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KINETICS OF SYNAPTIC TRANSFER FROM RECEPTORS TO GANGLION CELLS IN TURTLE RETINA

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SUMMARY

1. Synaptic transfer between the retinal input and output was studied in turtle eyecups by injecting rectangular current pulses into a single cone or rod while recording externally from a ganglion cell.

2. When a receptor was activated with weak steps of polarizing current, the probability of obtaining a ganglion cell impulse rose after an S-shaped delay to a peak at about 0·1 sec and then declined. This suggests that the transmission chain behaves like an electrical band-pass filter containing delay and differentiating elements.

3. To further characterize the kinetics of excitation in the subthreshold region, the duration and polarity of the polarizing current pulses were varied while determining the magnitude of the threshold current and the delay to the ganglion cell impulses. The results of these experiments were described with linear models which assume that synaptic transfer occurs over a cascade of first-order delay stages and a single differentiating stage.

4. The pathways which relay off responses to light from rods and red-sensitive cones were formally similar, but the time scale in the rod path was several times slower. The path carrying off responses from the red-sensitive cones was faster than the on path. These kinetic differences indicate that independent pathways mediate each of the three categories of response and suggest that the kinetics of each path are 'matched' to the input signals generated by light.

5. The strength–latency relations for the responses of on-centre ganglion cells to flashes and steps of light were approximately predicted from the description of synaptic transfer developed here and the description of visual transduction in red-sensitive cones from a previous study.

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6. It is suggested that the retinal paths have kinetics which might be useful in transmitting light-evoked signals whilst attenuating noise present near the input.

INTRODUCTION

The preceding paper (Baylor & Fettiplace, 1977) showed that electrical current applied through a micro-electrode can substitute for light in evoking synaptic transfer from a retinal receptor to a ganglion cell. With light as the stimulus, the input signal driving the pathway is the slow photocurrent generated by visual transduction. In turtle cones, for example, the electrical response to a dim flash has a long S-shaped delay and a mean duration of about 175 msec (Baylor, Hodgkin & Lamb, 1974), while in turtle rods the responses are several times slower (Baylor & Hodgkin, 1973; Schwartz, 1973; Copenhagen & Owen, 1976; Schwartz, 1976). With extrinsic current, the form of the input signals can be simplified to step functions and their duration can be shortened into a range normally prohibited by the slowness of the visual transduction mechanism. The present paper describes the use of this strategy to examine the kinetics of synaptic transfer from receptors to ganglion cells. It will be shown that small signals in a receptor are transformed by a long composite delay as well as a differentiation which prevents a steady response. The time scale of these processes is shown to differ between several functionally distinct pathways. It is suggested that the retinal pathways may have kinetics suitable for sifting signals from noise before impulses are generated in ganglion cells.

METHODS

The experimental methods were the same as those described in the previous paper (Baylor & Fettiplace, 1977). Experiments were performed on eyecups from both *Pseudemys scripta elegans* and *Chelydra serpentina*, the latter being preferred for experiments on rods. There was no evidence that the rods or their output pathways behaved differently in the two animals.

Eqn. (11) was integrated using a fourth-order Runge–Kutta method. This and the subsequent convolution were performed numerically on a small laboratory computer. The time intervals in the calculations were successively halved until the solution was not significantly affected.

RESULTS

General description of the kinetics of synaptic transfer

Fig. 1*A* shows the average response of an on-centre ganglion cell to rectangular pulses of hyperpolarizing current passed in a red-sensitive cone. The histogram gives the mean number of impulses per trial per 10 msec bin on the same time scale as the stimulus, which is represented

above. After a delay the impulse density rose to a peak at 100–110 msec and then declined to the original level even though the stimulus persisted. Records of the cone's membrane potential showed that the voltage changes were completed with a time constant of about 10 msec and that the potential relaxed by less than 7 % from its peak value. The relation between the stimulus and the discharge of impulses suggests two processes, a delay which slowed the onset of the response and a differentiation which blocked its later phase. The same features were evident when light rather

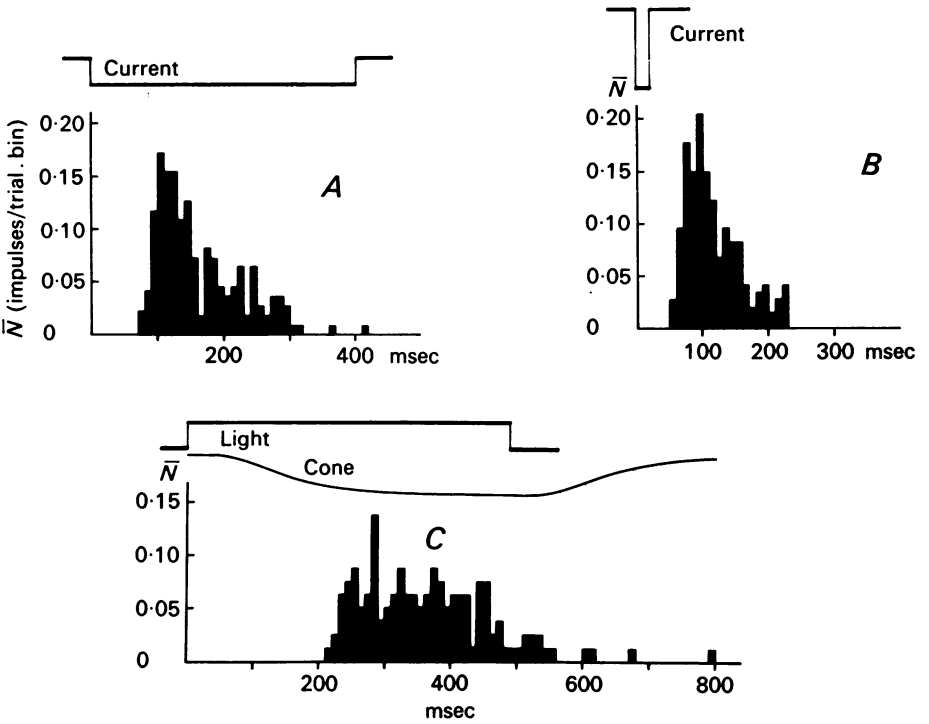

Fig. 1. Distributions of latencies of impulses generated in an 'on' centre ganglion cell by injection of hyperpolarizing current into a red-sensitive cone (*A* and *B*), or stimulation of the retina with a spot of light, 160 μm diameter, centred on the same cone (*C*). $\bar{N}$, mean number of impulses per trial per 10 msec bin, plotted against time after onset of stimulus. *A*, 110 trials with 400 msec current pulses of intensity 1·0–1·5 $\times$ $^{-10}$A; mean response in the ganglion cell 1·61 spikes/trial. *B*, seventy-three trials with 20 msec current pulses of intensity 6·0–9·0 $\times$ 10^{-10}A; mean response 1·48 spikes/trial. *C*, eighty trials with 640 nm steps of light. Mean response in the ganglion cell 1·79 spikes/trial. Stimulus intensity 56–72 photons μm^{-2} sec^{-1}. The middle line is the expected form of the linear step response in th e cones, calculated as described in the text. Saturating light response, U_{max}, in cone 16 mV; input resistance R_m 86 MΩ; Temperature 21·4° C.

than current was used as the stimulus. Fig. 1*C* shows the response of the ganglion cell when the retina was stimulated with a 160 μm spot of red light. With light, as with current, the discharge of spikes was transient, but the peak light response occurred about 150 msec later than the peak current response. This difference presumably reflects the delay in the electrical response of the cones to light. The smooth curve in Fig. 1*C* shows the expected form of the cones' light response, calculated from (see Baylor *et al.* 1974)

$$\bar{u} = (1 - e^{-\alpha t})^6, \quad (1)$$

where $\bar{u}$ is the scaled amplitude of the hyperpolarization and α is a rate constant taken as 15·2 sec^{-1}. This curve reaches its maximum rate of change at 118 msec after the onset of the step of light.

