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ANALYSIS OF ELECTRICAL NOISE IN TURTLE CONES

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SUMMARY

1. Properties of the light-sensitive voltage noise in cones in the retina of the turtle, *Pseudemys scripta elegans*, have been studied by intracellular recording.

2. Suppression of the noise by light was a function of the hyperpolarizing response of a cone but not of the size or pattern of illumination.

3. Power density spectra of the noise were fitted in many cones by the product of two Lorentzians with characteristic time constants τ_1 and τ_2 averaging 40 and 7 msec respectively. The spectra of some cells were peaked and could be fitted by a resonance curve.

4. Spectra in dim light exhibited decreased low frequency power. They could often be fitted by a product of two Lorentzians using the same value of τ_2 as used in darkness but decreasing τ_1 and the zero frequency asymptote. An e-fold reduction in τ_1 occurred with lights which hyperpolarized by 4–7 mV.

5. Injection of hyperpolarizing currents of about 0·1–0·2 nA into weakly coupled cones reduced the noise, and also reduced the sensitivity to dim flashes.

6. The variance-voltage relation during steady illumination of different intensities differed from cone to cone. Dim lights increased the noise in some cells and decreased it in others, but moderately bright lights which gave steady responses of more than about one third maximal reduced the noise in all cells.

7. When the cell was transiently depolarized during the differentiated component following steady illumination, the noise was less than it was after prolonged darkness.

8. In the after-effect of bright light, the time course of recovery of noise was the same as that of flash sensitivity and voltage. The noise was reduced

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e-fold for hyperpolarizations averaging 3 mV while for sensitivity this reduction occurred for 1·3 mV. For a given hyperpolarization the noise was lower during the after-effect than during steady dim illumination.

9. When a series of dim flashes was delivered to a cone, no significant increase in variance over the dark noise was detected during the photoresponse. This implies that each photoisomerization evokes no more than about 1·5 μV at the peak of the response in a coupled cone, corresponding to about 50 μV in an isolated cone.

10. The elementary shot events underlying the noise are about 100 μV in amplitude in an isolated cone, have a characteristic time constant of 16–60 msec and reflect unit conductance fluctuations of about 16 pS (S, Siemen $\equiv \Omega^{-1}$).

11. It is concluded that the noise source is internal to the cones. We postulate that the noise arises from opening and closing of the light-sensitive ionic channels in the outer segment, and that in darkness there is a residual concentration of the blocking substance which on average closes up to about one third of the channels. It seems likely that the unit event involves a considerable number of blocking molecules and ionic channels.

INTRODUCTION

Cones in the turtle retina are noisy in darkness and become quieter during bright illumination (Simon, Lamb & Hodgkin, 1975). The magnitude of the intrinsic noise (variance in darkness minus variance in bright light) varies over a 50-fold range from cone to cone (Lamb & Simon, 1976*b*), and this has been attributed to variation in the degree of electrical coupling between neighbouring cones, all of which act as similar noise sources. The purpose of this paper is to investigate the nature of that source and its relevance to phototransduction. The results suggest that the noise arises from fluctuations in the number of conducting light-sensitive ionic channels in the outer segment, and on this basis certain properties of the transduction mechanism can be investigated. Recent results from turtle rods (Schwartz, 1977) indicate the existence of noise with similar properties to that in cones, but suggest that it may have a different origin.

Preliminary power spectral measurements have been presented previously (Lamb & Simon, 1976*a*).

METHODS

Recording and stimulation. The methods of stimulating and recording from cones in the isolated eye-cup of the turtle, *Pseudemys scripta elegans*, were the same as described by Lamb & Simon (1976*b*). An exception was that the shutter in the second optical beam was operated by a powerful stepping motor (Tormax 020–004,

IMC Magnetics Corp., Maywood, Calif.) which provided a rise time and fall time of about 0·6 msec with a highly reproducible stimulus duration, as this was found to be important in the repetitive flash experiments.

Power spectral analysis. Data recorded on magnetic tape were digitized by the methods of Lamb & Simon (1976*b*). Power spectra were calculated by the fast Fourier transform method according to the procedure of Bendat & Piersol (1971, p. 327). As a rule each record contained 2048 samples at 2·5 msec intervals. The calculated spectral values were usually averaged over groups of five adjacent frequency points, and over as many records as were available. Before digitizing, the signal was filtered with a 1 sec RC low cut (half-power at 0·16 Hz) and a 3-pole Butterworth low pass filter with a half-power frequency of about 100 Hz, and no correction has been made to spectra for these effects.

RESULTS

Localization of the noise source

It is important to determine whether the source of noise is ternal to cones or whether it arises from synaptic input from other cell . Lamb & Simon (1976*b*) showed that the noise was largest in those cones which were not coupled to others so that it could not have arisen in cone-cone junctions. The only reported synaptic input to turtle cones is from horizontal cells (Baylor, Fuortes & O'Bryan, 1971) and there is no evidence to suggest input from other cell types. As turtle horizontal cells have large receptive fields and do not respond effectively to small areas of illumination (Simon, 1973), the relationship between noise suppression and pattern of illumination should reveal whether these cells are involved.

It was consistently found that small centred areas of bright illumination completely suppressed the dark noise (Simon *et al.* 1975; Lamb & Simon, 1976*b*) and this itself is strong evidence that horizontal cells are not involved. The effect of stimulus pattern was examined more fully for the isolated red-sensitive cone of Fig. 1. In *A* a 6 μm diameter spot and a 210 μm spot caused about the same degree of quieting when the relative intensities were adjusted to elicit responses of similar amplitudes. The intensity required with the very small spot was about 18 times greater than with the larger spot, and this is almost certainly attributable to attenuation as a result of light scatter (see Baylor *et al.* 1971). In Fig. 1*B* the responses to both a 6 μm spot and to an annulus (630 μm inner diameter, 1300 μm outer diameter) were again very similar with regard to both noise and response amplitude. In this case the annulus was about 4 times brighter than the small spot because the cone, located at its centre, was stimulated only by scattered light.

The extremely narrow spatial profile of this cell (see Lamb & Simon, 1976*b*, Fig. 1*B*) suggested that it was an isolated cone receiving no input from other cones, and it seems reasonable to assume that responses of

similar amplitude indicate the absorption of similar numbers of photons. On this basis the central incident intensity was the same for the stimulus pair in *A* and for the pair in *B*. Any cell with a larger receptive field, centred at the same position, would therefore receive relatively greater stimulation for the pattern of larger area. Hence all other nearby cells must have responded in a different way to stimulus pairs which gave identical responses in the isolated cone. As the cone noise was similar in

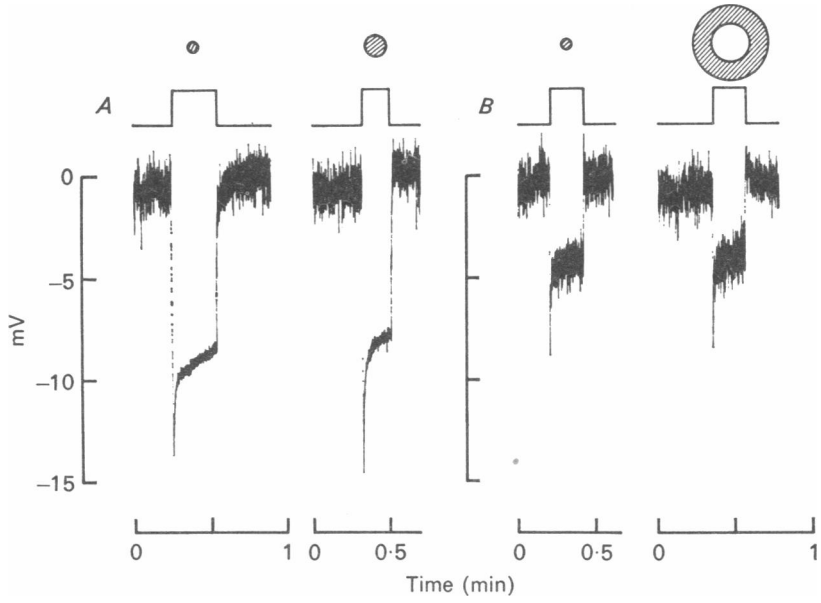

Fig. 1. Responses of an isolated red-sensitive cone to different patterns of light (639 nm, monitored at top). *A*, left: 6 μm diameter spot, $8{\cdot}4 \times 10^5$ photons μm^{-2} sec^{-1}; right: 210 μm spot, $4{\cdot}7 \times 10^4$ photons μm^{-2} sec^{-1}. *B*, left: 6 μm spot, 10^4 photons μm^{-2} sec^{-1}; right: annulus (inner diameter 630 μm, outer diameter 1300 μm), $4{\cdot}7 \times 10^4$ photons μm^{-2} sec^{-1}. The spatial profile of this cell is shown in Fig. 1*B* of Lamb & Simon (1976*b*).

each response pair despite different responses in other cells, we conclude that the noise does not depend substantially on the activity of any other cell type and that it is intrinsic to the cone.

To obtain an approximate idea of the relative responses of horizontal cells to stimuli of the three geometries, use is made of eqn. (6) of Lamb (1976). This shows that, within the linear range, a horizontal cell with a very large receptive field (λ = 1000 μm) will give responses roughly in the ratio 1:500:5000 for a 6 μm spot, 210 μm spot and the annulus mentioned above each of the same intensity. For a very small field horizontal cell (λ = 100 μm) the ratio would be about 1:200:50. Taking into account

the differences in applied intensity these ratios become roughly 1:30:20,000 and 1:12:200 respectively, showing that all horizontal cells would have responded much less to the small spot than to either the larger spot or the annulus despite the changed intensity.

