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CONES EXCITE RODS IN THE RETINA OF THE TURTLE

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SUMMARY

The intracellular responses of rods in the retina of the turtle, *Chelydra serpentina*, were studied with brief flashes of monochromatic light.

1. Flashes of red or green light applied over an area 25 μm in diameter produce responses with the same shape. With such restricted stimuli, the spectral sensitivity of a rod agrees well with the absorption spectrum of the porphyropsin pigment contained in its outer segment.

2. With stimulating spots more than 500 μm in diameter, dim flashes of red or green light produce responses having different shapes. When the spectral sensitivity of a rod is tested using dim lights of large diameter, the sensitivity to red light is much greater than predicted by the absorption spectrum of porphyropsin.

3. The shape of the response produced by large diameter spots of dim, red light resembles that of cones.

4. Increasing the diameter of a dim, red spot beyond 500 μm markedly alters the amplitude and shape of responses from horizontal cells but does not significantly affect the response of rods.

It is concluded that rods receive an excitation from neighbouring cones. This interaction is unlikely to be mediated by type I luminosity horizontal cells but may be mediated by either direct connexions between cones and rods or by an interneurone with a small receptive field.

INTRODUCTION

The responses of individual rods are modified by synaptic interaction with neighbouring neurones. In two preceding papers (Schwartz, 1973*b*, 1975) the responses of rods to dim, green light were determined. When light was restricted to the impaled cell the shape of the response was

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similar to that of cones but 2–4 × slower and 2 × larger in amplitude. When neighbouring rods were also illuminated this response was modified in two ways: following a flash the response was initially enhanced to increase the amplitude nearly tenfold and then after a delay disenhanced to reduce responsiveness. These effects were attributed to an interaction from peripheral retina mediated by neighbouring rods within a distance of 250 μm.

During the present experiments the responses of rods to different wave-lengths of light were studied. The results show that responses produced by large diameter spots are not independent of wave-length. Interaction adds for dim, red light a hyperpolarization which follows the time course of the response of cones. It is concluded that red-sensitive cones excite rods. This increases the responsiveness of rods to wave-lengths of light which they themselves do not optimally absorb.

METHODS

The procedure used for stimulating and intracellular recording from retinal cells of the turtle, *Chelydra serpentina*, were the same as described previously (Schwartz, 1973*a*, 1975). Rods were penetrated sclerad to horizontal cells in the receptor layer and identified by their great sensitivity to large diameter spots of 500 nm light, their characteristic response shape and slow recovery following moderately intense illumination, and by their sensitivity over only a small area to a dim 25 μm spot. These properties have been previously established as characteristic of rods (Schwartz, 1973*b*). Responses were measured as the deviation from the membrane potential during darkness. The signal-to-noise ratio of responses smaller than 4 mV was improved by averaging with a Hewlett–Packard Model 5480A Signal Analyzer. All stimuli were flashes 20 msec in duration. Monochromatic lights were obtained by inserting narrow-band interference filters (Baird-Atomic, less than 50 % of total transmission at $\pm$ 15 nm from λ_{max}) into the light path. The total irradiance transmitted by each filter was measured by a silicon photodiode (United Detector Technology, Inc., Optometer model 40A) placed at the normal location of the retina and converted to photons μm^{-2} $flash^{-1}$ by assuming all of the energy to be at the wave-length of maximum transmission.

RESULTS

The intensity-amplitude relation for large diameter spots

The relation between stimulus intensity and the peak amplitude of response produced by spots of large diameter depends upon wave-length. In Fig. 1 the circles are the peak amplitude of responses for different intensities of a 500 μm diameter spot of 510 nm light. The continuous line was drawn to smoothly approximate the data. Also shown as the squares are the peak amplitudes for different intensities of 690 nm light. A simple shift of the continuous line along the log intensity axis (as indicated) does not fit the data at 690 nm. There is a deviation for dim light where the interrupted line better describes the data.