Response histograms similar to that in Fig. 1*A* were observed in other experiments in which there were sufficient trials, the peak responses occurring between 50 and 125 msec after the polarizing current was switched on or off. For most pairs of cells, however, there were not enough trials to determine a full histogram and instead the mean impulse latency L_∞, was determined with long pulses of near threshold intensity. Since the latency distributions were asymmetric and skewed towards longer times, the mean latency will be greater than the time to peak impulse density. This can be illustrated by the histogram in Fig. 1*A*, where the peak was between 100 and 110 msec, while the mean latency was 163 msec.

Fig. 2 shows histograms of the mean impulse latencies from experiments on the 'off' and 'on' pathways from red-sensitive cones as well as the 'off' pathway from rods. In the experiments on the 'off' paths, spikes were generated by the make of depolarizing pulses and/or by the break of hyperpolarizing pulses; latencies obtained with each pulse polarity are shown separately. Some cells receiving from red-sensitive cones gave only single impulses at a very short and nearly constant latency of 45–55 msec. Results from these ten cells are cross-hatched. Since the four such cells tested did not respond to antidromic stimulation of the optic nerve (see Baylor & Fettiplace, 1977), it is possible that all ten may have been amacrine cells. In a given experiment there was considerable fluctuation in the timing of impulses from trial to trial and the standard deviations of the latencies were usually about a third of the mean. Similarly, as is clear from Fig. 2, there was also dispersion in the mean latencies obtained from different experiments on each pathway. Nevertheless there was evidence that the distributions of latencies differed systematically between pathways, the off path from red-sensitive cones being faster than the other two. Table 1 gives the grand means and S.D.s of the collected mean latencies from experiments on each pathway. It can be noted that

the latencies in the off path from red-sensitive cones (excluding the very fast cells) were about 1·5 times shorter than those in the cone on path and the rod off path.

The different kinetics of the on and off cone pathways were evident in the responses of on/off ganglion cells which received from the same

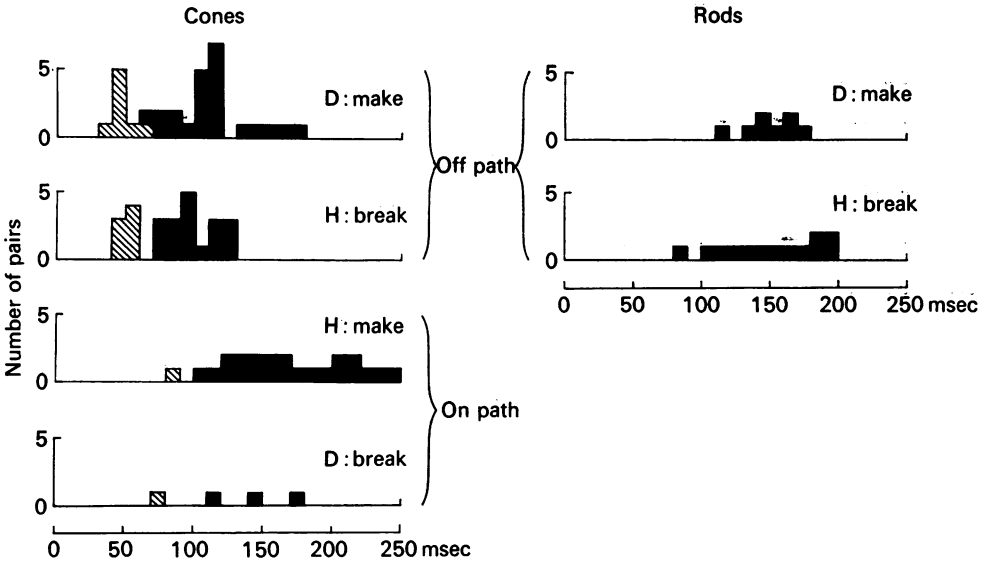

Fig. 2. Collected mean impulse latencies for three synaptic pathways from photoreceptors to ganglion cells. Latencies determined with threshold current pulses 400 msec long in the cones and 600–900 msec long in the rods; current polarities and response patterns as shown. Cross-hatched results from experiments in which the impulse-producing cell gave a single spike of fixed latency (see text). Temperature 19–22° C.

TABLE 1. Collected latencies of impulses generated by electrical polarization of receptors

Receptor	Pathway	Latency (msec) Make	Break
Red-sensitive cone	Off	113 ± 29 (23)	98 ± 17 (18)
	Off*	48 ± 9 (8)	51 ± 6 (7)
	On	173 ± 41 (22)	145 ± 29 (3)
Rod	Off	149 ± 19 (8)	152 ± 36 (13)

'Pathway' indicates whether impulses were generated by positive-going stimuli ('off') or by negative-going stimuli ('on'). 'Make' and 'break' give the phase of the pulse at which impulses were generated. The numbers are collected grand mean latencies ± 1 standard deviation. The figures in brackets show the number of pairs of cells examined. * Indicates cells giving only single impulses per trial.

cone over both pathways. In one experiment, for example, activation of the 'off' path gave latencies of 84 ± 8 msec (make of depolarizing pulses, mean $\pm$ standard error of the mean) and 75 ± 5 msec (break of hyperpolarizing pulses) while for activation of the same cell over the 'on' path the latencies were 145 ± 6 msec (make of hyperpolarizing pulses) and 144 ± 17 msec (break of depolarizing pulses). Similar differences in latencies were observed in five other experiments.

In an experiment in which the off pathways from a rod and a red-sensitive cone were compared in the same retina, the cone-ganglion cell pair had latencies of 95 ± 3 msec (make of depolarizing pulses) and 90 ± 3 msec (break of hyperpolarizing pulses). Activation of another ganglion cell over the rod pathway gave latencies of 150 ± 10 msec (depolarizing pulses) and 137 ± 6 msec (hyperpolarizing pulses).

Characterization of synaptic transfer in the threshold region

The long delay between the onset of a step of current in a receptor and the initiation of impulses in a ganglion cell suggests that the processes of synaptic transfer are comparable in time scale to the signals generated in the receptors by light. It was thus of interest to determine whether the pathways selectively filter input signals on the basis of their duration. In many experiments the threshold current was always higher for signals of 20 msec duration than for signals lasting 100 msec or more. The 400 msec pulses of Fig. 1*A*, for example, gave a mean of 1·61 spikes per trial with currents between 1 and $1{\cdot}5 \times 10^{-10}$ A. Interposed trials with 20 msec pulses (Fig. 1*B*) gave a mean of 1·48 spikes per trial with pulses six times stronger. The higher threshold for short pulses indicates that, during the delay, the pathway integrated the input.

