of the snapping turtle. *J. Physiol.* **259**, 251–282.

DACHEUX, R. (1982). Connections of the small bipolar cells with photoreceptors in the turtle. An electron microscope study of Golgi-impregnated, gold-toned retina. *J. comp. Neurol.* **205**, 55–62.

DETWILER, P. B., HODGKIN, A. L. & LAMB, T. D. (1983). A note on the synaptic events in hyperpolarizing bipolar cells of the turtle's retina. In *Photoreceptors*, ed. BORSELLINO, A. & CERVETTO, L. New York: Plenum Press.

FAMIGLIETTI, E. V., KANEKO, A. & TACHIBANA, M. (1977). Neuronal architecture of on and off pathways to ganglion cells in the carp retina. *Science, N.Y.* **198**, 1267–1269.

FURUKAWA, T., HAYASHIDA, Y. & MATSUURA, S. (1978). Quantal analysis of the size of excitatory post-synaptic potentials at synapses between hair cells and afferent nerve fibres in goldfish. *J. Physiol.* **276**, 211–226.

HAGINS, W. A. (1965). Electrical signs of information flow in photoreceptors. *Cold Spring Harb. Symp. quant. Biol.* **30**, 403–418.

KATZ, B. & MILEDI, R. (1972). The statistical nature of the acetylcholine potential and its molecular components. *J. Physiol.* **224**, 665–699.

LAMB, T. D. & SIMON, E. J. (1977). Analysis of electrical noise in turtle cones. *J. Physiol.* **272**, 425–468.

LASANSKY, A. (1978). Contacts between receptors and electrophysiologically identified neurones in the retina of the larval tiger salamander. *J. Physiol.* **285**, 531–542.

MARCHIAFAVA, P. L. (1978). Horizontal cells influence membrane potential of bipolar cells in the retina of the turtle. *Nature, Lond.* **275**, 141–142.

NEHER, E. & STEVENS, C. F. (1977). Conductance fluctuations and ionic pores in membranes. *Ann. Rev. Biophys. Bioeng.* **6**, 345–381.

PICCOLINO, M. & GERSCHENFELD, H. M. (1980). Characteristics of ionic processes involved in feedback spikes of turtle cones. *Proc. R. Soc.* B **206**, 439–463.

RICE, S. O. (1944). Mathematical analysis of random noise. *Bell Syst. tech. J.* **23**, **24**, 1–162, reprinted in *Selected Papers on Noise and Stochastic Processes* (1954), ed. WAX, N. New York: Dover.

RICHTER, A. & SIMON, E. J. (1975). Properties of centre-hyperpolarizing, red-sensitive bipolar cells in the retina of the turtle. *J. Physiol.* **148**, 317–334.

SAITO, T., KONDO, H. & TOYODA, J-I. (1979). Ionic mechanisms of the two types of on-centre bipolar cell in the carp retina. I. The responses to central illumination. *J. gen. Physiol.* **73**, 73–90.

SCHAFFER, S., RAVIOLA, E. & HEUSER, J. E. (1982). Membrane specialization in the outer plexiform layer of the turtle retina. *J. comp. Neurol.* **204**, 253–267.

SCHNAPF, J. & COPENHAGEN, D. R. (1982). Differences in kinetics of rod and cone synaptic transmission. *Nature, Lond.* **296**, 862–4.

SCHWARTZ, E. A. (1974). Responses of bipolar cells in the retina of the turtle. *J. Physiol.* **236**, 211–224.

SCHWARTZ, E. A. (1977). Voltage noise observed in rods of the turtle retina. *J. Physiol.* **272**, 217–246.

SIGWORTH, F. J. (1981). Interpreting power spectra from nonstationary membrane current fluctuations. *Biophys. J.* **35**, 289–300.

SIMON, E. J., LAMB, T. D. & HODGKIN, A. L. (1975). Spontaneous voltage fluctuations in retinal cones and bipolar cells. *Nature, Lond.* **256**, 661–662.

TOYODA, J. I. (1973). Membrane resistance changes underlying the bipolar cell response in carp retina. *Vision Res.* **13**, 283–294.

VAN MEETEREN, A. (1978). On the detective quantum efficiency of the human eye. *Vision Res.* **18**, 257–268.

WEILER, R. & MARCHIAFAVA, P. L. (1981). Physiological and morphological study of the inner plexiform layer in the turtle retina. *Vision Res.* **21**, 1635–38.

WERBLIN, F. S. (1977). Synaptic interaction mediating bipolar response in the retina of the tiger salamander. In *Vertebrate Photoreception*, ed. BARLOW, H. B. & FATT, P., pp. 205–230. London: Academic Press.