The intensity change necessary to produce responses of equal peak amplitude at different wave-lengths has been used frequently to characterize the spectral sensitivity of a receptor. However, Fig. 1 demonstrates that for large diameter spots the intensity–amplitude relation of rods is different for different wave-lengths of light. Therefore, the intensity difference necessary to produce the same criterion amplitude at two different wave-lengths depends upon the criterion amplitude selected. For example, the curves in Fig. 1 differ at a criterion amplitude of 13 mV by 3·3 log units (as indicated by the solid arrow) whereas for a criterion amplitude of 0·75 mV the curves differ by only 1·9 log units (as indicated by the dashed arrow).

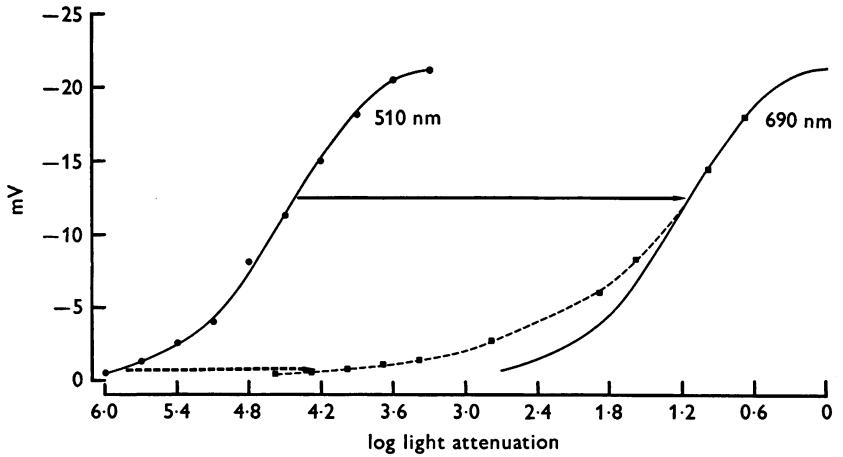


Fig. 1. The log intensity-amplitude relation of rods produced by spots of large diameter depends upon wave-length. Peak amplitude is plotted as ordinate against log intensity as abscissa. Circles are for a 500 μm diameter spot of 510 nm light; squares are for a 500 μm diameter spot of 690 nm light. The continuous line was drawn to smoothly approximate the data at 510 nm. A simple shift of the continuous line along the log intensity axis does not fit the data at 690 nm. For a criterion amplitude of 13 mV the curves differ by 3·3 log units (as indicated by the solid arrow) whereas for a criterion amplitude of 0·75 mV the curves differ by only 1·9 log units (as indicated by the dotted arrow). Log 0 intensity corresponds to $4{\cdot}4 \times 10^5$ photons μm^{-2} at both wave-lengths.

The reciprocal of the intensity change necessary to obtain criterion responses is plotted on a logarithmic scale for several wave-lengths in Fig. 2. Filled circles are for a 13 mV criterion and open circles are for a 0·75 criterion. The data were obtained from results similar to Fig. 1. Responses smaller than 4 mV were obtained by averaging approximately twenty sequential trials (as in Fig. 3). Also shown in Fig. 2 as the continuous

and interrupted line is the expected absorption of a porphyropsin pigment with a maximum absorption at 515 nm.

Microspectrophotometry of single, rod outer segments from retinae of the turtle, *C. serpentina*, indicates that these contain a porphyropsin pigment with a λ_{max} at approximately 518 nm (Liebman, 1972). The solid line is the porphyropsin nomogram of Bridges (1967) for a λ_{max} of 515 nm. The published values of this nomogram only extend to approximately 5% absorbance which for a λ_{max} of 515 nm occurs at about 620 nm. It is, therefore, necessary to extend the curve so that it may be compared to the data obtained with deep red light. For this purpose unpublished data of the hydroxylamine difference spectrum of the porphyropsin pigment isolated from the tench, *Tinca tinca* (with λ_{max} = 530 nm), were graciously provided by Dr F. Crescitelli. These data were rescaled to a λ_{max} of 515 nm and are plotted as the interrupted line.