A possible objection to this interpretation is that activation of the cone might evoke local responses in the horizontal cell terminals which affect

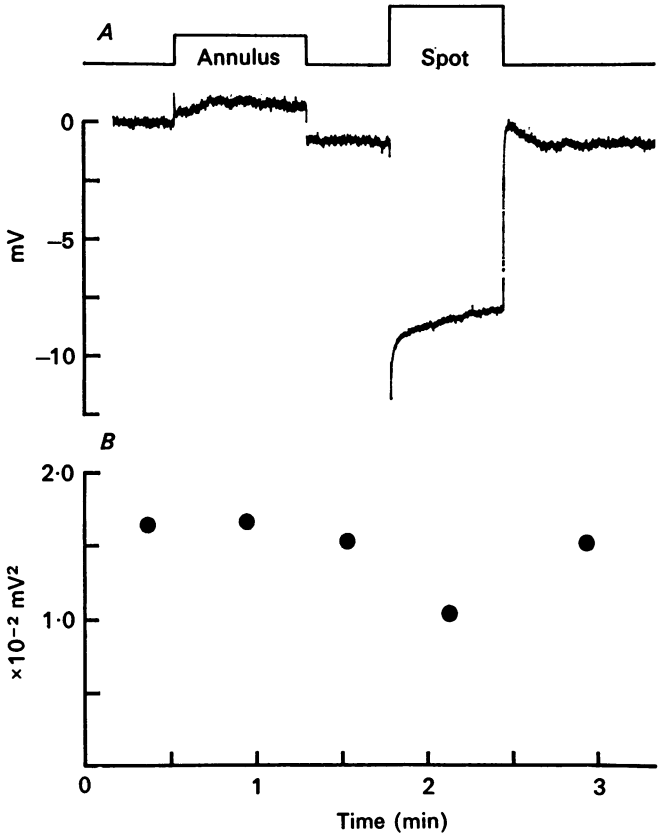

Fig. 2. Responses of a green-sensitive cone to a red annulus and to a green centred spot. *A*, annulus, inner diameter 385 μm, outer diameter 1300 μm, $2{\cdot}3 \times 10^7$ photons μm^{-2} sec^{-1} at 742 nm. Centred spot, 105 μm diameter, $7{\cdot}6 \times 10^6$ photons μm^{-2} sec^{-1} at 558 nm. *B*, each point is the mean variance measured over the indicated period of light or dark.

synaptic transmission without propagating to the cell body, but this notion is not consistent with the experiment of Fig. 2. In this green-sensitive cone a deep red annulus caused a depolarization of a few mV as a result of hyperpolarization of luminosity horizontal cells (Fuortes, Schwartz & Simon, 1973) but caused no significant change in noise. In

contrast a small green spot produced the usual hyperpolarizing response and a significant reduction in noise.

Power spectrum of the noise

The power spectrum of the voltage noise in a weakly coupled red-sensitive cone is plotted in Fig. 3 for darkness (●), dim light (+) and bright light (○). The points near 50, 100 and 150 Hz are the result of mains interference and its harmonics, and although unseemly provide a useful

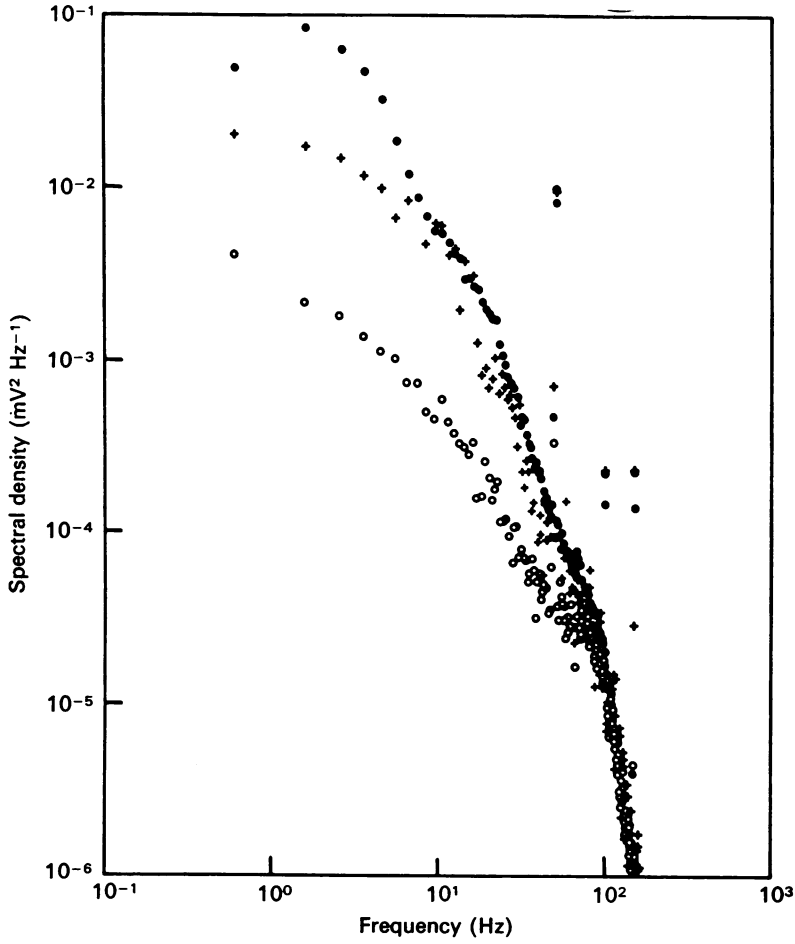

Fig. 3. Power spectral density of voltage noise in an isolated red-sensitive cone (cell 2 of Tables 1 and 2, and cell 1 of Table 1 in Lamb & Simon, 1976*b*). ●, darkness: variance 0·378 mV^2, record length 159 sec. +, dim light, $4{\cdot}7 \times 10^4$ photons μm^{-2} sec^{-1}, 639 nm: 0·154 mV^2, 10 sec. ○, bright light, $1{\cdot}9 \times 10^7$ photons μm^{-2} sec^{-1}, 639 nm: 0·031 mV^2, 31 sec. Stimulus diameter 6 μm. Points near 50, 100 an 150 Hz result from mains interference.

frequency calibration check. The low-pass active filter attenuated heavily above about 100 Hz to prevent 'aliasing' errors resulting from frequency components higher than the Nyquist limit, in this case 200 Hz for a sampling interval of 2·5 msec. On many occasions the noise was higher in bright light than outside the cell (not measured in this case), but in other cases the two spectra were closely similar. We believe changes in electrode properties on withdrawal to be responsible for the discrepancy, and although it is possible that some small amount of physiological noise remains in bright light, we have used this spectrum as a base line to subtract from spectra in darkness and in dim lights. The term 'difference spectrum' will be used loosely to refer to any spectrum from which the bright light spectrum has been subtracted. In most cases the bright light spectra were predominantly $1/f$, which is characteristic of the noise seen with glass micro-electrodes (De Felice & Firth, 1970). Above about 80 Hz the spectra in Fig. 3 converge and are dominated by electrode noise, so that it is pointless to attempt to extend the analysis to higher frequencies by employing a shorter sampling interval.

The difference spectra obtained from Fig. 3 are plotted in Fig. 4, and below 50 Hz the form is little changed from the raw spectra because of the moderately large variance ratio. The curves, which have been fitted by eye to both sets of points, are described by the equation

$$S(f) = \frac{S_0}{[1+(2\pi f\tau_1)^2]\,[1+(2\pi f\tau_2)^2]}, \tag{1}$$

the product of two Lorentzians. $S(f)$ is the power spectrum as a function of frequency f, S_0 is the low frequency asymptote and τ_1 and τ_2 are time constants. In Fig. 4 τ_2 is 7·5 msec for both curves and S_0 and τ_1 are respectively $9{\cdot}5\times10^{-2}$ mV^2 Hz^{-1} and 52 msec in darkness and $1{\cdot}5\times10^{-2}$ mV^2 Hz^{-1} and 24 msec for the steady light which hyperpolarized the cell by 6·5 mV and reduced the variance to 38 % of its dark level.

The form of spectrum, calculated on the assumption that each elementary event has the time course of the observed dim flash response, failed to fit the data of Fig. 4 or of any other cell. The expected spectrum in that case is the square of the modulus of the Fourier transform of the flash response. In darkness the flash response is given by eqn. (41) of Baylor, Hodgkin & Lamb (1974*a*), and by transforming their eqn. (34) the spectrum may be shown to be

$$S(f) = S_0 \prod_{i=1}^{n} \frac{1}{\left[1+\left(\frac{2\pi f\tau}{i}\right)^2\right]}, \tag{2}$$

where n is the number of stages involved in the formation of the active substance and τ is a time constant of about 60 msec. Such a spectrum

falls at high frequency with a slope of $2n$ decades per decade and, for τ = 60 msec (Baylor *et al.* 1974*a*) has a half-power frequency of about 2 Hz. This spectrum is shown by the interrupted curve in Fig. 4, and bears little resemblance to the observed spectrum.

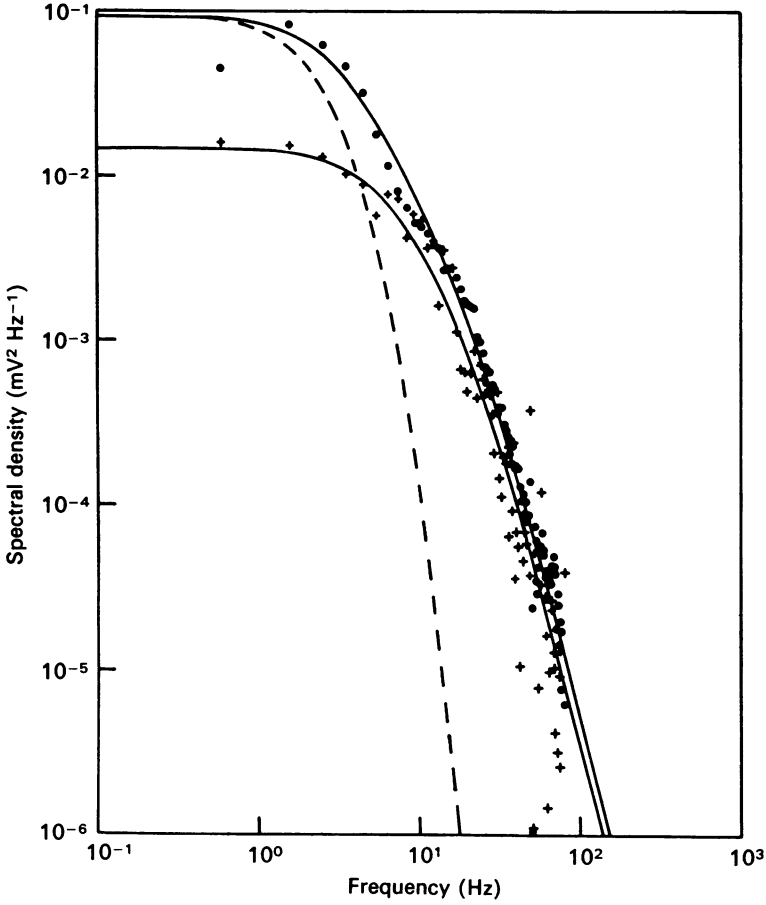

Fig. 4. Difference spectra for the cone of Fig. 3 compared with eqn. (1). ●, spectrum in darkness minus spectrum in bright light, $S_0 = 9{\cdot}5 \times 10^{-2}$ $mV^2\,Hz^{-1}$, τ_1 = 54 msec, τ_2 = 6 msec. +, dim light minus bright light, $S_0 = 1{\cdot}5 \times 10^{-2}$ $mV^2\,Hz^{-1}$, τ_1 = 26 msec, τ_2 = 6 msec. With dim light the steady hyperpolarization was 6·5 mV.