Further information about the kinetics of transfer was obtained by systematically varying the duration of the input pulses while determining the size of the threshold current and the delay to the impulses. Results from an experiment on the 'off' pathway between a rod and a ganglion cell are shown in Fig. 3. Above is a double logarithmic plot of the size of the threshold current as a function of the pulse duration. Filled circles represent depolarizing pulses which excited at make, open circles hyperpolarizing pulses which excited at break. With pulses of both polarities the threshold current for long pulses (rheobase) was about $6\text{–}7 \times 10^{-11}$ A. As the pulse duration was shortened, increasing currents were needed to bring the ganglion cell to threshold. Such results can be characterized empirically by drawing a line with a slope of -1 through the points for short pulses and extrapolating it to an intersection with a horizontal line drawn at the rheobase current. The position of the intersection on the abscissa will be termed the utilization time. As shown by the dotted lines

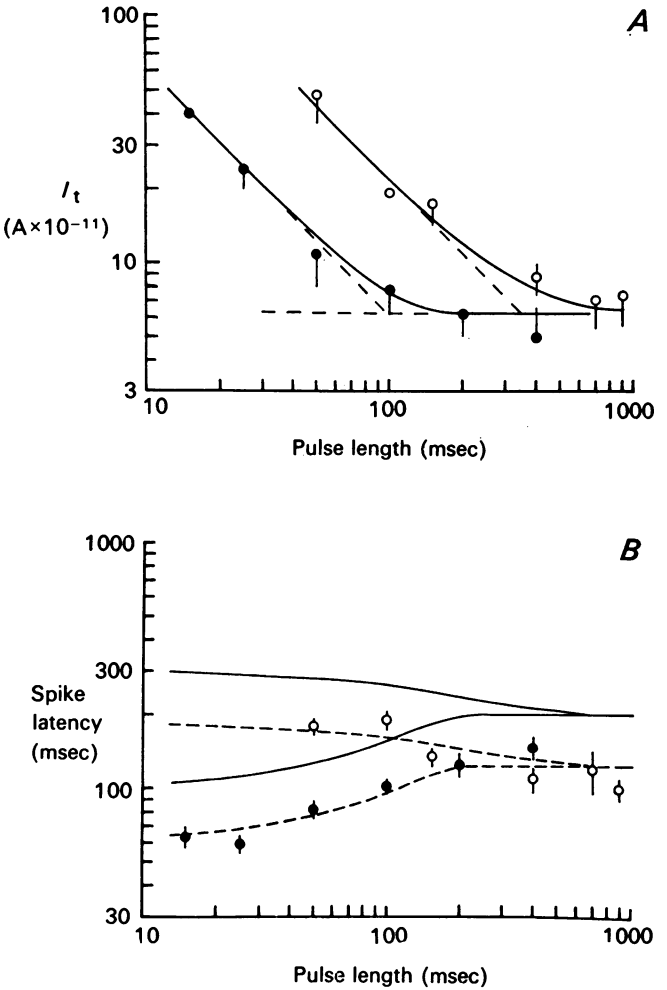

Fig. 3. Strength-duration (*A*) and latency-duration (*B*) relations for the off pathway from a rod to a ganglion cell. *A*, threshold current plotted against duration of the current pulse. Open circles, hyperpolarizing current which gave break responses, filled circles, depolarizing current which gave make responses. Vertical bars are estimates of the uncertainty in determining the thresholds. *B*, mean spike latency plotted against the duration of threshold current pulses. The points are means from ten to twenty trials, the bars 1 S.E. of the mean. *Chelydra* preparation. Rod U_{max} 15 mV, R_m 83 MΩ, flash sensitivity S_t 2786 μV photon^{-1} μm^2, time to peak linear response 600 msec. Continuous lines based upon eqns. (2), (3) and (4) with $n = 8$, $\beta = 25$, $a = 0{\cdot}66$ sec^{-1}. Interrupted lines in *B* are the continuous lines shifted downward (see text). Temperature 21·4° C.

in Fig. 3*A*, the utilization times for this pair were 96 msec (filled circles) and 350 msec (open circles). The pathway thus discriminated against depolarizing pulses shorter than 96 msec and hyperpolarizing pulses shorter than 350 msec.

Information complementary to the strength–duration relations was provided by measuring the latency to the impulses as a function of the pulse duration, as illustrated in Fig. 3*B*. In this pair, the impulses had a mean latency of 148 ± 67 msec (mean $\pm$ S.D.) for long depolarizing pulses and 107 ± 61 msec for long hyperpolarizing pulses. The latency shortened with shorter depolarizing pulses (filled circles), while it lengthened with shorter hyperpolarizing pulses (open circles). The maximum fractional change in latency was similar for both directions of current.

The continuous lines in Fig. 3*A* and *B* were derived from a model of synaptic transfer described in the next section.

Quantitative description of synaptic transfer

The object of this section is to derive a quantitative description of the experimental strength–duration and latency–duration relations determined with currents of each polarity. Fluctuations will be ignored. It will be assumed that the delay arises in one or more low-pass filters, the differentiation from a single high-pass filter. For simplicity the filters are taken to be linear and independent; it is imagined that they may represent cell time constants, synaptic release or transmitter action kinetics. Provision for multiple delays is needed because of the experimental observation (see Fig. 1*B*) that the peak ganglion cell response occurred well after the end of a short make current pulse. The ganglion cell is assumed to have a fixed threshold d mV positive to the potential at rest and to give impulses when threshold is reached.

A general form of equation which incorporates these ideas and allows for variation in the rate constants of the filters is

$$v(t) = cI(e^{-at} - e^{-bt})^{n-1}, \tag{2}$$

this being the step response of a cascade of $(n-1)$ low-pass filters and a single high-pass filter, where the rate constants of the n elements in the chain are in arithmetic progression. In eqn. (2) $v(t)$ is the voltage output representing the synaptic excitation of the ganglion cell, I is the current passed in the cone with outward current positive, and c is a sensitivity constant which assumes positive values in the off pathways and negative values in the on pathways. The rate constants of the n filter elements range from α_1 to α_n, with values

$$\alpha_1 = (n-1)\,b, \quad \alpha_n = (n-1)\,a. \tag{3}$$

The range of the rate constants is thus determined by the ratio b/a, which for convenience will be denoted by β.

If the step response of the synaptic pathway is $v(t)$, then the response $g(t)$ to a rectangular pulse of duration T delivered at $t = 0$ will be given by

$$\begin{aligned} g(t) &= v(t), \quad 0 \leqslant t \leqslant T \\ g(t) &= v(t) - v(t-T), \quad t > T \end{aligned} \tag{4}$$

and the response to a unit impulse will be

$$g(t) = \frac{dv}{dt}. \tag{5}$$

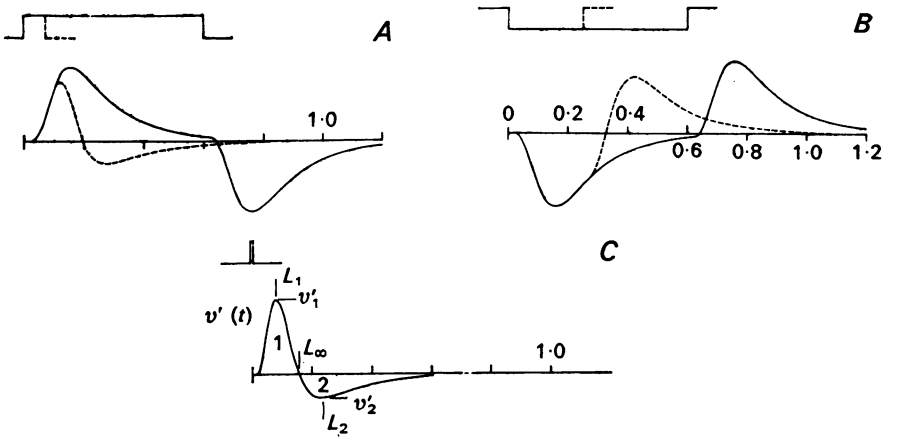

Fig. 4. Theoretical plots of an off-centre ganglion cell's synaptic excitation as a function of time during passage of currents into a receptor. In *A* the stimuli are long or short depolarizing pulses, in *B* hyperpolarizing pulses of two durations. In *C* the current is a depolarizing unit impulse. The curves in *A* and *B* were calculated from eqns. (2), (3) and (4) in the text with $n = 8$ and $\beta = 20$. The time axes are in units of a^{-1}. Several features of the unit impulse response, $v'(t)$, are given in *C*. L_∞ is the latency to the peak excitation with a long depolarizing step, as in *A*, or at the end of a long hyperpolarizing step, in *B* (latency measured from end of pulse). L_1 is the limiting latency of the make response to a short shock, L_2 the limiting latency of the break response to a short shock.