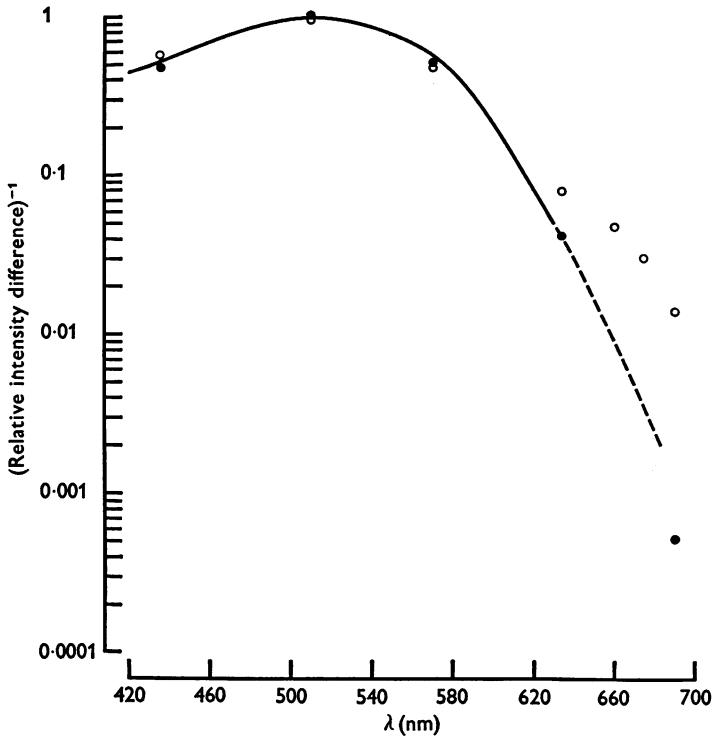


Fig. 2. Spectral sensitivity for stimuli of large diameter depends upon the criterion voltage. The reciprocal of the relative intensity change necessary to produce a criterion voltage is plotted on a logarithmic scale as ordinate against wave-length as abscissa. The filled circles are for a criterion voltage of 13 mV; the open circles are for a criterion voltage of 0·75 mV. The continuous and interrupted line indicates the expected result if sensitivity was determined only by the porphyropsin pigment contained in the rod outer segments (see text). Same cell as in Figs. 1 and 3.

The intensity changes necessary to produce large responses (13 mV criterion) closely agree with the pigment absorption curve. The intensity changes necessary to produce small responses (0·75 mV criterion) deviate significantly from the pigment absorption curve. The deviation is such that less light than predicted is required to produce small responses at the red end of the spectrum. Results similar to those obtained with large amplitude responses have been noted previously by Fain & Dowling (1973) in retinae of the mudpuppy, *Necturus maculosus*, and by Baylor & Hodgkin (1973) in retinae of the red-eared turtle, *Pseudemys scripta elegans*. The deviation with small amplitude responses has not been reported previously.

The shape of responses produced by large diameter spots of different colours

The preceding results demonstrate that for large diameter spots the responses of rods deviate from those predicted by the porphyropsin absorption curve. At dim intensities the amplitudes of responses to red light are much greater than predicted. Therefore, responses to dim, red light were studied carefully and compared with those obtained with light near the wave-length of optimal absorbance. Fig. 3*A* shows average responses to increasing intensities of 510 nm light. The intensity was doubled for each trace proceeding from the top downward. All of the responses have a similar time course with a peak at approximately 300 msec. Fig. 3*B* shows responses to increasing intensities of 690 nm light. The intensity was increased four times for each trace proceeding from the top downward. The shapes of responses produced by 690 nm light are markedly different from responses of equal amplitude produced by 510 nm light. For very dim, red light the peak amplitude occurred at approximately 150 msec. As intensity was increased a second wave appeared and the peak shifted to a longer interval. The log intensity-amplitude relation for these responses is shown in *C*. A simple shift along the log intensity axis cannot bring the data at the two wave-lengths into coincidence.