Raw and difference spectra for a tightly coupled cone are shown in Fig. 5 for comparison. The ratio of raw spectra (A) between darkness and light is considerably smaller than for the previous cell and reflects the smaller variance ratio. This limits the maximum useful frequency to about 60 Hz in the difference spectrum and points beyond this have not

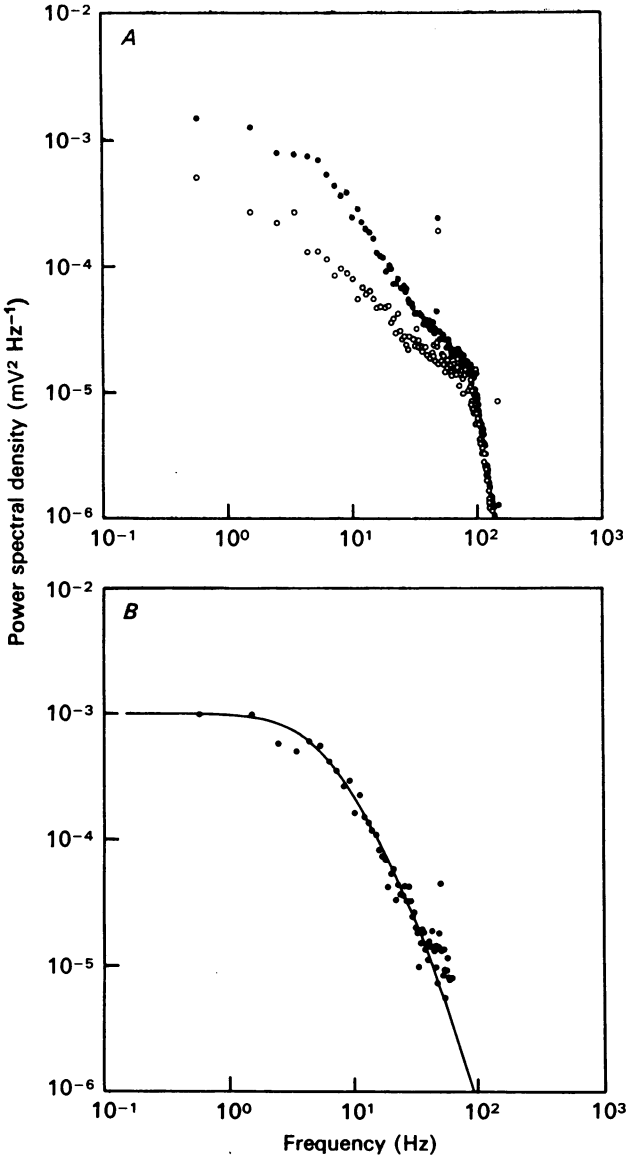

Fig. 5. Raw and difference spectra for a tightly coupled red-sensitive cone (cell 24 of Table 1). *A*, raw spectra. ●, darkness: variance 0·012 mV^2, record length 123 sec. ○, bright light: 0·0042 mV^2, 67 sec. *B*, difference spectrum of dark minus light. Curve plots eqn. (1) for $S_0 = 10^{-3}$ mV^2 Hz^{-1}, $\tau_1 = 29$ msec, $\tau_2 = 3$ msec.

been plotted. Eqn. (1) has again been fitted by eye to the difference spectrum and this gave $S_0 = 10^{-3}$ mV^2 Hz^{-1}, $\tau_1 = 29$ msec, $\tau_2 = 3$ msec.

In twenty-five red-sensitive cells in which difference spectra were measured, eqn. (1) provided a good fit on sixteen occasions. Values of τ_1 ranged from 16 to 60 msec with a mean of 40 msec, while τ_2 ranged from 2 to 14 msec with a mean of 7·4 msec. The fits were obtained by eye and would allow a variation of 20 % or so in the parameters, but it is clear that the time constants in different cells vary over a much wider range than this. Fig. 6 illustrates spectra from four different cells which span much of the range encountered, and Table 1 summarizes all cells for which satisfactory difference spectra were obtained. In Fig. 6, *A* and *B* are from noisy cells while *C* and *D* are from quiet cells. In *A* a moderately large τ_1 of 42 msec was required while in *B* the shortest τ_1 of 16 msec was used. The points in *D* for a green-sensitive cone could not be fitted with a product of Lorentzians, but instead it was found that the peaked spectrum could be described fairly well with a resonance curve. The curve is for a spectrum

$$S(f) = S_0\left(\frac{a^2+b^2}{a}\right)^2 \frac{a^2+(2\pi f)^2}{[a^2+b^2-(2\pi f)^2]^2+(4\pi a f)^2} \tag{3}$$

which corresponds to an impulse response of the form

$$e^{-at} \cos bt. \tag{4}$$

With the values $a^{-1} = 44$ msec and $b^{-1} = 36$ msec used in Fig. 6*D* the curve displays a peak of about twice the low frequency asymptote. This was the most pronounced example in five red- and three green-sensitive cones which showed a definite peak in the vicinity of 5–10 Hz. The spectra of the four remaining red-sensitive cones could not satisfactorily be fitted. Of the total of four green-sensitive cones only one was fitted by eqn. (1), the other three being peaked.

The usual effect of dim lights, which caused a moderate variance reduction, was to depress the spectrum at low frequencies without causing substantial change in the high frequency behaviour (see for example Fig. 4 and Fig. 6*C*). It was found that as a rule such spectra could be fitted with a product of two Lorentzians using the same value of τ_2 as used in darkness but decreasing S_0 and τ_1. The reduction of τ_1 with light-induced voltage was quite steep, and an e-fold change occurred for a hyperpolarization of about 4–7 mV in different cells (see Table 1). In one cell (Table 2, cell 3), which in darkness had a spectrum fitted by eqn. (1), the reduction of low frequency components by dim light led to a peaked spectrum. This may be related to the fact that in dim light the flash response typically has a biphasic nature (Baylor & Hodgkin, 1974).

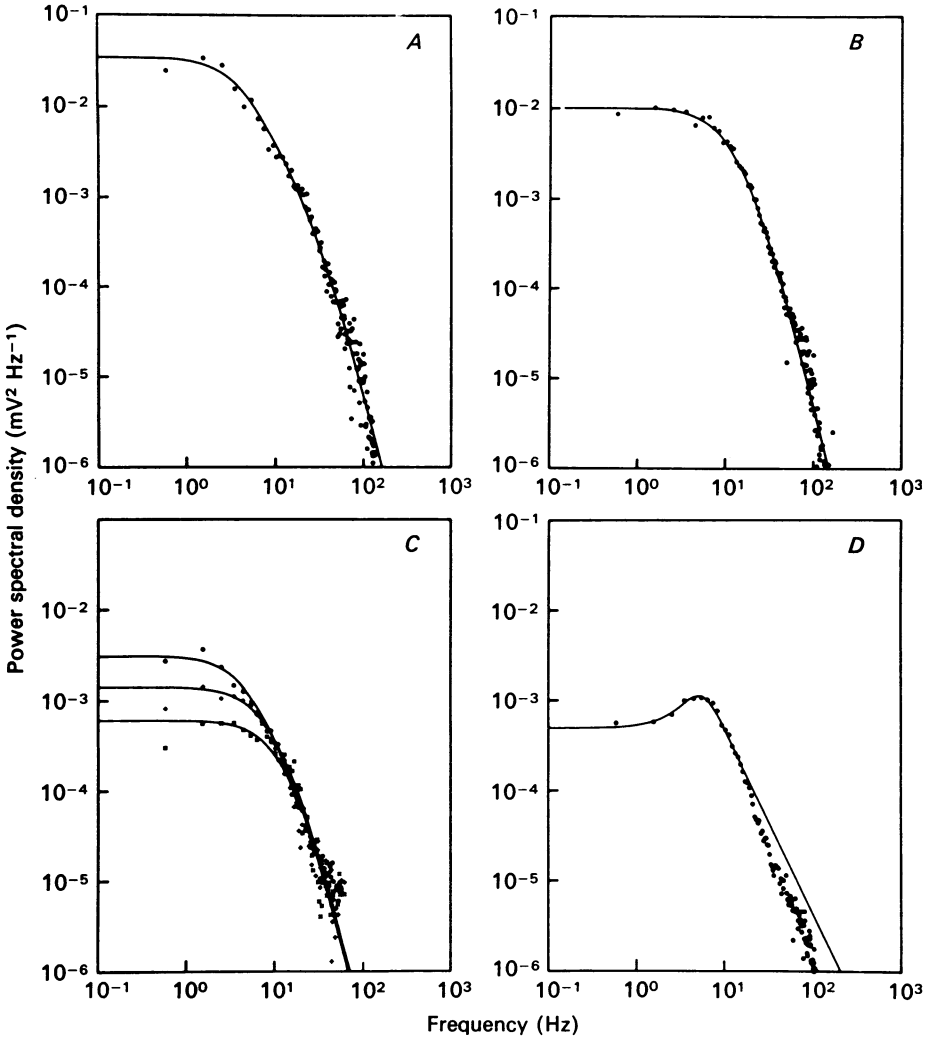

Fig. 6. Difference spectra for four cones. *A*, *B* and *C* are for red-sensitive cones, and *D* is for a green-sensitive cone. The respective values of: variance (with bright light subtracted), record length, record length in bright light, and S_0, τ_1, τ_2 in eqn. (1) for the curves in *A*–*C* are: *A*, 0·17 mV^2, 72 sec, 31 sec, $3{\cdot}4 \times 10^{-2}$ mV^2 Hz^{-1}, 42 msec, 4 msec. *B*, 0·11 mV^2, 148 sec, 26 sec, 10^{-2} mV^2 Hz^{-1}, 16 msec, 7 msec. *C*, ●, (dark) 0·016 mV^2, 210 sec, 97 sec, $3{\cdot}1 \times 10^{-3}$ mV^2 Hz^{-1}, 39 msec, 8 msec. +, (dim) 0·010 mV^2, 51 sec, 97 sec, $1{\cdot}4 \times 10^{-3}$ mV^2 Hz^{-1}, 23 msec, 8 msec; 3·5 mV steady hyperpolarization. ×, (dim) 0·0070 mV^2, 46 sec, 97 sec, 6×10^{-4} mV^2 Hz^{-1}, 16 msec, 8 msec; 5·7 mV steady hyperpolarization. *D*, 0·013 mV^2, 394 sec, 77 sec, 5×10^{-4} mV^2 Hz^{-1}; see text for curve.