For a rectangular input pulse the synaptic excitation $g(t)$ can be calculated from (2) and (4). The sensitivity of the pathway is proportional to the peak positive value of the excitation, g_{max}. Therefore, for a constant threshold d in the ganglion cell, a plot of d/g_{max} against T gives the form of the strength–duration curves. The latency–duration relations were calculated by finding the times to g_{max}.

Fig. 4 illustrates the model excitation in an off centre ganglion cell.

In Fig. 4*A* the long depolarizing pulse generates a transient excitation followed by an underswing at the break. The response to the shorter pulse (interrupted line) reaches a smaller peak value and the time to the peak excitation occurs earlier. With long hyperpolarizing pulses (Fig. 4*B*), the response is inverted and excitation occurs at break. Shortening the hyperpolarizing pulse reduces the peak positive value of the excitation. Fig. 4*C* shows the form of the response to a positive unit impulse and explains several limiting values in the strength–duration and latency–duration relations. With a long pulse the time to the peak excitation occurs at the crossover point L_∞. For short pulses the latency of the make response shortens to L_1, while the latency of the break response lengthens to L_2. The utilization times t_u (make) and t_u (break) are

$$t_u(\text{make}) = \frac{1}{v_1'}\int_0^L v'(\tau)\,d\tau, \tag{6}$$

$$t_u(\text{break}) = \frac{1}{v_2'}\int_{L_\infty}^\infty v'(\tau)\,d\tau. \tag{7}$$

Since the model has no steady-state response, the integrals above (areas 1 and 2 in Fig. 4*C*) have equal magnitudes, and the ratio of break and make utilization times, corresponding to the horizontal separation of strength–duration curves on a double log plot, is simply v_1'/v_2'. The interval between t_u(make) and t_u(break) may be considered to represent a window within which the duration of an input signal should fall if it is to be transmitted with least attenuation.

In fitting eqn. (2) to the experimental results, it was necessary to select values for β, the ratio of largest and smallest rate constants, and n, the number of rate constants. These parameters could not be fixed independently and were chosen by successive approximation. Increasing β or reducing n increases the separation in the strength–duration relations and the maximum variation in the latency. As mentioned earlier, mean impulse latencies rather than peak impulse densities were usually determined. This will give an underestimate of n and an overestimate of β.

The off pathway from cones

Fig. 5 shows collected results from experiments on the off pathway from red-sensitive cones to ganglion cells. The strength–duration relations shown in Fig. 5*A* were obtained with depolarizing current (filled symbols, four pairs) and hyperpolarizing current (open symbols four pairs), and the currents have been normalized to their rheobase values. For only one of the pairs was it possible to obtain both relations. The utilization times were about 30 msec for depolarizing current and 100 msec for hyperpolarizing current.

Fig. 5*B* shows latency–duration measurements from the same experiments as in 5*A*. The mean latencies for long pulses, L_∞, varied considerably between experiments, ranging from 75 to 147 msec although strength–duration relations on the same cells varied much less. Because of this variation, the latencies for each experiment were normalized to the

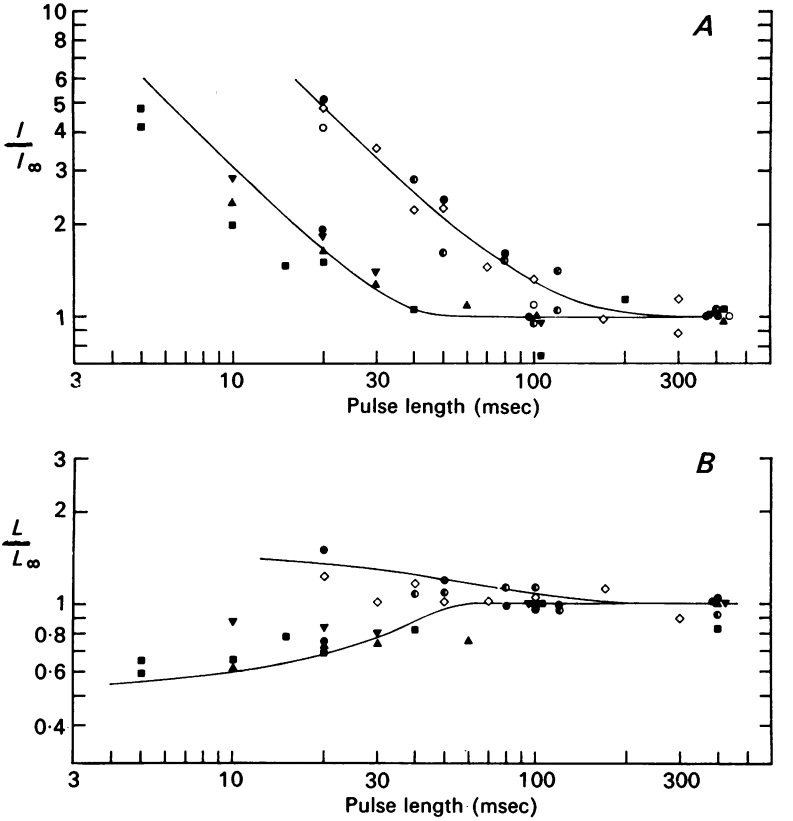

Fig. 5. Collected strength-duration (*A*) and latency-duration results (*B*) for the off pathway from red-sensitive cones. Filled symbols determined with depolarizing current, open symbols with hyperpolarizing current. For each set of points the threshold currents have been scaled to the rheobase current, I_∞, and the mean impulse latencies have been scaled to the latency L_∞, for a long pulse. Values for L_∞ ranged from 82 to 145 msec, and I_∞ ranged between 4·4 and 62×10^{-11}A. The lines are based upon eqn. (2) with $n = 8$, $\beta = 20$, $a = 2{\cdot}5$ sec^{-1}. Temperature 19–22° C.

limiting value obtained with a long pulse. The continuous curves were drawn from eqns. (2), (3) and (4) with $n = 8$, $a = 2{\cdot}50$ sec^{-1} and $\beta = 20$. This fit corresponds to time constants of 2·9, 3·3, 3·9, 4·8, 6·3, 8·9, 15·4, 57·1 msec. In the linear case considered any of these time constants could

represent that of the differentiating process, and the sequence of the filter stages in the cascade does not affect the overall performance. Although the number and magnitude of the time constants are not unique for describing the results, the fit does demonstrate one way in which the slowness of the path might arise. It is interesting that all the time constants except the longest are in the range of membrane time constants or synaptic delays. A discrepancy between experiment and theory was that the expected peak excitation in the ganglion cell had a latency L_∞ of 63 msec, while the experimental latencies were 113 ± 24 msec (grand mean $\pm$ S.D., make of depolarizing pulses) and 93 ± 15 msec (break of long hyperpolarizing pulses). The discrepancy is probably due in part to using mean latencies rather than times to the peak impulse density.