The probability for the absorption of light by a visual pigment is determined by its absorption spectrum. The effect of each quantum once absorbed is believed to be independent of wave-length. This assumption has been referred to as the principle of univariance (Naka & Rushton, 1966). Since large diameter spots of red or green light produce responses with different time courses, these responses should, assuming univariance, be determined in part by different pigments. The responses to large diameter spots of dim, green light are similar in time course to that previously reported for rods (Baylor & Hodgkin, 1973; Schwartz, 1975). The responses to very dim, red light (top trace of Fig. 3*B*) are similar in time course to that of cones (Baylor & Hodgkin, 1973). As the red light

is brightened the early response to a very dim, red light grows only slightly while a large, slow wave similar to that produced by green light is added. As the light intensity is further increased the difference in wave-shape produced by green and red lights is diminished. At moderate intensities these colours produce responses which differ only in their time of onset but have a similar peak amplitude and time course of repolarization (Fig. 4).

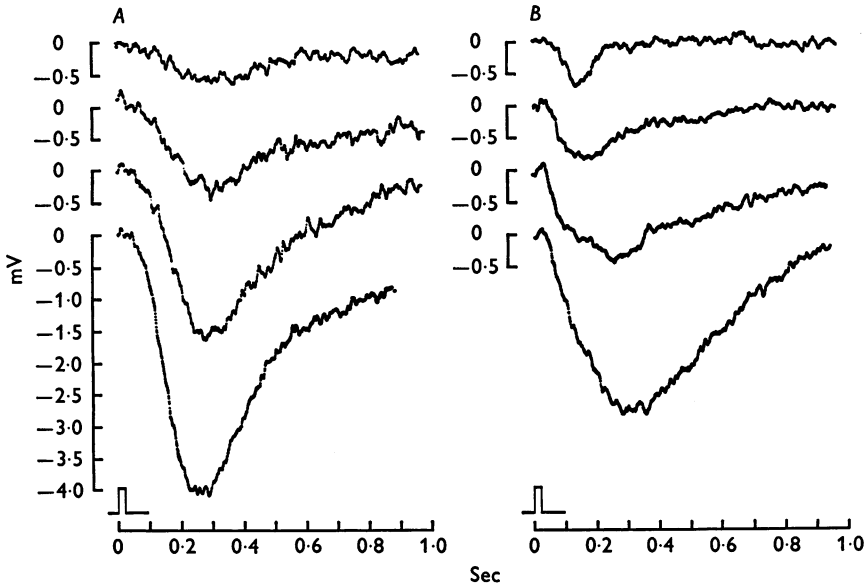


Fig. 3. The shape of the response to a large spot of dim light depends upon wave-length. *A* shows average responses to a 500 μm diameter spot of 510 nm light. The irradiance for the top trace was 1·4 photons μm^{-2} and was increased 2× for each subsequent trace proceeding downward. *B* shows average responses to a 500 μm diameter spot of 690 nm light. The irradiance for the top trace was 34 photons μm^{-2} and was increased 4× for each subsequent trace proceeding downward. *C* (on following page), the peak amplitude of responses in *A* and *B* are plotted as ordinate against log irradiance as abscissae. The plot is an enlargement of the data which was obtained at dim irradiance and was also presented in Fig. 1.

Interaction mediated by neighbouring cones

The preceding results could, perhaps, be explained if each rod contained in addition to porphyropsin a small fraction of a red-absorbing pigment which when bleached produced a response similar to that of red-sensitive cones. This conjecture is vitiated by the following experiment. In Fig. 5 are shown the average responses to a 25 μm diameter spot of either 510 nm (part *A*) or 690 nm light (part *B*). These small diameter

stimuli restrict the light as nearly as possible to the impaled cell. Both stimuli produced responses of very similar time course. The difference in intensity was 3·4 log units which is in good agreement with that predicted from Fig. 2 for the relative difference in the efficiency of absorption by