TABLE 1. Power spectral measurements

Cell	$\sigma^2(V)_{D-L}$ (mV^2)	T_D (sec)	S_0 ($\times 10^{-3}$ $mV^2\,Hz^{-1}$)	τ_1 (msec)	τ_2 (msec)
			Red-sensitive cones		
1	0·041	82	8·5	36	12
2	0·35	159	95	54	6
3	0·11	148	10	16	7
4	0·075	133	10	21	10
5	0·012	307	1·7	—	
6	0·010	451	1·1	—	
7	0·15	113	23	22	14
8	0·070	82	20	60	6
9	0·11	20	21	40	8
10	0·032	92	8·1	51	10
11	0·011	143	1	Peaked	
12	0·012	72	1·4	23	2
13	0·012	225	1	Peaked	
14	0·11	25	10	Peaked	
15	0·43	72	120	60	12
16	0·038	133	11	44	11
17	0·014	174	2	—	
18	0·11	118	10	Peaked	
19	0·011	174	1·5	—	
20	0·0065	189	1	—	
21	0·016	210	3·1	39	8
22	0·018	82	4	46	5
23	0·17	72	34	42	4
24	0·0075	123	0·93	29	3
25	0·0088	179	1·8	44	2
26	0·064	92	16	53	5
Mean				40·0	7·4
			Green-sensitive cones		
27	0·014	159	1	Peaked	
28	0·019	297	1	Peaked	
29	0·011	394	0·5	Peaked	
30	0·012	82	1·2	19	6

$\sigma^2(V)_{D-L}$ is the intrinsic voltage variance (dark minus bright light); T_D is the record length in darkness used in calculating the spectrum; S_0, τ_1 and τ_2 are the parameters in eqn. (1) which gave the best fit by eye to the points in the difference spectrum. Dashes indicate that eqn. (1) could not be fitted satisfactorily.

Time course of the elementary event

The fact that the spectrum of the noise is not described by the square of the Fourier transform of the small signal response indicates that the noise is not made up of elementary events having the shape of the flash response. The spectrum in the majority of cells was instead well described by a product of two Lorentzians. On the assumption that the cell's electrical

behaviour can be represented as a pure resistance-capacitance, the voltage noise resulting from conductance fluctuations would be filtered by a single RC, accounting for one Lorentzian. The shorter time constant averaged 7·4 msec, and we ascribe this to the cell's capacitive filtering because it is in reasonable agreement with measurements made with current injection (Baylor *et al.* 1974*a*). Although intercellular coupling will complicate the capacitive filtering, calculations showed this to be a fairly small effect, principally causing the apparent time constant of the network to be shorter than the RC of individual cells.

TABLE 2. Power spectra in dim light

Cell	$\sigma^2 (V)_{diff}$ (mV^2)	T_D (sec)	S_0 ($\times 10^{-3}$ mV^2 Hz^{-1})	τ_1 (msec)	τ_2 (msec)	U_s (mV)	U_{max} (mV)
2	0·35	159	95	54	6	0	
	0·12	10	15	26	6	6·5	13·5
3	0·11	148	10	16	7		
	0·059	36	2	Peaked		3·7	10
7	0·15	113	23	22	14	0	
	0·045	46	5·1	14	14	1·2	7
21	0·016	210	3·1	39	8	0	
	0·010	51	1·4	23	8	3·5	11
	0·0070	46	0·6	16	8	5·7	
29	0·011	394	0·5	Peaked		0	
	0·010	61	1	14	14	0·5	8
	0·0064	36	0·5	11	11	2·8	

Cell numbers are those used in Table 1. U_s is steady hyperpolarization in the appropriate dim light; U_{max} is the maximum steady hyperpolarization in bright light.

The longer time constant averaging 40 msec is then attributed to the frequency behaviour of the noise current and (as the driving voltage is nearly constant) of the noise conductance. Its interpretation depends on whether the elementary event represents, for example, closure of a single channel or perhaps the response to a single blocking particle. As 40 msec is considerably less than the removal time constant $\bar{\kappa}_{12}^{-1} = 100$ msec of Baylor, Hodgkin & Lamb (1974*b*), the spectral data does not appear consistent with their last stage long and rapid reversible binding model. Part of the discrepancy might have arisen because of insufficient dark adaptation in our case, but unfortunately we did not routinely measure the limiting time to peak of the dim flash response, and so the data are not directly comparable. The difficulty with a final stage time constant in darkness of 40 msec is that it is unlikely to be able to explain the speeding up of the response with adapting lights (see Baylor *et al.* 1974*b*).

Variation of noise with voltage

Dependence of noise on injected current

A straightforward way to examine the relation between noise and voltage is to polarize the cell by injecting current through the recording electrode, but in practice this experiment proved unsatisfactory. In the first place the electrode usually generated considerable noise when passing current, and secondly there was uncertainty in the degree of polarization as a result of difficulty in balancing the bridge circuit. Electrode noise was particularly severe in tightly coupled cells because of their lower voltage variance and because the required currents were larger. An additional problem is that in a strongly coupled network, current injected at a point is a very unsatisfactory method of uniformly polarizing the cell membranes (see Jack, Noble & Tsien, 1975).

In spite of these difficulties one result which we were able to obtain consistently was that hyperpolarizing currents (i.e. inward membrane current) of about 0·1 nA could substantially reduce the noise in weakly coupled cells. In addition, a few experiments suggested that injection of currents of up to 0·1 nA of either polarity during the application of bright lights produced almost no change from the low noise level. This and other experiments described in the following sections indicated that although the noise displays a voltage sensitivity it is not solely voltage dependent. We were not able to obtain reproducible measurements either of power spectra during current passage or of variance with positive current.

Voltage-dependence of sensitivity

A possible way that hyperpolarization could decrease the noise is by desensitizing the cone. Fig. 7 is an example of an experiment in which the flash sensitivity of a coupled red-sensitive cone was measured during passage of current through the recording electrode. The DC levels during current passage have been shifted arbitrarily, and each tracing is the computer average of eleven to seventeen responses. The excellent stability of the cell is clear as the experiment was performed in the indicated sequence. Current of $-0{\cdot}1$ nA decreased the small-signal response by 25 % while $+0{\cdot}1$ nA increased it by 30 %. This experiment was repeated in four other cells and hyperpolarizing currents of 0·1 nA produced desensitization of the small-signal response of 17, 13, 0 and 36 % respectively. With depolarizing currents the cell in Fig. 7 provided the largest sensitivity increase observed; in the other cells changes of $+22$, 0, -50 and -45 % were found.

A quantitative estimate of the effect of current passage into a cell in a

tightly coupled network can be calculated from the distributed model of the cone array (Lamb & Simon, 1976*b*). We will assume that the sensitivity of a cell is a function of voltage and that, for small responses, the desensitization is proportional to hyperpolarization. Calculations then show that, relative to an isolated cell, the effectiveness of a given current in changing the sensitivity of a coupled cell is the same function of the

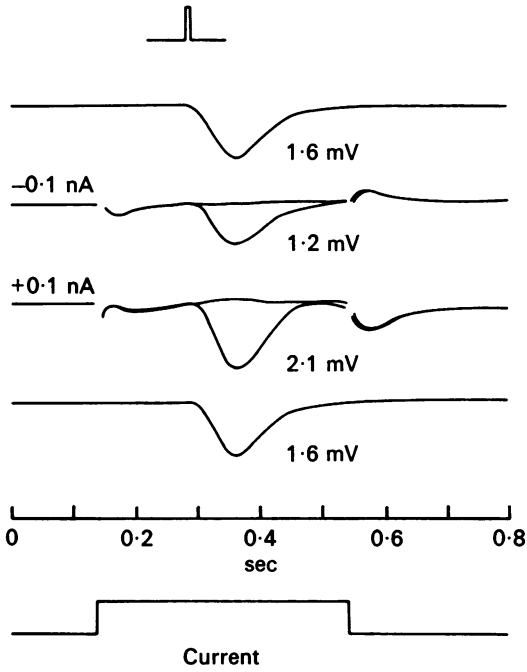

Fig. 7. Effect of injected current on flash sensitivity. Each trace is the computer average of eleven to seventeen responses, and records were obtained in the sequence from top to bottom. During current passage responses were recorded with and without flashes, and the DC levels are arbitrarily shifted. Voltages are measured peak responses. Flash 10 msec, 639 nm, 220 photons μm^{-2}, 105 μm diameter spot, red-sensitive cone, U_{max} 12·5 mV.

tightness of coupling as is the dark noise. Denoting i as the current required to produce a given change in flash sensitivity in a coupled cell and i_{isol} as the equivalent value in an isolated cell, it is found that

$$\frac{i}{i_{isol}} = 4\pi\left(\frac{\lambda}{D}\right)^2 \tag{5}$$

analogous to eqn. (6) of Lamb & Simon (1976*b*) where λ was defined as the network length constant and D the mean cell spacing. Assuming an input resistance of 200 MΩ for an isolated cell, a current of $-0{\cdot}1$ nA would hyperpolarize it by 20 mV. In a coupled network with $\lambda/D = 1{\cdot}5$

(λ = 22·5 μm) the input resistance is decreased seven-fold (see Lamb & Simon, 1976*b*) so the central cell would be polarized 3 mV by the same current, but this current would only be as effective in reducing sensitivity as a *uniform* polarization of about 0·7 mV (eqn. (5)). For an observed desensitization of 25% this corresponds to an e-fold reduction in sensitivity for a hyperpolarization U_e of 2·4 mV. This is similar to the effectiveness of light-induced polarization in reducing sensitivity (Baylor & Hodgkin, 1974) and suggests that part of the reduction during steady light is caused by voltage alone. However, as length constants were not measured for these cells the calculated value of U_e is only approximate.