In one experiment a pair of strength–duration and latency–duration relations were determined on a cell which gave single impulses at very short latency and thus may have been an amacrine. Excitation in this cell was about twice as fast as in the cells mentioned above. For the break responses to hyperpolarizing current L_∞ was 52 ± 9 msec and the utilization time 45 msec. For depolarizing pulses exciting at make L_∞ was 47 ± 8 msec and the utilization time 15 msec. The speed of response in these cells might result from rapid accommodation in the spike-generating mechanism.

The off pathway from rods

Physical systems for processing signals in the presence of noise are often designed in accordance with the properties of the signals to be encountered. Since the rods' light responses are slower than those of the cones, the latency distributions of Fig. 2 suggest that a similar principle might apply in the retina. Further measurements on the rod off pathway are presented in Figs. 3 and 6. The continuous curves in Fig. 3 are based on eqns. (2), (3) and (4) with $n = 8$, $a = 0{\cdot}660$ sec^{-1} and $\beta = 25$. The vertical position of the experimental latency–duration relations differed appreciably from the theoretical curves (solid lines). The dashed lines are the theoretical curves shifted down so that L_∞ is 124 rather than 203 msec; after this arbitrary adjustment the points are fairly well fitted. The reason for the difference between experiment and theory is not clear; one possible factor would be a non-linearity in the suprathreshold responses which brings the impulses to earlier times than the peak excitatory synaptic drive.

Collected results from a group of similar experiments are shown in Fig. 6. The filled symbols were obtained from make responses to depolarizing current, the open symbols from break responses to hyperpolarizing current. The currents have been scaled to the rheobase values and the

latencies to the estimated L_∞'s. The horizontal positions of individual break strength–duration relations varied considerably even though the make relations were in good agreement; sets of open symbols have been shifted horizontally by factors of 0·7–2·0 to bring them to the position

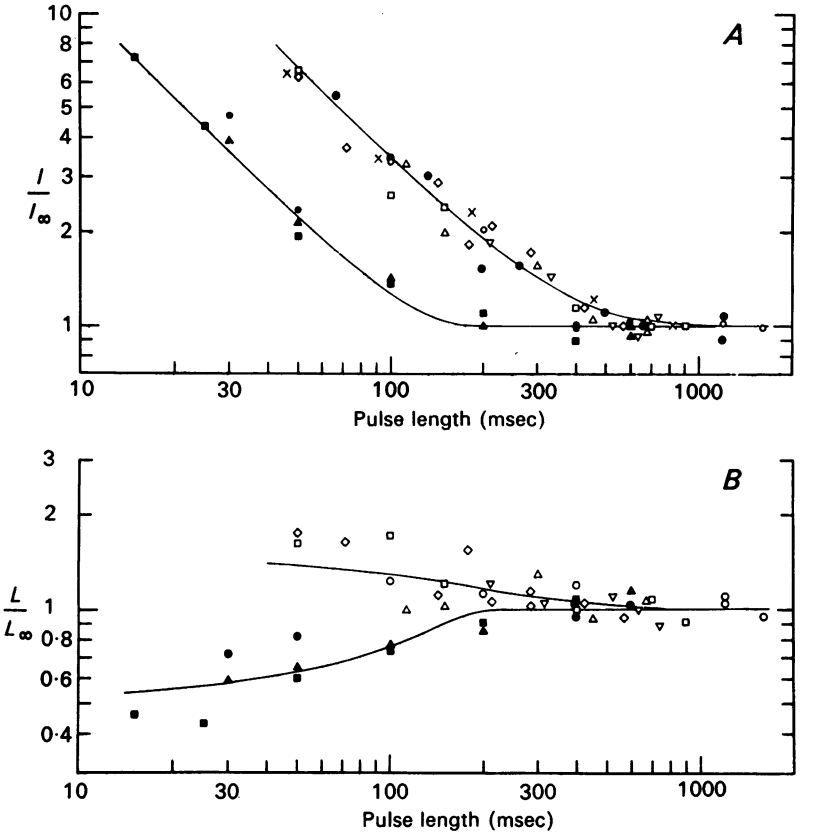

Fig. 6. Collected strength-duration (*A*) and latency-duration results (*B*) for the off pathway from rods, symbol conventions as in Fig. 4. Each set of points normalized to the rheobase current, I_∞ or limiting latency, L_∞. Each set plotted by open symbols in *A* has also been scaled on the abscissa to the best estimate of the mean; the multiplicative scaling factors were in the range 0·71–2·0. Open and filled symbols of the same kind are from the same pairs of cells. Theoretical curves based upon eqn. (2) with $n = 8$, $\beta = 20$, $a = 0{\cdot}714\ \text{sec}^{-1}$. Temperature 19–22° C.

of the mean. The mean utilization times were 110 msec (depolarizing current) and 350 msec (hyperpolarizing current). The continuous curves drawn through the points were based on eqns. (2), (3) and (4) with $n = 8$, $\beta = 20$, and $a = 0{\cdot}714\ \text{sec}^{-1}$. This fit is formally identical to that used

for the cone off path in Fig. 5, but the value of a is 3·5 times smaller. The flash responses of the rods used in the experiments of Fig. 6 were 3 to 6 times slower than those of cone responses under the same conditions. In the experiment of Fig. 3, for example, the time to the peak rod response to a dim flash was 600 msec and the integration time of the flash response (see Baylor & Hodgkin, 1973) was 820 msec. Corresponding figures for a red-sensitive cone would be about 120 and 175 msec.

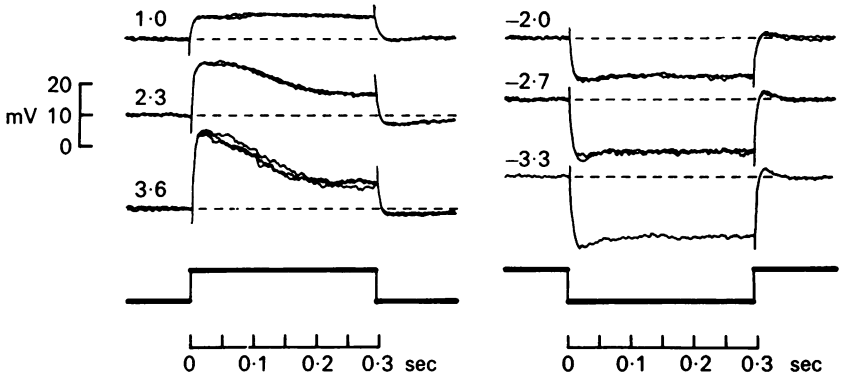

Fig. 7. Relaxation of voltage response of a red-sensitive cone on injection of depolarizing (left) and hyperpolarizing currents. The number at the left of each trace gives the current intensity in A $\times 10^{-10}$. From one to three tracings have been superimposed at each current intensity. Cone U_{max} 14·5 mV, R_m 73 MΩ. Timing of current pulse shown below.

The difference between experimental and theoretical latencies was noted again in the rod collected results. The fit described above gives L_∞ as 220 msec, while the experimental values ranged from 148 to 173 msec for depolarizing pulses and from 107 to 196 msec for hyperpolarizing pulses.