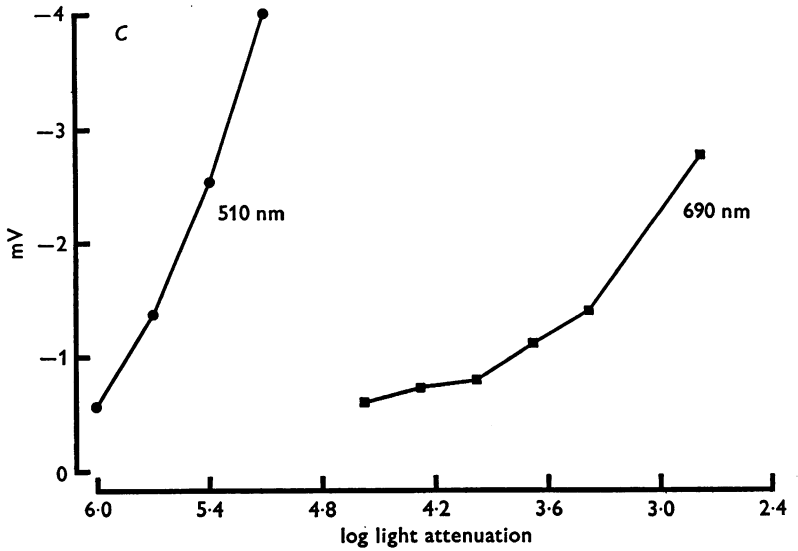


Fig. 3*C*. For legend see facing page.

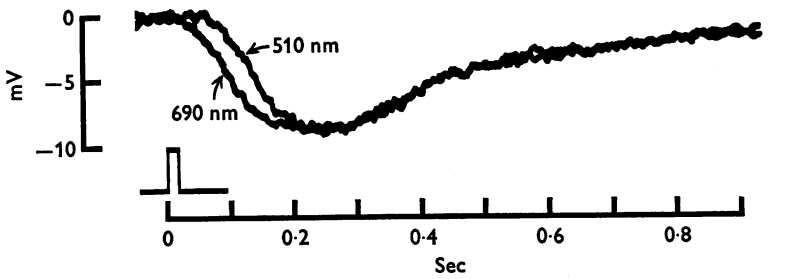


Fig. 4. The difference in the shape of the response produced by large diameter spots of different colours of moderate intensity. Super-imposed are the responses produced by 500 μm diameter spots of 510 and 690 nm wave-length light. Responses have been selected for equal peak amplitudes. These responses deviate only during their time of onset. Red light produced a more rapid hyperpolarization.

the porphyropsin pigment at these two wave-lengths. This and the coincidence of the response shapes at these two wave-lengths indicates that for small diameter spots univariance pertains, i.e. excitation by a small spot depends only on the number of photons absorbed and not wave-length. When the spot was enlarged, however, a very different situation occurred.

C is the response when the 510 nm wave-length stimulus of part *A* was enlarged from 25 μm to 500 μm diameter and the nominal intensity dimmed 1·8 log units. *D* is the response when the 690 nm wave-length stimulus of part *B* was similarly enlarged to 500 nm and dimmed 1·8 log units. Unlike the responses to the 25 μm spots, the responses to the 500 μm diameter spots are not similar. The response to the 690 nm wave-length light begins earlier and reaches a greater peak hyperpolarization. The observations that for small diameter spots the responses are of the same shape and differ in responsiveness according to the porphyropsin nomogram but