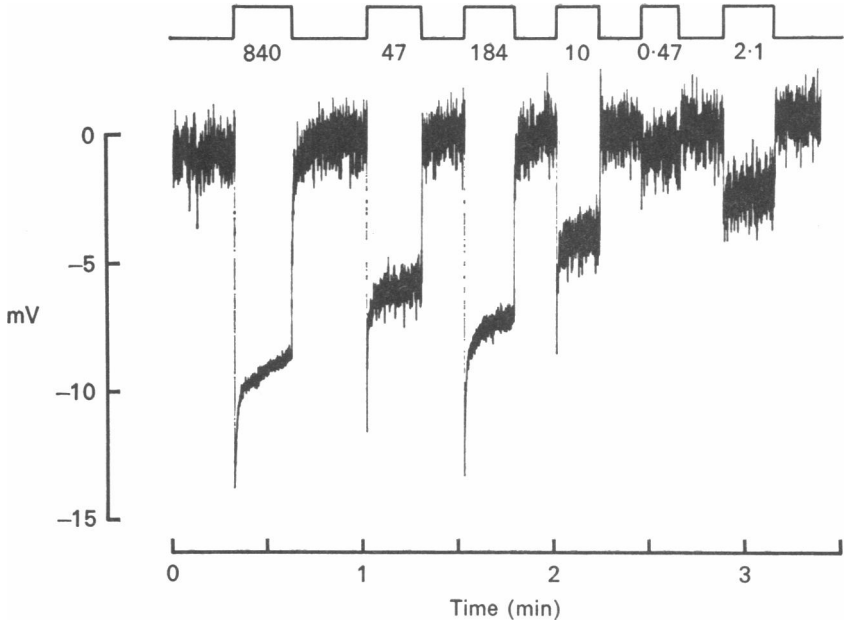

Fig. 8. Response of an isolated red-sensitive cone to lights of different intensities. Stimulus 639 nm, 6 μm diameter centred spot; photon flux is given by numbers near light monitor in units of 10^3 photons $\mu m^{-2} sec^{-1}$. Same cell as Fig. 2. Light flux and measured variances: dark, 0·418 mV^2; 840, 0·029 mV^2; dark, 0·381 mV^2; 47, 0·168 mV^2; dark, 0·406 mV^2; 184, 0·056 mV^2; dark, 0·410 mV^2; 10, 0·310 mV^2; dark, 0·416 mV^2; 0·47, 0·475 mV^2; dark, 0·462 mV^2; 2·1, 0·460 mV^2; dark, 0·384 mV^2.

Results with bright flashes were in better agreement with those reported by Baylor & Fuortes (1970) as negative currents usually increased the response. Also, positive currents always decreased the response, but this effect could occur even when the test flash was presented several hundred milliseconds after current passage, implying that factors other than driving force contributed to the response reduction.

Relation between noise and light-evoked hyperpolarization

This section examines the noise magnitude during steady lights of different intensities. The response of an isolated red-sensitive cone to lights of various intensities is illustrated in Fig. 8. In this cell dim lights which evoked steady responses of only a few millivolts changed the noise very little, but the larger responses were associated with a graded and progressive quieting.

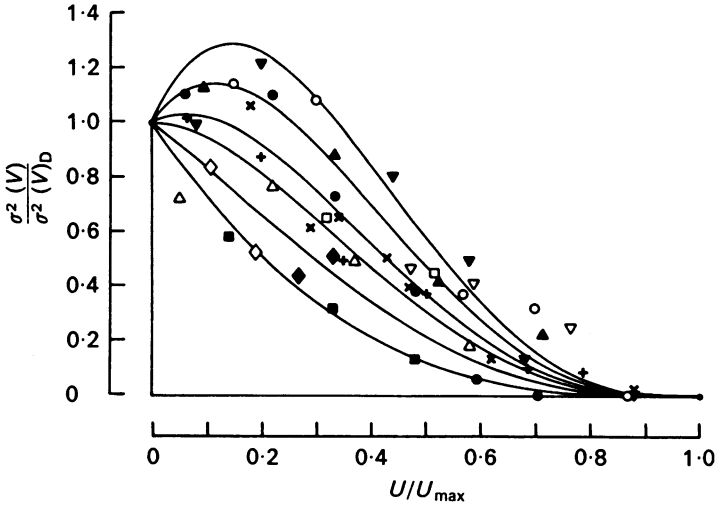

Fig. 9. Relation between intrinsic noise and light-induced hyperpolarization. Symbols are for ten cones; ● is the isolated red-sensitive cone of Fig. 8; ×, + are green-sensitive cones; the remainder are coupled red-sensitive cones. The variance in bright light has been subtracted from all points, and the remaining intrinsic noise normalized with respect to its value in darkness. Steady hyperpolarization U is normalized with respect to its value in bright light. Curves are drawn from eqns. (6) and (7) with V_D/V_{max} from top to bottom: 0·12, 0·15, 0·2, 0·25, 0·4, approaching 1.

For two green-sensitive and ten red-sensitive cones, including both weakly and strongly coupled cells, which were exposed to different intensities the measured relations between $\sigma^2(V)$ and U are plotted as the symbols in Fig. 9. This experiment is difficult to perform satisfactorily as the cell must be held with great stability for a considerable time. Ideally the variance should be measured over sections of at least 20 sec duration in alternate periods of darkness and steady test light. This is necessary to ensure that the cell's intrinsic variance is not changing and to enable measurement of the steady hyperpolarizations from darkness. The base line noise in bright light has been subtracted from all variances, which requires good electrode stability, and each point has been normalized so

that the variance in darkness and the steady voltage in bright light are unity. One obvious feature of the plot is that different cells exhibited different behaviour; in particular, for dim lights there was a noise increase in some cells while in other cells all intensities decreased the variance. Although some of this variability can be attributed to the large statistical error inherent in noise measurements (see Lamb & Simon, 1976*b*, p. 267) it appears that the diversity is a real phenomenon. The family of curves in Fig. 9 is derived from a model in the next section. The points for most of the cells are fitted moderately well by one or other of the curves, and the implications of this are mentioned on p. 456.

Quantitative interpretation of the variation of noise with light

It is of interest to know whether the variation of noise with light intensity can be described in a simple manner, and whether quantitative information about the underlying events can be obtained. The noise has been shown to be internal to the cones, and in the absence of evidence to the contrary we will investigate the proposition that the source involves fluctuations in the total conductance of the light-sensitive channels in the outer segment.

Several distinct mechanisms are possible for the ultimate source of noise. (1) There may be an inevitable low rate of events indistinguishable from photoisomerizations which, on the model of Baylor *et al.* (1974*a*), give rise to the chain of reactions leading to blocking molecule production and closure of ionic channels. (2) There may be an alternative source of the blocking molecules, such as leakage across the cell membrane, which leads to random arrival of either 'packets' or molecules of blocking substance in the outer segment. (3) The number of conducting channels may fluctuate spontaneously as a consequence of the statistical nature of reactions with blocking molecules, the concentration of which is approximately constant. Finally, a combination of all these may occur.

Analysis of the first mechanism depends on whether the chain of reactions in the model of Baylor *et al.* (1974*a*) leads either to elementary voltage events each having a wave form identical with the small signal response, or alternatively to a release of discrete 'particles' as a result of stochastic reactions in the chain, and therefore to events of rectangular shape (i.e. the presence or absence of a particle). In the latter situation it will be important to know whether each 'isomerization' gives rise to a single particle or to a multitude of particles which independently propagate through the chain. In the case of a single generated particle, it may be shown that the chain of events is irrelevant, and that the situation is identical with one in which the random source leads directly to production of blocking particles. In other words, the mean, variance and

power spectrum of the number of blocking particles in the cell is independent of whether the source is, for example, random leakage of individual molecules across the membrane or the random release of single particles followed by random transitions through a series of intermediate states.

The situations may be analysed by a combination of the methods used by Katz & Miledi (1972) and Anderson & Stevens (1973) (see Conti & Wanke, 1973). The third mechanism, stochastic reaction of channels with a *constant* concentration of blocking molecule, corresponds to the potassium channel kinetics case treated by Hill & Chen (1972) with $x = 1$. In all cases it must be borne in mind that the relation between voltage and light intensity (and also blocking particle concentration) is approximately Michaelis (see Baylor *et al.* 1974*a*).

Presentation of a theoretical analysis of each of these cases is beyond the scope of this paper, but some useful results are given below. As in the case considered by Katz & Miledi (1972) mechanisms (1) and (2) predict a variation of noise with light-induced voltage given by

$$\sigma^2(V) = f \Delta V_0 V\left(1 - \frac{V}{V_{\max}}\right)^3. \tag{6}$$

Here $\sigma^2(V)$ is the voltage variance, f is a 'shape factor' (see Katz & Miledi, 1972), ΔV_0 is the amplitude of the elementary event in the limit of very few events, V is voltage displacement from the level which would exist in the absence of events and $V_{\max}$ is the maximum value of V in strong light. In fact we measure hyperpolarization U from the dark voltage V_D and this is related to V by

$$\frac{U}{U_{\max}} = \frac{V - V_D}{V_{\max} - V_D}. \tag{7}$$

For many purposes the elementary event of interest is that response elicited by an additional event in darkness, ΔU_D, rather than the value ΔV_0 which applies in the absence of other events. In darkness eqn. (6) can then be shown to give

$$f \Delta U_D = \frac{\sigma^2(V)_D \, V_{\max}}{U_{\max} \, V_D}. \tag{8}$$

The mean number of simultaneous events in darkness, N_D, can also be shown on this model to be

$$N_D = f \frac{U_{\max}^2}{\sigma^2(V)_D} \left(\frac{V_D}{V_{\max}}\right)^2. \tag{9}$$

For rectangular events the shape factor f is unity and for events having the form of eqn. (43) of Baylor *et al.* (1974*a*) f may instead be shown to be

$$f = \frac{n^n}{2(2n-1)\,(n-1)^{n-1}} \tag{10}$$

$$\simeq 0{\cdot}68, \quad \text{for } n = 5, 6, 7, 8,$$

TABLE 3. Characteristics of models

	Model		Predictions			
	Mechanism of particle generation	Mechanism of binding to channels	Shape factor f	Event represents	Power spectrum	Noise *vs.* response relation
1.	'Dark photoisomerizations'					
(*a*)	Event shape is flash response	—	~0·68	Peak photon response	Eqn. (2)	Eqn. (6)
(*b*)	Stochastic chain					
	(i) Many particles released (independent propagation through chain)	—	?	?	?	?
	(ii) One particle released	Rapid reversible (deterministic)	1	Response to one additional particle	Lorentzian (ignoring capacitance)	Eqn. (6)
2.	'Leakage'					
	(i) Individual particles					
	(ii) 'Packets'	Rapid reversible (deterministic)	1	Response to one additional packet	Lorentzian (ignoring capacitance)	Eqn. (6)
3.	Any, but dark concentration approx. constant	Stochastic	1	Response to closure of one additional channel	Lorentzian (ignoring capacitance)	Equation not given

where n is the number of reaction stages in their model. Analysis of mechanism (3) leads to a different form of the relation between variance and light induced voltage, but eqn. (8) still applies with $f = 1$ and with ΔU_D representing the effect of closing one additional channel in darkness.