The general conclusion would be that the time scales of the rod and cone off pathways differ by about the same factor as the single photon effects in the rods and cones.

Relaxation of the electronic potentials in the receptor

While passing long current steps into receptors, we sometimes noticed a sag in the potential during the pulse, with a subsequent rebound past the resting potential when the current was switched off. The sag was observed in both rods and cones, but in the rods it was several times slower, thus paralleling the kinetic differences of the rod and cone off pathways. The extent of the effect varied considerably from one experiment to another. In the cones it was rarely significant for potential changes

less than 5–10 mV and was not a requisite for observing the differentiation in the synaptic pathways. In some rod experiments, however, a noticeable relaxation was present even to very small potential changes of about 1 mV.

Fig. 7 shows records from a red-sensitive cone which exhibited a pronounced sag in the potential evoked by steps of current. For currents which depolarized by more than about 8 mV, the potential declined to a plateau with a time constant of 48–58 msec; the extent of the decline

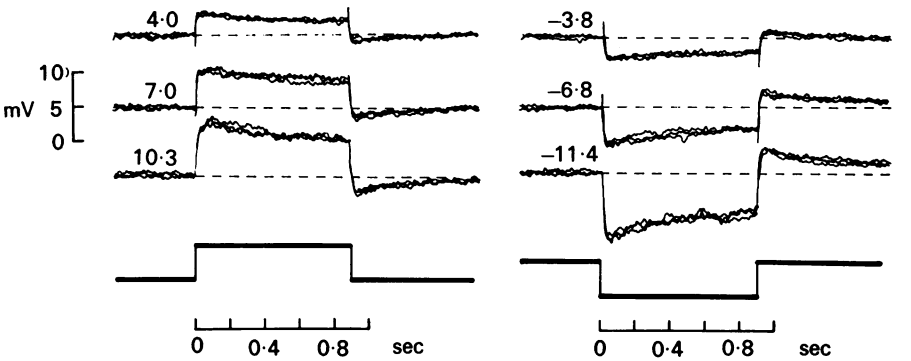

Fig. 8. Relaxation of the voltage response of a rod on injection of depolarizing (left) and hyperpolarizing currents. The number at the left of each trace gives the current intensity in $A \times 10^{-11}$. At each current intensity, three tracings have been superimposed. Timing of current pulse drawn below. Snapping turtle preparation; rod U_{max} 20 mV, R_m 74 MΩ, flash sensitivity 2033 μV photon^{-1} μm^2, time to peak of the linear flash response 600 msec. The voltage responses were recorded through a low pass filter of time constant 6 msec. Temperature 21·7° C.

was more pronounced with larger depolarizations. A smaller sag back towards the resting potential was seen with hyperpolarizing currents and the time constant of the process was similar. The sag could not be observed when the electrode was outside the cone and thus was not due to rectification in the electrode. In six experiments on red-sensitive cones a relaxation of similar magnitude was observed with currents which produced potential changes of 10–30 mV with pulses of both polarities. The time constant of the relaxation ranged from 35 to 86 msec with a mean of 54 msec. An example of the same phenomenon in the voltage response of a rod is illustrated in Fig. 8. The droop was comparable in size with both pulse polarities and had a time constant of about 360 msec. In measurements on 5 rods, the time constants ranged from 118 to 400 msec with a mean of 228 msec.

The 'on' pathway from cones

Fig. 9 shows collected strength–duration measurements from four experiments on the 'on' pathway from red-sensitive cones to ganglion cells. Hyperpolarizing currents were passed in the cone and gave make responses in the ganglion cells. Utilization times estimated by the lines ranged between 89 and 182 msec. The limiting latencies L_∞, in the four experiments varied from 123 ± 40 msec (mean $\pm$ S.D.) to 219 ± 52 msec. With 20–30 msec threshold pulses the latencies shortened to values 0·54–0·62 times those of L_∞.

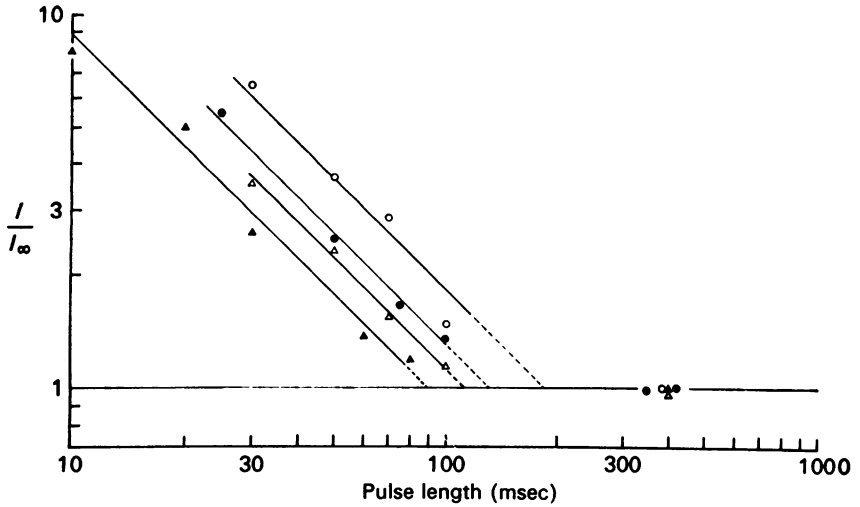

Fig. 9. Collected strength–duration relations for the 'on' pathway from red-sensitive cones; make responses to hyperpolarizing current. The ordinate is the threshold current divided by the rheobase current, I_∞. Lines with a slope of -1 are extrapolated to intercept the horizontal line drawn at the rheobase to obtain the utilization time. This parameter and I_∞ were: ▲, 89 msec, 2×10^{-11}A; △, 112 msec, $13{\cdot}5 \times 10^{-11}$A; ●, 132 msec, $5{\cdot}2 \times 10^{-11}$A; ○, 182 msec, $13{\cdot}8 \times 10^{-11}$A. Temperature 20–21 °C.

In one experiment it was possible to determine the relations with both polarities of current, giving the results shown in Fig. 10. The strength–duration relations shown above were separated by only a factor of 1·4, the utilization times being 120 msec (make responses to hyperpolarizing pulses) and 170 msec (break responses to depolarizing pulses). The latency–duration relations shown below crossed over one another so that with long pulses the latency for make responses was longer than that for break responses; this indicates a non-linearity in the pathway. The smooth curves drawn through the points were based on the idea that the excita-

tion passes through a chain of four exponential delays and a single differentiating stage, each with a time constant τ. The step response of this chain is

$$v(t) = cI(t/\tau)^4 e^{-t/\tau}, \tag{8}$$

where c is a sensitivity constant, I the current passed in the cone and v the output voltage as a function of time. To account for the small

