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DETECTION AND RESOLUTION OF VISUAL STIMULI BY TURTLE PHOTORECEPTORS

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SUMMARY

1. Hyperpolarizing responses up to 30 mV in amplitude were recorded from cones and from certain cells believed to be rods in the isolated retina of the swamp turtle, *Pseudemys scripta elegans*.

2. The responses evoked by weak flashes of light reach their maximum in 100–140 msec in red-sensitive cones, 140–180 msec in green-sensitive cones, and 300–600 msec in the rod-like cells (20° C).

3. The cone response evoked by weak flashes of light is linearly related to light intensity and obeys the superposition principle in that the response to a very weak step of light is the integral of the response to a very weak flash.

4. On the basis of their spectral sensitivities cones can be divided into three distinct classes, namely red-sensitive cones whose relative quantum sensitivity is maximal at 630 nm, green-sensitive cones with a maximal sensitivity at 550 nm and blue-sensitive cones with a maximum at 460 nm.

5. The difference between the spectral sensitivity of rods with a maximum at about 520 nm and green-sensitive cones (λ_{max} = 550 nm) is consistent with the view that both receptors contain a 518_2 retinal pigment as reported by Liebman & Granda, but that light is filtered by an orange oil droplet in green-sensitive cones.

6. The spectral sensitivities of both red- and green-sensitive cones agree well amongst themselves at long wave-lengths but differ markedly in the extent of the reduction at short wave-lengths. This variation is attributed to differences in the extent to which light is filtered through the coloured oil droplets.

7. There is a significant positive correlation between the absolute sensitivity of red- and green-sensitive cones and the reduction in sensi-

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tivity at short wave-lengths. This would be explained if a greater fraction of the light passes through the oil droplet in the most sensitive cells.

8. The absolute flash sensitivities of the most sensitive receptors were about 250 μV photon^{-1} μm^2 in red- and green-sensitive cones, 120 μV photon^{-1} μm^2 in blue-sensitive cones, and 1300 μV photon^{-1} μm^2 in rods.

9. If the effective collecting area (which includes factors for absorption etc.) is taken as 10 μm^2 in a red-sensitive cone the peak hyperpolarization produced by 1 photon would average 25 μV.

10. Provided that small spots of light are used, individual receptors obey the 'univariance principle' and the response produced by light of strength I', and wave-length λ_1 can be matched by a light of strength kI' and wave-length λ_2, where k is the same for all values of I'.

11. A small proportion of cones behave like isolated units in that they have very sharp sensitivity-profiles and obey the univariance principle with respect to the position as well as to the wave-length of light.

12. The majority of red and green cones have more diffuse sensitivity-profiles, sometimes with bumps on the descending limb, and behave as though cones with the same spectral sensitivity were electrically coupled to one another.

13. The relation between the area of illumination and flash sensitivity agreed approximately with that calculated from the spatial profile.

INTRODUCTION

The general aim of the present work is to analyse the electrical change produced by applying flashes or steps of light to the photoreceptors of the swamp turtle *Pseudemys scripta elegans*. The retinal cells of *Pseudemys* are unusually large, and stable intracellular records of cone responses can be obtained for periods of up to an hour (Baylor & Fuortes, 1970; Baylor, Fuortes & O'Bryan, 1971). In this preparation, as in several other vertebrate retinae, it has been found that light generates a graded hyperpolarization, accompanied by a decrease in membrane conductance (see Tomita, 1970). These results, as well as measurements of the external currents flowing around rods (Hagins, Penn & Yoshikami, 1970) and observations on the osmotic properties of isolated rod outer segments (Korenbrot & Cone, 1972) are consistent with the view that light acts by blocking ionic channels in the outer segment.

The kinetic properties of the transduction mechanism and its behaviour in light and dark adaptation will be examined in later papers. Here, the object is to determine the spectral and spatial resolution of the receptors and to measure their absolute sensitivity. Without this information, which is interesting in itself, it would be difficult to know whether to analyse

responses with large or small spots of light or to form any idea of the magnitude of the voltage change produced by absorption of a single photon. It is also of interest to see whether variations in the structure and visual pigment of receptors are correlated with differences in electrical behaviour.

METHODS

Preparation. Red-eared swamp turtles, *Pseudemys scripta elegans*, with shells 17–25 cm long, were obtained from the Connecticut Valley Biological Supply Co., Southampton, Mass., U.S.A. They were shipped by air freight and kept in the laboratory in a large tank containing a pool of shallow water, and fed on ox hearts. Isolated eyecup preparations were made using the method described by Baylor *et al.* (1971). It was found that an eye left in the pithed head and stored in the refrigerator for several hours yielded a retina which behaved identically to one taken from a fresh eye; use of both the fresh and refrigerated eye thus doubled the amount of information obtained per animal.