It is important to note that this treatment has not taken account of changes in the time course of the small signal response, of any voltage dependence of sensitivity, or of the effects of the cell's capacitive time constant, and is therefore at best an approximation. Table 3 summarizes the predictions of the various models.

TABLE 4. Variation of noise with steady light

Cell	$\sigma^2(V)_{D-L}$ (mV²)	U_S (mV)	U_p (mV)	V_D/V_{max}	$f\Delta U_D$ (μV)	N_D/f
● 1	0·35	13·5	15·5	0·18	125	22
△ 2	0·12	10	14	0·27	32	119
▲ 3	0·014	10·5	15	0·15	6·2	362
◇ 4	0·012	8·5	13·5	Large	—	—
▽ 5	0·014	8·5	14	—	—	—
◆ 6	0·092	8·5	14	0·5	13	533
□ 7	0·017	11	19	0·2	4·5	849
■ 8	0·024	10·5	17	Large	—	—
○ 9	0·011	11·5	20	0·12	4·6	524
▼ 10	0·059	11	20	0·13	2·3	115

Symbols are those used in Fig. 9. $\sigma^2(V)_{D-L}$ is intrinsic voltage variance; U_S is maximum steady hyperpolarization; U_p is maximum peak hyperpolarization. V_D/V_{max} is the fractional hyperpolarization in darkness estimated from the fit of the curves in Fig. 9 to the points. $f\Delta U_D$ is then calculated from eqn. (8), and $N_D f$ from eqn. (9).

The curves in Fig. 9 were calculated from eqns. (6) and (7) for six values of V_D/V_{max}, the fractional hyperpolarization in darkness. For nine of the ten sets of experimental points one or other of the curves provides a reasonable fit, allowing for the considerable uncertainty in each point. A satisfactory fit could not be obtained for one cell (▽) and for two others (◇, ■) the required value of V_D/V_{max} approached unity, which seems unphysical. The values of V_D/V_{max} estimated to provide the best fit for each of the seven remaining cells are given in Table 4, and from these the respective values of $f\Delta U_D$ were calculated from eqn. (8). As the shape factor f should not differ greatly from unity these are estimates of the elementary event magnitude ΔU_D. In the isolated cone (cell 1 of Table 4) the calculated value is 125 μV, and in other cells smaller values were found, presumably as a result of electrical coupling. Taking the input resistance of an isolated cone to be 200 MΩ (Lamb & Simon, 1976*b*) and the driving potential on the channel to be 40 mV (Baylor & Fuortes, 1970), the

elementary conductance change giving a voltage of 125 μV is 16 pS ($= 1{\cdot}6 \times 10^{-11}\ \Omega^{-1}$).

Most of the values of V_D/V_{max} are between 0·12 and 0·27, meaning that in darkness most cells are hyperpolarized about one tenth to one quarter of their maximum range, or about 2–5 mV. This means that about one tenth to one third of the channels are closed in darkness by residual transmitter. By lowering extracellular calcium concentration Yoshikami & Hagins (1973) found a three- to five-fold increase in light response in

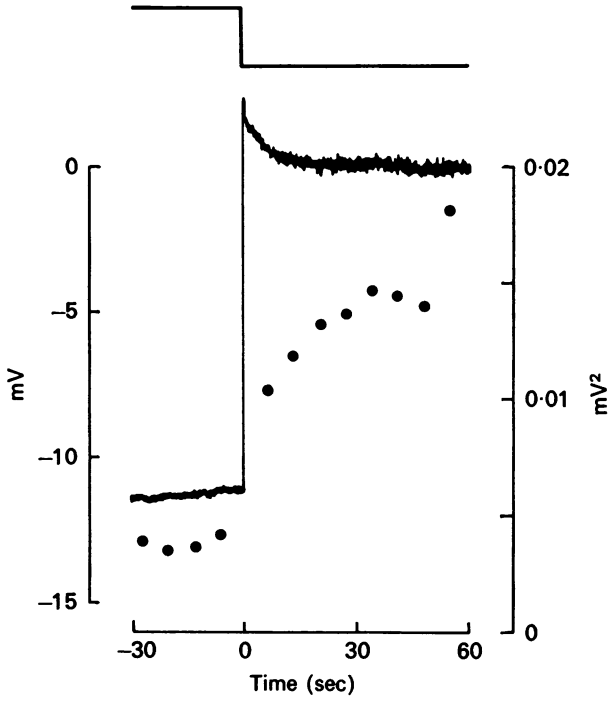

Fig. 10. Noise during the differentiated component. Bright light (639 nm, $4{\cdot}1 \times 10^6$ photons μm^{-2} sec^{-1}, 105 μm diameter, monitored at top) had been applied for about 130 sec and was turned off at time zero. The continuous curve is cell voltage DC coupled (scale on left), and points are voltage variance (scale on right) measured over 5·12 sec records. The high gain signal was recorded AC coupled with a 1 sec time constant, and on replay was filtered with an additional 0·25 sec RC high-pass filter to remove the effect of rapid drift.

rat rods, apparently resulting from an increased standing dark current. Such a change would be expected if light-sensitive channels were closed in darkness with normal extracellular calcium but not with low calcium, but the effect is larger than would be expected if only a third of the channels were closed.

Noise recovery following illumination

Differentiated component. When a prolonged step of light of appropriate intensity is turned off, there is a transient overshoot of the dark membrane potential which Baylor & Hodgkin (1974) called the 'differentiated' component. We have found that the noise is lower during this time than following complete recovery even though the cell is relatively depolarized. The record shown in Fig. 10 illustrates this property. Immediately after switching off the light the variance was 30% lower than the level it reached eventually and this is significant at the 5% level. In other experiments the noise was lower in both the depolarized and hyperpolarized transient periods following brighter light. Although it is a poorly understood phenomenon, the differentiated component is a useful illustration that the noise is not a function of voltage alone.

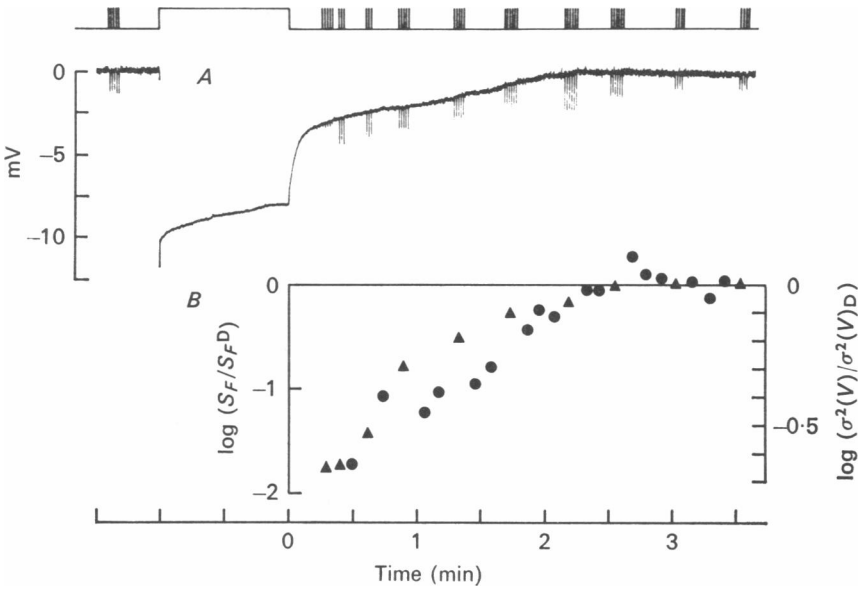

Fig. 11. Recovery of noise and flash sensitivity in a green-sensitive cone following a bright light. A, voltage record; dim flashes were delivered at intervals to determine sensitivity S_F. *B*, recovery of log S_F (▲) and log $\sigma^2(V)$ (●). Variance in bright light has been subtracted. Points are normalized with respect to their mean dark levels S_F^D and $\sigma^2(V)_D$. Step, white light equivalent to 10^8 photons μm^{-2} sec^{-1} at 558 nm, 105 μm diameter spot; test flashes, 558 nm, 10 msec, 105 μm spot.

Bright light. The green-sensitive cone in Fig. 11 was exposed to a very bright white light (monitored at top) for about 1 min. After the light was turned off, it took several minutes for the voltage to recover to the

original dark level. During this recovery phase flashes were delivered periodically so that flash sensitivity S_F and voltage variance $\sigma^2(V)$ could be measured in alternate periods. Part *B* of the Figure is a semilogarithmic time plot of S_F (▲) and $\sigma^2(V)$ (●) normalized to their respective dark values, and shows that the noise recovers with about the same time course as voltage and sensitivity.