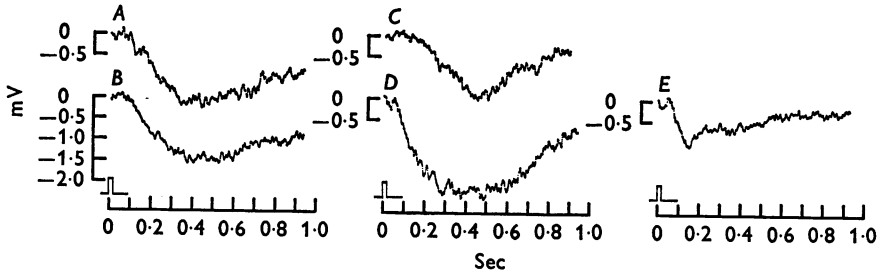


Fig. 5. Univariance pertains for small but not large spots. *A* is the response to a 25 μm diameter, 510 nm wave-length spot which delivered (after correction for loss due to light scatter; see Schwartz, 1973*b*) 7·0 photons μm^{-2}. *B* is the response to the same diameter stimulus of 690 nm wave-length light which delivered $1{\cdot}7 \times 10^4$ photons μm^{-2}. The responses in *A* and *B* can be exactly super-imposed. *C* is the response after the 500 nm light of *A* was enlarged to 500 μm diameter, and the nominal intensity dimmed 1·8 log units (to deliver 0·44 photons μm^{-2}). *D* is the response after the 690 nm light of *B* was enlarged to 500 μm diameter and dimmed 1·8 log units (to deliver $1{\cdot}1 \times 10^3$ photons μm^{-2}). After enlarging and equally attenuating the stimuli of *A* and *B* to obtain those of *C* and *D*, the shapes and amplitudes of the responses differ. The response to red light has an earlier onset and reaches a greater peak hyperpolarization. *E* is the response to a 500 μm diameter, 690 nm wave-length spot which delivered 68 photons μm^{-2}. This response is believed to be determined almost entirely by synaptic interaction from red-sensitive cones.

that for large diameter spots the responses are of dissimilar shape and disproportionately large for red light, indicate that the difference is best accounted for by a neural interaction from neighbouring cells. The faster time course and increased amplitude in red light can be accounted for if rods receive an excitation from red-sensitive cones. This input from red-sensitive cones can be isolated by attenuating the intensity of the 690 nm light. The response in *E* was produced by a flash delivering 68 photons μm^{-2} of 690 nm wave-length. From the product of the spectral sensitivity of red-sensitive cones at 690 nm (Baylor & Hodgkin, 1973, Fig. 9), their scaled amplitude and collecting area (Schwartz, 1973*b*,

1975; Baylor & Hodgkin, 1973), and the intensity of the stimulus, it can be estimated that for this stimulus the red-sensitive cones each absorbed approximately 200 photons and produced a response of approximately 3–4 mV peak amplitude. This estimate agrees very well with responses actually observed in red-sensitive cones during this study. In contrast, each rod may be expected to have absorbed 0·4 photons and to have generated in this case a response of approximately 0·1 mV. The peak amplitude actually observed was 0·9 mV. In addition, the peak amplitude occurred at approximately 140 msec and in this regard resembles the responses of red-sensitive cones. It is, therefore, concluded that this dim, red flash delivered too few absorbable photons to the rod pigment to produce a sizeable response in rod outer segments but that, nonetheless, it was sufficiently bright to produce sizeable responses in red-sensitive cones. These, then, synaptically activated rods.