The eyecup was mounted in a moist chamber gassed with moistened 95% O_2, 5% CO_2, as shown in Fig. 1*A*. The mechanical stability of the isolated tissue was improved by glueing the bottom of the eyecup to the chamber with a drop of Ringer containing about 5% (w/v) dissolved gelatin.

For changing the temperature of the eye, warm or cool liquid, selectable at a 3-way tap, was circulated through the hollow walls of a modified chamber. The temperature of the eye was read with a calibrated thermistor placed just under it. These experiments are described in a later paper.

Electrical recording. Recordings were made with gently tapered micro-pipettes, pulled on a Livingston-type puller (Takahashi Seiki Kogyo Co. Ltd, Tokyo, Japan) and filled with 4 M potassium acetate, using the glass-fibre method (Tasaki, Tsukahara, Ito, Wayner & Yu, 1968). Satisfactory electrodes had resistances, measured in the tissue, of 160–250 MΩ. The retina was penetrated from the vitreal surface, at the position shown in Fig. 1*B*. As indicated in Fig. 1*A*, the electrode was mounted and advanced vertically, but entered the tissue about 45° from normal to its surface. As a 'good' electrode was moved through the retina, a characteristic sequence of membrane potentials and responses to light was observed. At the inner surface of the retina, the electrode often impaled cells with large resting potentials (up to −85 mV) but negligible responses to light; these 'silent potentials' are presumably glial cells. At the ganglion cell layer, units were penetrated which gave synaptic potentials and action potentials in response to test flashes; these are presumably ganglion or amacrine cells. At the inner nuclear layer, large 'silent potentials' were again seen, as well as occasional cells with smaller resting potentials (*ca.* 40 mV), graded depolarizations or hyperpolarizations to light, and a prominent surround antagonism. These cells are thought to be bipolar cells by analogy with cells recorded from and stained by Kaneko (1970) and Werblin & Dowling (1969). A further movement of the electrode almost invariably led to the penetration of a horizontal cell. These gave graded hyperpolarizations of up to −60 mV amplitude, with a membrane potential in darkness of about −20 mV. Although most were of the 'luminosity' type, responding to all colours with hyperpolarizations, some were 'chromatic', giving a graded depolarization with red light and hyperpolarization with green or white light. After recording from up to three distinct horizontal cells, the electrode again consistently recorded a large 'silent potential'. After gently tapping the manipulator and advancing further, this potential was lost, and the electrode was in the receptor layer. To penetrate a receptor, the electrode was