The triangles and circles of Fig. 12 respectively plot the dependence of $\sigma^2(V)$ and S_F on hyperpolarization for the experiment shown in Fig. 11. In agreement with Baylor & Hodgkin (1974), there is a linear relation

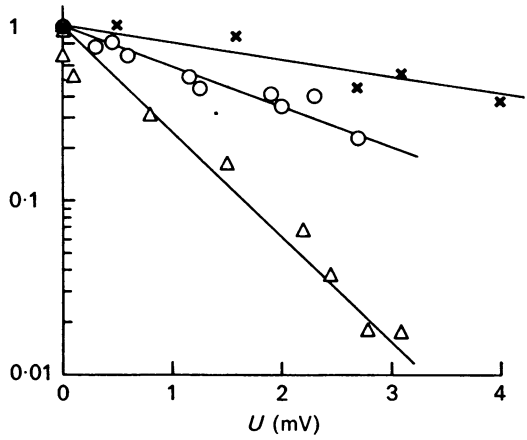

Fig. 12. Sensitivity and intrinsic variance as semilogarithmic functions of hyperpolarization *U*. △, sensitivity and ○, variance during the after-effect of light. ×, variance during steady dim light. Ordinate values are normalized with respect to those in darkness. Straight lines correspond to an e-fold change for 0·72 mV, 1·9 mV and 4·3 mV. Same cell as Fig. 11.

between log S_F and the steady response *U* for small hyperpolarizations. For a sample of eight cones the exponential parameter determined by the slope of this relation averaged 1·3 mV (range 0·7–2·2 mV), close to the value of 1·5 mV measured by Baylor & Hodgkin (1974, Table 2) for the 100 sec component of recovery. The circles were also fitted by a straight line, indicating a roughly linear relation between log $\sigma^2(V)$ and *U*, but with a smaller slope than for sensitivity. This exponential voltage parameter for variance determined in the same eight cones averaged 3·0 mV (range 1·7–5·1 mV) and the ratio of the two parameters averaged 2·3 (range 1·7–3·4). This result reflects the fact that the noise is less affected following an adapting light than is sensitivity.

The crosses in Fig. 12 plot $\sigma^2(V)$ against *U* in the same cell during periods of steady illumination. There is less quieting for a given hyperpolarization in steady light than during the after-effect of bright light.

This type of behaviour was typical and, although in some cells the noise in dim light was increased above the dark level, the noise during recovery from bright light was always lower than that in darkness. At a hyperpolarization of 2 mV in this cell, for example, there was about 0·004 mV^2 more noise in steady light. A possible reason for the higher noise is the continual arrival of photons during illumination. If at a given hyperpolarization the additional noise in steady light added linearly to that during the after-effect then the extra noise would correspond to an elementary event corrected to darkness of

$$\frac{0{\cdot}004\ \mathrm{mV}^2}{2\ \mathrm{mV}}/(1-\tfrac{2}{11})^3 \simeq 3{\cdot}7\ \mu\mathrm{V}.$$

Electrical coupling reduced the dark variance in this cell some 30-fold from that in an isolated cell, so that this figure would correspond to an event of about 110 μV in an isolated cell. As this is of the same order of magnitude as the event size of the dark noise it may be that the additional noise is indeed the result of photon events.

Variability of responses to repetitive flashes

Baylor & Hodgkin (1973) suggested that the magnitude of the response to a single photoisomerization could be determined from the variability of the responses to a series of identical flashes. As the number of photons in a flash is random with a Poisson probability distribution, the variance of the actual number absorbed will equal the mean of the number absorbed. Consequently, for small responses, the ratio of the variance of the voltage response to the mean response should equal the peak quantal voltage ΔU_ϕ elicited by a single isomerization.

We have attempted to detect such 'photon noise' in the responses of turtle cones, but somewhat surprisingly have been unable to demonstrate an increase in variance over the level in darkness. An example of such an experiment is illustrated in Fig. 13 where the average (upper trace) and variance (lower trace) of the voltage response to 120 consecutive identical flashes are displayed as functions of time. From inspection of the variance trace it appears that there is no consistent variance increase coincident with the peak of the flash response, and this is investigated by the following statistical test.

We wish to test the null hypothesis that the variance near the peak of the flash response is unchanged from its value before the flash, and for this we use the properties of the F-distribution (Bendat & Piersol, 1971). For two independent estimates, s_1^2 and s_2^2, of the same variance σ^2, with n_1 and n_2 degrees of freedom respectively, there is a probability α that the ratio s_1^2/s_2^2 will exceed $F_{n_1, n_2; \alpha}$. In Fig. 13 the variance at each point (at

2 msec intervals) has 119 degrees of freedom, but adjacent points are not independent so that when the variance is averaged over a certain time the degrees of freedom do not simply add. On the argument of Lamb & Simon (1976*b*, p. 268), the number of degrees of freedom in a single sweep is equal to the record length T divided by the time constant τ characterizing the noise. Hence we estimate that the number of degrees of freedom in an averaged estimate of the variance is

$$(\text{number of sweeps} - 1)\, T/\tau, \quad \text{for } T > \tau,$$

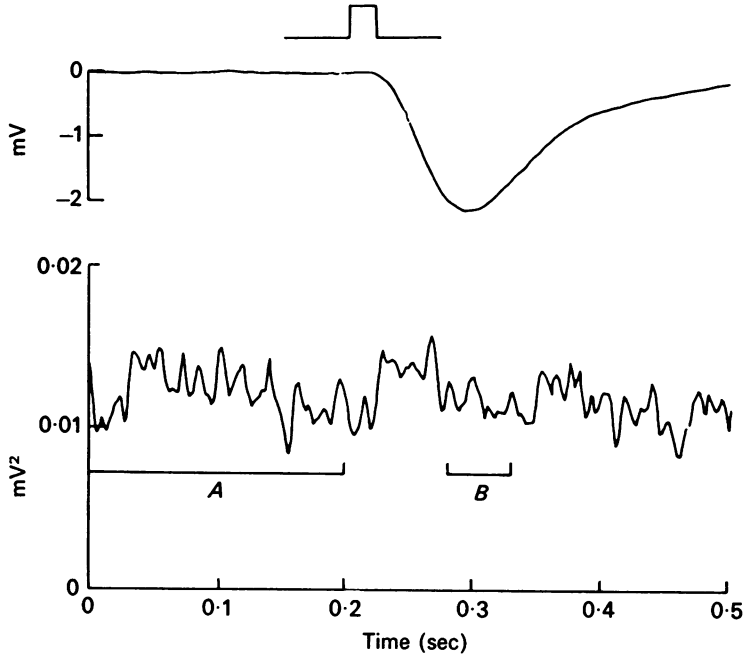

Fig. 13. Variability of responses to repetitive flashes. Upper trace average response, lower trace voltage variance, computed for 120 consecutive responses at 2 sec intervals, AC coupled with 1 sec time constant and sampled at 2 msec intervals. The rising phase of the response has deliberately been avoided in region *B* because of slight variability in the time of occurrence of the flash (see text). Flash 20 msec, 643 nm, 40 photons μm^{-2}, 61 μm diameter spot. Red-sensitive cone 4 of Table 5.

where T is the time over which the variance is averaged. In Fig. 13 the two regions selected for comparison are indicated *A* and *B*, and are of 200 and 50 msec duration respectively; the rising phase of the response has deliberately been avoided as a slight variation (of maximum 2·5 msec) in the time of occurrence of the flash caused an artificial variance increase at this time. The value of τ in this cell was found from power

spectral measurements to be 29 msec (Table 1, cell 24). The mean variances in the respective regions are 0·0122 mV^2 with about 820 degrees of freedom and 0·0117 mV^2 with about 205 degrees of freedom. The value of $F_{205,\,820;\,0\cdot05}$ is about 1·192 so that an increase would be significant at the 5 % level only if $\sigma^2{}_B/\sigma^2{}_A$ exceeded 1·192. In fact for this cell there is a slight decrease so the null hypothesis is accepted. Accordingly we interpret this experiment as providing no evidence of increased variability of cell voltage coincident with the peak flash response.

By making the assumption that photon noise would add linearly to the dark noise, it is possible to estimate the maximum quantal size which could have existed without giving rise to a detectable noise increase. In the above experiment we should have been able to detect a noise increase of $0{\cdot}0122\ mV^2 \times (1{\cdot}192 - 1) = 0{\cdot}0023\ mV^2$. With correction (see Katz & Miledi, 1972) for non-linear summation of elementary events due to the Michaelis relation between voltage and intensity (Baylor & Fuortes, 1970) for a 2·1 mV response in a cell with peak response of 17·5 mV, the quantal voltage is

$$\frac{0{\cdot}0023\ mV^2}{2{\cdot}1\ mV}\left(\frac{17{\cdot}5}{17{\cdot}5-2{\cdot}1}\right)^3 = 1{\cdot}6\,\mu V.$$

This is of course the amplitude of a quantal event in the coupled network, and to estimate the value in an isolated cone the result is scaled by a factor based on the cell's measured length constant and dark noise (Lamb & Simon, 1976*b*). From the mean length constant of 23 μm and noise (dark minus light) of 0·0075 mV^2 we estimate that the scaling factor is between 25 and 53. We conclude from the absence of a variance increase in this experiment that, on the assumption of additive noise, the quantal sensitivity of an isolated cone is less than about 39–82 μV.

Data from repetitive flash experiments in five cells are collected in Table 5, and in none of these cells was a significant variance increase detected. The threshold level for detection of a significant increase depends on correct choice of the number of degrees of freedom n_1 and n_2, and this in turn depends on the time constant τ describing the noise (p. 460). The values of τ are not highly reliable but it was found that even with a 30 % change in τ, the variance ratios still showed no significant increase. Values are given in Table 5 for the estimated upper limit of ΔU_ϕ consistent with failure to detect a variance increase. The ΔU_ϕ of 25 μV per photoisomerization estimated by Baylor & Hodgkin (1973) by direct measurement applies to the case of uniform illumination, for which the effects o coupling may be ignored as adjacent cones undergo the same average voltage change, and so should be comparable to our estimate for isolated cells.