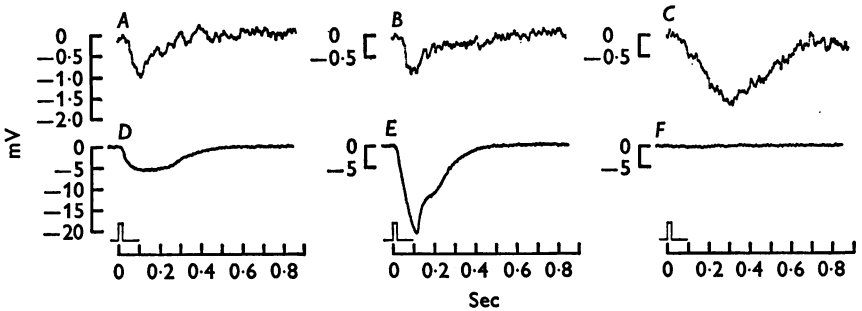


Fig. 6. The responses of rods to dim, red light do not correlate with the responses of horizontal cells. *A* is the average response of a rod to a 500 μm diameter, 690 nm wave-length spot which delivered $1{\cdot}4 \times 10^2$ photons μm^{-2}. *B* is the response when this stimulus was enlarged to 2000 μm diameter. *C* is the average response when the light was changed to 510 nm wave-length and delivered 1·4 photons μm^{-2}. *D*, *E* and *F* are responses from a luminosity horizontal cell. *D* is for a stimulus identical to *A*; *E* is for a stimulus identical to *B*; *F* is for a stimulus similar to *C* but 8× brighter.

The excitation mediated by cones may be considered to occur via direct cone-rod connexions or indirectly via horizontal cells. The following experiment partly differentiates between these two possibilities. In Fig. 6, *A* is the response of a rod to a 690 nm wave-length, 500 μm diameter spot; *B* is the response when this stimulus was enlarged to 2000 μm diameter; finally *C* is the response when the colour was changed to dim, 510 nm wave-length light. Also shown in Fig. 6 are responses from a luminosity type I horizontal cell produced by comparable stimuli. *D* is the response to a 690 nm wave-length, 500 μm diameter spot identical to that used in *A*; *E* is the response when this stimulus was enlarged to

2000 μm diameter as in *B*; *F* is the response when the colour was changed to 510 nm wave-length but is 0·9 log units brighter than in *D*. The responses from the rod were not significantly different when the stimulus was enlarged from 500 μm (part *A*) to 2000 μm (part *B*). In contrast, the responses from the horizontal cell increased nearly three times with the same change in stimulus diameter (parts *D* and *E*). This is consistent with the large receptive fields of these horizontal cells in turtle retinae (Simon, 1973). The change in horizontal cell response was not accompanied by an increase in the amplitude of the rod response. In several additional cells it was observed that when a very dim, red light was enlarged from 500 to 2000 μm the response amplitude was either unchanged or occasionally slightly smaller. Hence, it is unlikely that the horizontal cells which mediate the recurrent inhibition of cones also mediate an excitation of rods. The interaction between cones and rods is, therefore, mediated by either direct cone–rod connexions or by interneurones with a small receptive field.

DISCUSSION

The results presented suggest that rods receive a synaptic excitation from red-sensitive cones. Small diameter fields of red or green light produce rod responses of identical shape and the irradiance necessary to produce equal amplitudes is predicted by the absorption spectrum of the porphyropsin pigment contained in the rod outer segments. Thus, light impinging on only a single rod produces a response in accordance with the principle of univariance. The situation for large diameter spots is, however, quite different. Rods are more responsive to large spots of red light than is predicted by the porphyropsin absorption spectrum. In addition, the time course of responses to dim, large spots of red or green light differs. The time course of responses to very dim, large spots of red light resembles the time course of responses from cones. These observations indicate that rods are excited by red-sensitive cones.

The excitation of rods by cones is not a peculiarity of the turtle retina. I have observed phenomena similar to that reported here in both the retinae of the salamander, *Ambystoma tigrinum*, and the toad, *Bufo marinus*. This interaction may be a general property of amphibian and reptilian retinae.

A summary of the known interactions which affect the response of rods in the turtle retina is indicated diagrammatically in Fig. 7. Cones interact within a radius of 50 μm to enhance their response (Baylor *et al.* 1971; Baylor & Hodgkin, 1973) and also receive a recurrent inhibition from horizontal cells (Baylor *et al.* 1971; Fuortes *et al.* 1973). These latter cells

are electrically coupled and their receptive fields are consequently very large (Watanabe & Tosaka, 1959; Naka & Rushton, 1967; Kaneko, 1971; Simon, 1973). Rods themselves interact within a radius of 250 μm (Schwartz, 1973*b*, 1975). This interaction has two effects: following a flash the response is initially enhanced to increase the amplitude nearly tenfold and then after a delay disenhanced to reduce responsiveness. Finally, as demonstrated in this report, rods, unlike cones, do not receive an inhibition from horizontal cells but do receive an excitation mediated by red-sensitive cones. The possibility of input from cones containing other pigments is uncertain.