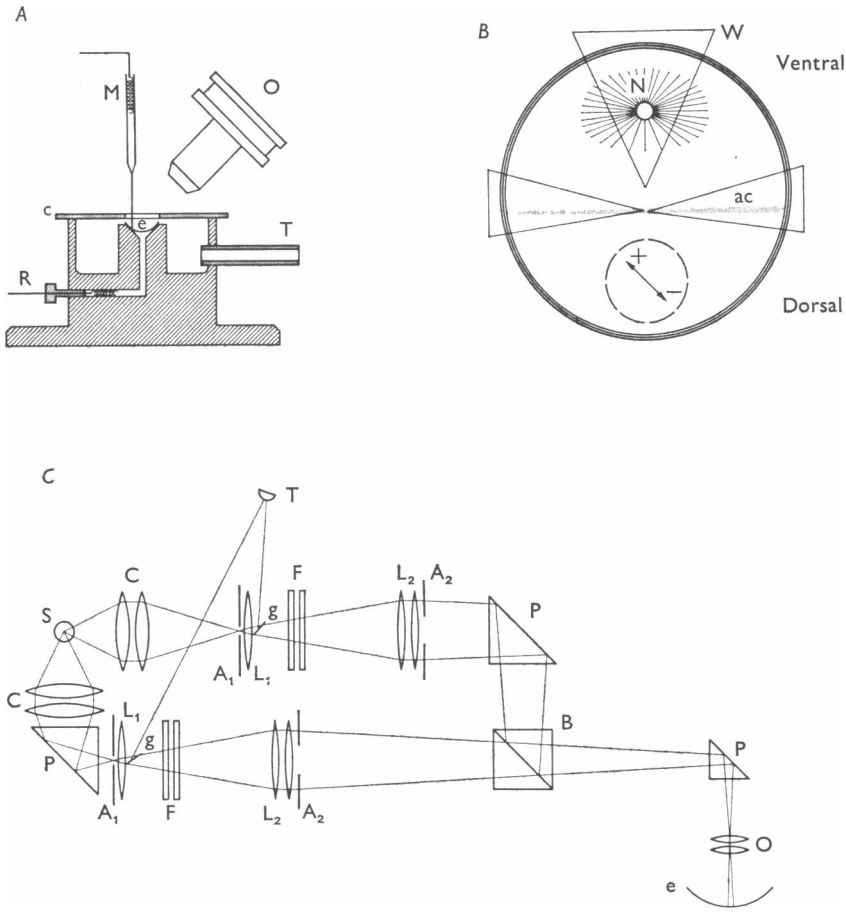

Fig. 1. *A*, diagram of cell and arrangement of micro-electrode, eye, and microscope objective used for stimulation. M, micro-electrode; O, microscope objective; c, coverslip with hole for electrode and stimulating beam; e, eyecup; T, inlet tube for moist O_2–CO_2; R, reference electrode. The space around the central pedestal in the chamber was packed with Kleenex moistened with Ringer, and electrical contact with the reference electrode was made through a column of Ringer in the hole under the eye.

B, diagram of the eyecup, showing region used for penetrations. N, optic nerve. ac, area centralis, visible under the dissecting microscope. W, wick of Kleenex used for draining retina; two other wicks over the area centralis are not labelled; the dashed circle indicates the approximate region in which most impalements were made. The arrow within this region indicates the direction in which movements of the stimulating spot were made in sensitivity profiles.

C, diagram of optical stimulator. S, source, tungsten-iodide lamp, General Electric Quartzline Q6.6A, T2 1/2, 1 Cl; C, condenser, focal length 4·5 cm; P, prism; A_1, electro-mechanical shutter; L_1, collecting lens, focal length 10 cm; g, glass plate reflecting lights to T, light monitor; F, filters, wave-length and neutral density; L_2, field lens, focal length 21·6 cm; A_2, variable field aperture, position controlled by micrometer drive; B, combining prism; O, Leitz ultrapak objective, × 6·5, working distance 16 mm; e, eyecup. The light was run from a regulated d.c. power supply.

advanced slightly until a shift of a few mV appeared in the voltage trace, and then an oscillating voltage was applied to the electrode tip by over-compensating the capacitance neutralization (see Baylor *et al.* 1971). Most of the cells thus penetrated were cones, recordings from which can be identified by physiological criteria verified by dye marking (Baylor & Fuortes, 1970; Baylor *et al.* 1971). A few of the cells impaled in the receptor layer had quite different properties, as will appear later. From the position of the electrode, the receptive fields, the spectral sensitivity and the slowness of the response these are tentatively identified as rods. They will be referred to as 'rods', with the reservation that until they are marked with a dye electrode this is not absolutely certain.

The electrode was connected to a high impedance negative-capacitance pre-amplifier (WPI-M4 ARME, WP Instruments, Inc., Hamden, Conn. U.S.A.) which had provision for passing a constant current through the recording electrode and balancing out the voltage drop in the electrode resistance. The capacity compensation was usually not used; the input time constant, without compensation and with the electrode in the tissue, was about 0·5–1·0 msec. This was ascertained by recording 40 mV spikes from a ganglion cell using a 200 MΩ electrode and no compensation, then fully compensating, which increased the spike amplitude to 70 mV. This change in amplitude and the shape of the uncompensated record were consistent with an uncompensated time constant of 0·8 msec.

The high resistance of the micro-electrodes introduced considerable noise in the electrical records. To improve resolution of small responses to dim lights, a Biomac 500 signal averaging computer was sometimes used. Alternatively, a series of single sweeps was photographed from the oscilloscope and later averaged by hand.

Light stimuli. Fig. 1*C* shows the design of the optical stimulator, for which we are indebted to Professor A. F. Huxley, F.R.S. It incorporated two beams, one of which (lowermost in Fig. 1*C*) was used routinely, and the other when a background light or second variable flash was needed. Light from the source passed through a condenser, shutter and filter box, and illuminated a variable field aperture mounted on a micrometer drive (Spindler–Hoyer precision cross carriage). The reduced image of the field aperture was formed on the retina with a microscope objective. An optical reduction of 30× was obtained with the field aperture in the position shown in the Figure. Sometimes it was placed on the preparation side of the combining prism, which allowed identical spots to be projected from the two beams; in this position the reduction was 20×.

The stimulus intensity was controlled with a series of neutral density filters (Bausch and Lomb, Inconel) which were individually calibrated through the visible region of the spectrum in a Zeiss spectrophotometer. Reflexions between filters placed in series were avoided by positioning the filters at slight angles from the vertical, in alternating fashion. The performance of the filters in the box was checked with a silicon photo diode (Pin -5, United Detector Technology Inc., Santa Monica, California, U.S.A.) at the position of the retina. The filter box also contained a calibrated Chance glass heat filter which was kept in the beam when using white light.