TABLE 5. Repetitive flash experiments

Cell	Sweeps	U_{flash} (mV)	U_p mV)	$S_F(\mu V$ photon^{-1} μm^2)	τ_1 (msec)	$\sigma^2(V)_A$ (mV^2)	$\sigma^2(V)_B$ (mV^2)	F	$\sigma^2(V)_{det}$ (mV^2)	ΔU_ϕ (μV)	λ_{mean} (μm)	$\sigma^2(V)_{D-L}$ (mV^2)	$\Delta U_{\phi iso}$ (μV)
1	217	2·2	15	5	(20)	0·0187	0·0185	1·134	0·0025	1·8	20·5	0·010	46–73
	214	3·4				0·0204	0·0195	1·134	0·0027	1·7			43–69
2	170	1·6	13	4	(25)	0·0145	0·0162	1·148	0·0022	2·0	22	0·0065	50–120
3	120	3·2	10	53	(20)	0·0530	0·0561	1·164	0·0087	8·6	10·5	0·031	43–112
4	120	2·1	17·5	99	29	0·0122	0·0117	1·192	0·0023	1·6	23	0·0075	41–82
	140	1·1				0·0121	0·0133	1·175	0·0021	2·3			57–121
5	50	1·9	14	11	19	0·0176	0·0214	1·255	0·0045	3·7	21	0·015	74–124

U_{flash} is the peak of the average flash response for the given number of sweeps; U_p is the peak flash response with bright light; S_F is the flash sensitivity. τ_1 is the time constant of the dominant Lorentzian in the power spectrum; parentheses indicate that a good fit could not be made and give a rough estimate. $\sigma^2(V)_A$ and $\sigma^2(V)_B$ are the voltage variance over the regions before the flash and at the peak respectively; the duration of B was 50 msec and A varied between 100 and 200 msec. F is the 5 % point on the F-distribution for the appropriate number of degrees of freedom. $\sigma^2(V)_{det}$ is the minimum variance increase which would be detectable for this value of F, and ΔU_ϕ is the event size which would give rise to such a noise increase. λ_{mean} is the mean length constant and $\sigma^2(V)_{D-L}$ is the intrinsic voltage variance (dark-light) used to scale the event size to that in an isolated cone, $\Delta U_{\phi iso}$.

It is instructive to calculate the minimum detectable quantal size in an ideal experiment. Imagine an isolated cone with dark voltage variance of 0·4 mV^2, characteristic τ of 25 msec and maximal response of 20 mV held with perfect stability for 200 responses at 2 sec intervals (about 7 min) at the optimal quarter maximal response amplitude of 5 mV, the variance being calculated over 250 msec and 50 msec regions. Using the method on p. 460 and the value $F_{400,2000;0\cdot05} = 1\cdot133$, the quantal size which would just cause a detectable variance increase is

$$\frac{0\cdot4\ mV^2 \times 0\cdot133}{5\ mV}\left(\frac{4}{3}\right)^3 = 25\cdot2\ \mu V,$$

so that a value of 25 μV might be just resolvable. If, however, the photon noise did not add linearly to the dark noise, perhaps because the two involve a common mechanism, then the situation would be even less favourable.

Although in well controlled experiments we never detected a variance increase, spurious results were obtained in less well controlled attempts. It was found to be particularly important to ensure that the average response did not deteriorate or improve during the sequence, and it was usually necessary to select a sub-group of consecutive responses over which there was no gradual change. In practice a change of more than about 1 % between the first and second half of the series proved unacceptable. In early experiments it was found that the flash duration was not sufficiently stable, and the shutter motor was replaced by a powerful stepping motor which gave a highly reproducible flash. As mentioned above, a minor problem was experienced as a result of slight instability of the time of occurrence of the flash, but at worst this introduced a small variance peak coinciding with the rising phase of the response.

Our conclusions from the repetitive flash experiments in cones are that under practical conditions no increased voltage variance is detectable coincident with the peak of the flash response and that this is consistent with the quantal sensitivity to diffuse dim flashes being less than the order of 50 μV per photoisomerization. In rods the situation is quite different and the higher ΔU_ϕ can result in a pronounced variance increase (Schwartz, 1975; P. B. Detwiler and A. L. Hodgkin, personal communication; and our own unpublished findings).

DISCUSSION

Location of the noise source

There is strong evidence that the source of the cone dark noise is internal to the cone. Cone–cone interactions can be ruled out as the source because the noisiest cones are those which are isolated (Lamb & Simon, 1976*b*),

and horizontal cell feed-back, even of a localized kind in the synaptic terminal, can be ruled out because reduction of the noise is a function of the magnitude of the cone response but not of stimulus size or pattern. Accurate localization of the source within the cone has proved more difficult, but there is evidence that it arises from random closure of the light-sensitive ionic channels in the outer segment as postulated by Simon *et al.* (1975).

Much of the difficulty in interpretation arises from the voltage-dependence of the noise, in particular the observation that hyperpolarization by current as well as by light reduces the noise. We believe that this behaviour might be explained by a direct effect of voltage on the light-sensitive mechanism itself, but for a similar phenomenon in rods Schwartz (1977) proposes two separate components, one voltage sensitive and the other light induced. Several experiments indicate that, although strongly influenced by voltage, the noise is not purely voltage-dependent. Firstly, in a few experiments in weakly coupled cones positive currents did not bring back the noise in bright light even though in darkness negative currents of the same magnitude completely suppressed the noise. Secondly, during the differentiated component the noise was lower than in the dark steady state even though the cell was depolarized. Thirdly, during the slow hyperpolarizing phase following bright lights, the noise was lower for a given hyperpolarization than it was during steady dim light. Although these results might be explained by unidentified slow conductance changes, the simplest explanation is that the noise can be controlled by illumination as well as by voltage. Accordingly we have assumed that the noise is caused by fluctuation in the conductance of light-sensitive ionic channels in the outer segment, but we have no definite evidence in support of this. It is likely that focal extracellular recording of outer segment membrane current could resolve this problem.

Nature of the elementary event

On our assumption that the intrinsic dark noise results from opening and closing of the light-sensitive channels several ultimate mechanisms are possible, each basically involving the random nature of some process in the phototransduction mechanism. They include (i) random occurrence of identical events indistinguishable from the average response to a dim flash, (ii) random arrival of either 'packets' or molecules of blocking substance in the outer segment, and (iii) random interaction of blocking substance with channels. Each of these mechanisms implies the existence of a finite residual concentration of blocking substance in the outer segment in darkness.

The form of the measured power spectrum was inconsistent with the

prediction of the first model and so the idea that the noise is composed of events indistiguishable from the average response can be rejected. The form of the measured variance versus steady response relation was consistent with the second model (as well as the first) but it was difficult to determine whether or not it was consistent with the third model. Calculation of the average number of simultaneous events in darkness gave a value of about 22 in an isolated cone, which on the second model represents the mean residual number of either molecules or 'packets' of blocking substance. In the case of molecules, and estimating the outer segment internal volume to be 2×10^{-14} l, this corresponds to a concentration of 2×10^{-9} M. Such a value seems too low for the residual concentration of a substance such as calcium ion, which in nerve has a resting level of about 5×10^{-8} M (DiPolo, Requena, Brinley, Mullins, Scarpa & Tiffert, 1976), and suggests that the elementary event may involve the release of a substantial number of blocking particles. In addition, the amplitude of the elementary voltage event, about 100μV, is so large that the cone would be half-maximally hyperpolarized with about 100–200 simultaneous events. It seems complicated to postulate that a single particle would close more than one channel on average, so that the total number of channels could not be greater than 200–400. This corresponds to a very low channel density of 0·06 μm^{-2}, which is again suggestive that each event involves more than one blocking particle.

An interesting finding is that the time integral of the elementary event (125 μV $\times$ 40 msec) is very similar to that of the photon event (25 μV $\times$ 170 msec) estimated by Baylor & Hodgkin (1973). It suggests that both the noise event and photon event may be closely related, despite their different time courses.

Speculative model

A speculative model which seems consistent with our observations is as follows. Photoisomerization of pigment leads by a series of random transitions to an active state, which in the case of cones represents the opening of a calcium channel or carrier in the membrane. This state ends spontaneously after an average of about 40 msec, during which time a substantial number of calcium ions enter the outer segment. Calcium closes light-sensitive sodium channels and is removed from the cell by a mechanism with a time constant substantially shorter than 40 msec. Even in darkness there is a finite probability that the active state will be entered by some pigment molecules. The mean time in the active state is reduced by either an increase in intracellular calcium or by hyperpolarization of the cell.

Such a scheme would account for (i) equality of the time integrals

of the noise event and photon event, (ii) the Lorentzian power spectrum of current fluctuations, (iii) variation of noise with response according to eqn. (6), (iv) the qualitative effect of background illumination in speeding the response, (v) the qualitative effect of extrinsic hyperpolarization in reducing noise and decreasing sensitivity to dim flashes, and (vi) the existence at a given hyperpolarization during dim light of a larger variance than after a bright light. In addition it is consistent with a resting calcium ion concentration of 10^{-8}–10^{-7} M if an average of 5–50 calcium ions entered during the active period. As stated on p. 456 the conductance change associated with an 'elementary event' is about 16 pS. If this event corresponded to closure of a number of ionic channels then the estimate of channel conductance would be reduced accordingly, and the total number of channels per cell would be increased. Using the above range of 5–50 calciums and assuming a one-to-one combination with channels the individual channel conductance would be 0·32–3·2 pS, which is more in line with the values found for sodium channels in nerve (see Almers & Levinson, 1975) than for acetylcholine-sensitive channels (Anderson & Stevens, 1973).

The equivalent 'dark light'

Irrespective of the mechanism of generation of the noise in darkness, an equivalent intensity of 'dark light' in a noiseless cone may be calculated. Baylor & Fettiplace (1977) have shown that the path from cone to ganglion cell acts as a bandpass filter, passing those frequencies corresponding approximately to the time course of the cone flash response. Accordingly it is reasonable to ignore the higher frequencies in the dark noise. In darkness an isolated cone exhibits a voltage variance of about 0·4 mV^2 with a characteristic time constant of about 40 msec, while the dim flash response has a spectrum with bandwidth of about 2 Hz. As a result we estimate that the variance of interest is halved to about 0·2 mV^2. For an event with a shape factor of 0·68 (see p. 453), a height of 25 μV and an integration time of 170 msec (Baylor & Hodgkin, 1973) this corresponds to an event rate of

$$\frac{0{\cdot}2\ \mathrm{mV}^2}{(25\ \mu\mathrm{V})^2 \times 0{\cdot}68 \times 170\ \mathrm{msec}} = 2800\ \mathrm{sec}^{-1}.$$

In other words the dark noise is approximately equivalent to a light giving 2800 photoisomerizations per sec per cone, which for a collecting area of 10 μm^2 (Baylor & Hodgkin, 1973) corresponds to an intensity of 280 photons μm^{-2} sec^{-1}. For comparison a steady light of about 2000 photoisomerizations per sec cone reduces a cone's sensitivity by half (Baylor & Hodgkin, 1973).

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