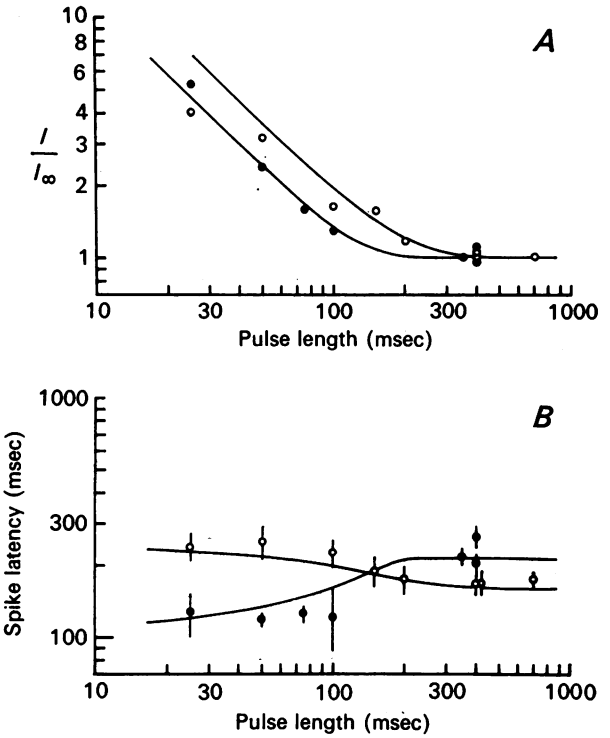

Fig. 10. Strength–duration (*A*) and latency–duration relations (*B*) for the on pathway from a red-sensitive cone to a ganglion cell. Filled circles: hyperpolarizing current giving responses at make; open circles: depolarizing current giving responses at break. The impulse latencies are means from ten to thirty trials, and the vertical bars are 1 S.E. of the mean. Cone U_{max} 15·5 mV, R_m 70 MΩ. Rheobase current, I_∞, $5{\cdot}5 \times 10^{-11}$ A (hyperpolarizing), 8×10^{-11} A (depolarizing). Temperature 20·4° C. Theoretical curves based on eqn. (8) as described in text.

degree of separation between the strength–duration curves and the crossing in the latency–duration relations the time constant was taken as 53 msec for hyperpolarizing currents and as 40 msec for depolarizing currents.

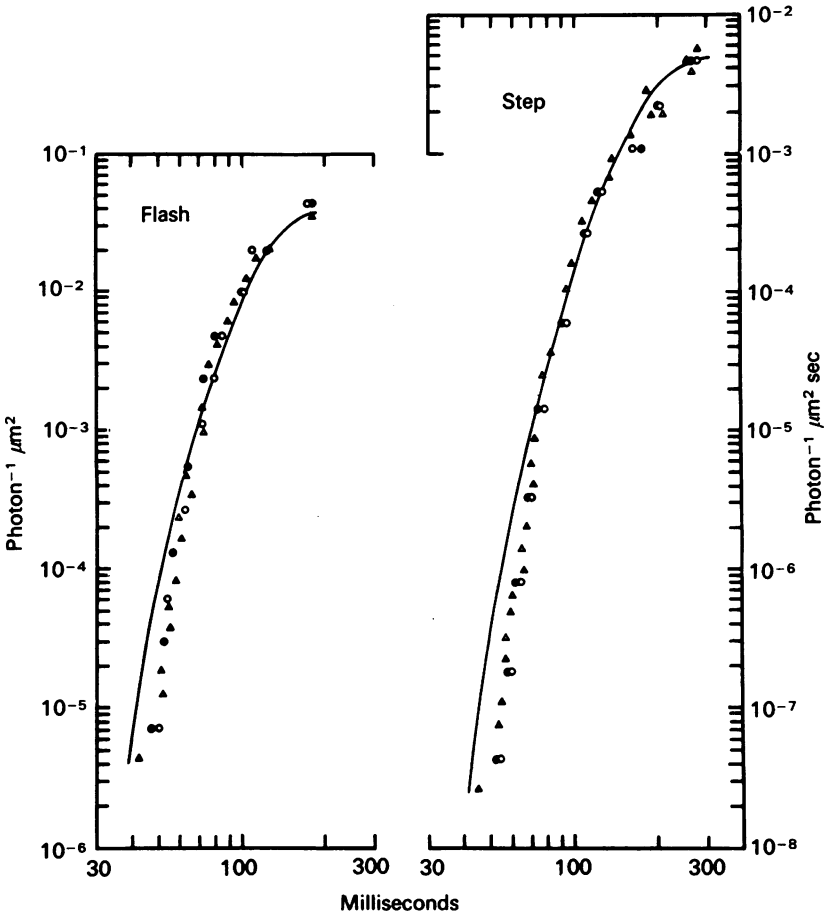

Fig. 11. Collected strength-latency relations for 'on' responses in four ganglion cells to 6 msec flashes (left) and steps (right) of red light. Reciprocal 640 mm light intensity plotted against time to first impulse with each point the mean from five to ten trials. Sensitivities and latencies have been scaled slightly to superimpose the points; each relation from a given cell was scaled by the same amount. The scaling factors and stimulating spot sizes were:

Symbol	Spot size (μm)	Latency	Sensitivity
○	270	1·11	1·0
●	270	1·11	1·0
△	320	1·0	1·56
▲	210	0·85	0·53

The continuous lines were calculated by the method described in the text. Temperature 20·1–21·6° C.

Latency of the ganglion cell response to light

The previous sections have shown that transmission over the retinal pathways is marked by a long delay. A ganglion cell's response to light should reflect this transmission delay as well as the lag in the electrical response of the receptor. The object of this section is to see whether the latency of the ganglion cell light response can be explained in terms of these components.

Fig. 11 shows collected strength–latency relations from four ganglion cells which gave on responses to flashes and steps of red light applied to the centre of their receptive fields. The ordinates give the reciprocal intensity of the flashes (left plot) and steps (right plot) on logarithmic scales, while the logarithmic abcissae give the mean latency to the first impulse, averaged from five to ten trials. It can be seen that as the stimulus intensity increased the latency decreased, rapidly at first and then more slowly.

The continuous curves were calculated in the following way. Assuming linear synaptic transfer the ganglion cell excitation $g(t)$ to a light applied at $t = 0$ should be given by

$$g(t) = u(i,t) * s(t), \tag{9}$$

where u is the cone response to light of intensity i, s is the impulse response of the pathway and $*$ denotes the operation of convolution. The latency to the first spike was found by solving $g(t)$ and finding the time required to reach a fixed threshold value. The synaptic impulse response was taken as

$$s(t) = \frac{\mu}{\tau} T^3 \mathrm{e}^{-T}(4-T), \tag{10}$$

where μ is a constant and T is t/τ. This is the derivative of the step response fitted to the results from the 'on' pathway. The variable u representing the cone response is highly non-linear with all but very dim lights. A set of equations which simulates the main features over the times considered is (see Baylor *et al.* 1974):

$$\tau_{\mathrm{L}} \frac{\mathrm{d}u}{\mathrm{d}t} + u\{f-(f-1)B\} = Bu_{\mathrm{L}}, \tag{11}$$

$$B = \frac{K_s i\phi(t)}{K_s i\phi(t) + u_{\mathrm{L}} f}, \tag{12}$$

$$\phi(t) = (1-\mathrm{e}^{-\alpha t})^6, \qquad \text{steps (13)}$$

$$\phi(t) = \Delta t 6\alpha \mathrm{e}^{-\alpha t}(1-\mathrm{e}^{-\alpha t})^5. \qquad \text{flashes (14)}$$

In eqn. (11) u is the hyperpolarization and u_{L} its maximum value, B the fractional closure of the light-sensitive conductance, τ_{L} the time constant

of the cell when $B = 1$, and f is a constant representing the factor by which the total cell conductance can be reduced in bright light. Eqn. (12) gives the dependence of B on light intensity, i, and time, where K_s is the step sensitivity constant of the cone. $\phi(t)$ is a dimensionless kinetic variable describing the concentration of blocking particles near the light-sensitive conductance as a function of time, and Δt in eqn. (14) is the duration of the flash. Values for the cone constants were selected from previous work as $f = 1{\cdot}64$, $u_L = 25$ mV, $\tau_L = 10$ msec, $\alpha = 20{\cdot}8$ sec^{-1}, and $K_s = 2{\cdot}5\ \mu$V photon^{-1} μm^2 sec (Baylor *et al.* 1974; Baylor & Fettiplace, 1975). μ and τ in eqn. (10) were selected empirically to fit the experimental step strength–latency relation. μ was chosen as $d/4{\cdot}587$ where d is the threshold depolarization in the ganglion cell, and τ was taken to be 35 msec. Having selected these values the entire theoretical flash relation was then fixed.