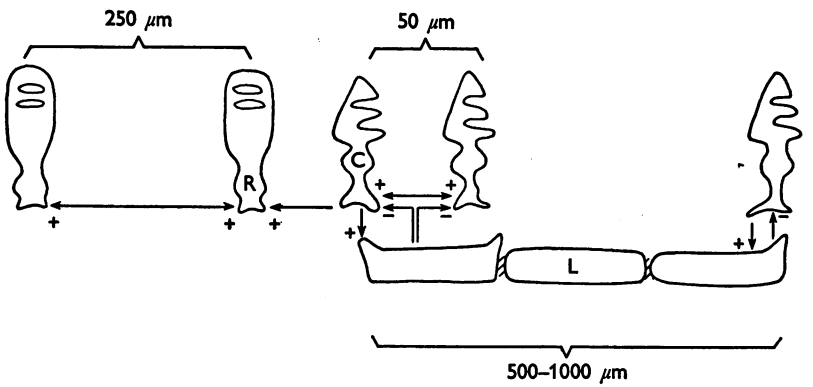


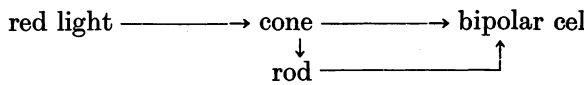
Fig. 7. Diagram of the synaptic interactions between rods, R; red-sensitive cones, C; and luminosity horizontal cells, L. + indicates that the pathway preserves the polarity of the presynaptic voltage change; – indicates that it is reversed. The interaction between cones and rods may not be direct but may involve an interneurone. See text for discussion.

The indirect responses of rods are quite different from those of cones. Cones receive an inhibition from horizontal cells (Baylor *et al.* 1971) which are themselves influenced by the pattern and colour of the stimulus (Fuortes & Simon, 1974). The result is that cones demonstrate a colour opponence which should increase the ability to discriminate between colours (Fuortes *et al.* 1973). In contrast, rods receive from cones an excitation for wave-lengths at which their own pigments are not themselves optimally sensitive. Thus, the responses of rods to large diameter fields of dim, red light are observed to be greater than predicted from the porphyropsin absorption spectrum. In this manner the responsiveness of rods to red light is increased and their ability to contribute to colour discrimination is decreased.

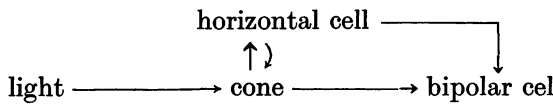
The enhancement and disenhancement produced by rod–rod interaction should affect both the responses generated by the absorption of light

within the rods themselves and also the synaptic excitation from cones. Consequently, rod–rod interaction must modify the excitation from cones in two important ways. First, it must enhance the input from cones; and, therefore, the responses contributed by cones in the absence of rod–rod interaction must be very small. Second, because disenhancement should affect signals arising from either source, chromatically bleaching rod or cone pigments may not achieve a selective isolation of the two components of the rod response.

The functional importance of rods as second order neurones for cones is entirely speculative. Perhaps the convergence of rods or cones onto bipolars and the organization of bipolars receiving from rods or exclusively from cones are so sufficiently different that the pathway involving rods is important for detecting even red light at visual threshold. Or, perhaps, recent anatomical observations are relevant. In teleosts the bipolar cells which receive from rods are also contacted by cones containing red pigment (Scholes & Morris, 1973). Thus, the circuit



indicates two pathways by which dim, red light can excite bipolar cells. Similarly, the circuit (see Schwartz, 1974; Richter & Simon, 1975)



indicates two pathways by which horizontal cells can influence bipolar cells. These two examples imply a possibility for considerable redundancy and parallel organization within the retina.

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