It can be seen that the theoretical curves fit the experimental measurements except toward the bottom of the relations, where the points fall to the right of the curves. The disagreement is of the type which would occur if one of the four integrating stages treated as a first-order element in the model were instead a composite delay. It is worth noting that the value for τ of 35 msec would predict L_∞ in a current passing experiment to be $4 \times 35 = 140$ msec and the 'make' utilization time 76 msec. These are not far from the values obtained experimentally.

DISCUSSION

A large body of previous work has shown that signals in the retinal receptors are modified by spatial filtering during transmission to the ganglion cells. The present experiments show that electrical changes in the receptors are also sifted by temporal processes which preferentially pass signals having durations of the order of 0·1 sec. This is comparable to the length of the voltage change evoked in a receptor by a brief flash of light or gap of darkness. The notion of a match between the temporal properties of encoder and transmission line is reinforced by the difference in the kinetics of the off pathways originating in the rods and cones. Although formally similar, these paths differed several fold in average time scale, paralleling the different speeds of visual transduction in the rods and cones.

Psychophysical measurements of amplitude sensitivity as a function of flicker frequency yield a value of about -10 for the limiting high frequency slope on double log plots (see De Lange, 1958; Kelley, 1972). This is similar to the initial steepness of the strength–latency relation on the left side of Fig. 11. It thus seems attractive to suppose that in man,

as well as the lowly turtle, the long retinal delay may reflect approximately equal contributions from receptor activity and the linkage to ganglion cells.

Organization of the pathways

The delay in the paths presumably arises at least in part from cell time constants, cable spread in elongated cellular processes, transmitter action kinetics, and perhaps also delays in the release and diffusion of transmitter substances. The differentiation is more obscure; it is not clear whether it results from a single localized process or a series of mechanisms distributed over several cells. In the rods and cones themselves there was evidence of a voltage-sensitive conductance which caused a partial relaxation in the voltage change evoked by a step of current (see also Baylor *et al.* 1974; Schwartz, 1976). This process developed at different rates in rods and cones and might play a role in the differentiation.

Tentative inferences about the wiring of the paths can be drawn from their kinetics. The different time scales and the distributed nature of the delay in the off pathways from rods and cones suggest that the channels are independent before convergence on ganglion cells. In terms of the wiring scheme proposed in the previous paper (Baylor & Fettiplace, 1977), this would imply anatomically that separate hyperpolarizing bipolars carry the rod and cone messages in the turtle retina. The differing speeds of synaptic transfer in the on and off pathways from the red-sensitive cones suggest that the depolarizing bipolars, thought to carry the on signals, may respond more slowly to voltage changes in a receptor than the hyperpolarizing bipolars.

Limitations of the models

The quantitative descriptions of retinal transmission presented here have several obvious limitations. In the first place it was assumed that the interaction between a receptor and a ganglion cell was linear in the subthreshold region. This assumption is the simplest to make but at best it is probably no better than a rough approximation. The discrepancies between the measured and theoretical latencies give a direct indication that strict linearity was not obeyed. Linearity would also imply that the outcome of double pulse experiments should be predictable from the effects of single pulses. A few experiments of this type suggested that the stimuli did not have independent additive effects, and that the 'differentiation' was accompanied by a sensitivity reduction.

Within the 'cascade' category of linear models, there are many combinations of time constants which would give nearly the same over-all

behaviour. Eqn. (2), originally derived by Professor A. L. Hodgkin in a different context (Baylor *et al.* 1974), and eqn. (8) have the advantage of simplicity and were selected on this basis.

Processing of signals and noise

Utilization times for the on synaptic pathway from red-sensitive cones were in the range 90–180 msec, while the mean duration of the linear flash responses of these receptors is about 170 msec (Baylor & Hodgkin, 1973). Signals of the average form generated by photoisomerizations should thus be relatively effective inputs for the pathway. Small input signals generated by light will be contaminated, however, by the spontaneous voltage noise recently discovered in turtle photoreceptors by Simon, Lamb & Hodgkin (1975). In an electrically isolated cone in darkness this noise would have an r.m.s. amplitude of about 630 μV (Lamb & Simon, 1976), while a single photoisomerization in an isolated cone is estimated to give a peak response of about 25 μV (Baylor & Hodgkin, 1973; Baylor & Fettiplace, 1975). Although the effect of a single photon would thus probably not be recoverable, it is possible that larger signals could be enhanced relative to the noise by temporal filtering in the synaptic pathway.

The question of how light-evoked signals and noise will be processed by the path can be approached as follows. For simplicity suppose that the signal has the form of the response to a dim flash, described by (Baylor *et al.* 1974)

$$u(t) \propto e^{-\alpha t}(1-e^{-\alpha t})^5, \tag{15}$$

where u is the hyperpolarization in mV, t is time from the flash, and α is a rate constant. The form of the power spectrum of this signal will be given by

$$S_s(\omega) \propto \frac{1}{(1+\omega^2\tau_1^2)(1+\omega^2\tau_2^2)\ldots(1+\omega^2\tau_6^2)}. \tag{16}$$

In eqn. (16) S_s is the power spectral density of the signal as a function of angular frequency, ω, and the τ's are time constants defined by

$$\tau_n = \frac{1}{(7-n)\alpha}, \tag{17}$$

where n assumes integer values from 1 to 6. From information kindly supplied by Drs Trevor Lamb and the late Elliot Simon, the form of the power spectrum of the cone noise will be approximated by

$$S_n(\omega) \propto \frac{1}{1+\omega^2/(2\pi f_0)^2}, \tag{18}$$

where S_n is the noise power spectral density and f_0, the half power frequency, is 5 Hz. Assuming that the synaptic pathway has the impulse

response given by eqn. (10), the form of the output power spectrum to a white noise input would be

$$S_f(\omega) \propto \frac{\omega^2}{(1+\omega^2\tau^2)^5}. \tag{19}$$

The factor by which the power signal-to-noise ratio will be changed by the filter is

$$R_P = \frac{\int_0^\infty S_s(\omega)\, S_f(\omega)\, d\omega}{\int_0^\infty S_n(\omega)\, S_f(\omega)\, d\omega} \times \frac{\int_0^\infty S_n(\omega)\, d\omega}{\int_0^\infty S_s(\omega)\, d\omega}, \tag{20}$$

and the amplitude signal-to-noise improvement figure, R_a, is

$$R_a = (R_P)^{\frac{1}{2}}. \tag{21}$$

Eqns. (16), (18) and (19) were evaluated and the integrals in (20) determined graphically. With values for α of 15 sec^{-1} and τ of 44 msec R_P was calculated as 2·05 and R_a as 1·43. The relatively small degree of improvement in the signal-to-noise ratio is principally due to the similar bandwidths of signal and noise. In the rod system, where signals and noise may have more divergent properties, synaptic filtering could give a larger effect. To evaluate this possibility it will be useful to examine the kinetics of synaptic transfer in the on path from rods.

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