Narrow spectral bands in the range of wavelengths between 400 and 805 nm were obtained with interference filters (Schott, Depal, band double filters) inserted in the light path between shutter and filter box; at this point the small divergence of the beam (5° half angle) had no effect on the filter behaviour. The interference filters themselves were calibrated in a Unicam SP-800 recording spectrophotometer and had half widths of 15–20 nm; the measured optical properties of the filters agreed closely with the manufacturer's specifications. The irradiance (erg cm^{-2} sec^{-1}) of the source through each interference filter was measured with a radiometer (YSI-Kettering Model 65) whose sensor was placed at the location of the retina. From

these measurements, and the transmission spectra of the interference filters, the spectral energy distribution of the source at the preparation was derived from the approximate relation

$$E(\lambda) = i_\lambda / \int T(\lambda)\, d\lambda, \quad (1)$$

where $E(\lambda)$ is the energy of the source at wave-length λ (erg cm^{-2} sec^{-1} nm^{-1}), which is assumed constant over the band-width of the filter, i_λ is the measured irradiance through the filter with central point λ, and $T(\lambda)$ is the transmission spectrum of the filter. The source energy was thus found to rise approximately linearly from $6{\cdot}0 \times 10^1$ erg cm^{-2} sec^{-1} nm^{-1} at 460 nm to $6{\cdot}4 \times 10^2$ erg cm^{-2} sec^{-1} nm^{-1} at 750 nm. This energy distribution, converted to photons and corrected for the effect of the heat filter, was used to calculate a factor which enables sensitivities at the optimum wave-length to be derived from measurements with white light (see p. 183).

The irradiance readings obtained with the interference filters were also converted directly to photons μm^{-2} sec^{-1} referred to the central points of the filters. These values were used in deriving sensitivities with monochromatic lights. As an example of the method used in determining the absolute sensitivity in this way, we consider red-sensitive cone 4 in Table 2. This cell gave 2·6 mV peak hyperpolarization for an 11 msec flash with the 644 nm interference filter and a nominal 2·7 log unit neutral filter attenuation in the optical path, as well as the beam combining cube. Calibration consisted of reading the irradiance of the source through the interference filter at the preparation; the neutral filters and combining cube were removed from the path to increase sensitivity. The spot of light on the sensor of the radiometer was 1·5 mm in diameter, the sensor itself was 3 mm in diameter. The reading obtained was $1{\cdot}83 \times 10^3$ erg cm^{-2} sec^{-1}, equivalent to $5{\cdot}95 \times 10^{14}$ photons cm^{-2} sec^{-1} at 644 nm. Additional measurements showed that (i) at 644 nm the nominal 2·7 log unit neutral filter attenuation was 2·884 log units, (ii) the combining cube had a density of 0·618 log units at 644 nm, and (iii) confirmed that in the calibration the ratio of the area of the radiometer sensor to that of the spot of light was 4·0. The sensitivity of the cell to flashes was then calculated as

$$S_F = 2{\cdot}6 \text{ mV}/(1{\cdot}1 \times 10^{-2} \text{ sec})\ (5{\cdot}95 \times 10^{14} \text{ photon cm}^{-2} \text{ sec}^{-1})\ (4)\ (10^{-0{\cdot}618})\ (10^{-2{\cdot}884})$$
$$= 31{\cdot}5\ \mu\text{V photon}^{-1}\ \mu\text{m}^2.$$

Red (Ilford 206) and green (Ilford 624) colour filters were useful in determining rapidly whether a cone was of the red-, green- or blue-sensitive types (see Results). The procedure was to compare responses, at a given neutral filter attenuation, with the red or green filter inserted in the beam. Red-sensitive cones gave much larger responses to the red light, while green-sensitive cones gave larger responses to the green light. Blue-sensitive cones, of which only three were studied, behaved similarly to green-sensitive cones in this test but had a much lower sensitivity to white light, owing in part to the relatively low output of the stimulator in the blue portion of the spectrum.

In determining the spatial resolution of cones, a spot of light 2·4–10 μm in diameter was applied at various positions on the retinal surface while recording the cell's responses. The quality of the image actually applied to the retinal surface and the precision with which a spot could be positioned, were checked by the following procedure. An electron microscope aperture 9 μm in measured diameter (about that of a cone) was placed in front of a photomultiplier. The stimulator was used to image a spot 7 μm in diameter on the metal surface bearing the hole and moved over it in a series of steps. At each position, the photomultiplier output recorded the light passing through the hole. The results obtained in this test are shown in Fig. 2. The symmetrical smooth curve drawn through the points is similar to that calculated for the area of overlap of 9 and 7 μm diameter circles, indicating that the applied

stimulus was very sharp. However, as will be seen later, optical scatter appears to blur the image falling on the receptors.

Stray light on the preparation was minimized by working in a dimly lit room and enclosing the eye and micromanipulator in a black box with a hinged lid which could be closed after obtaining a satisfactory intracellular recording.

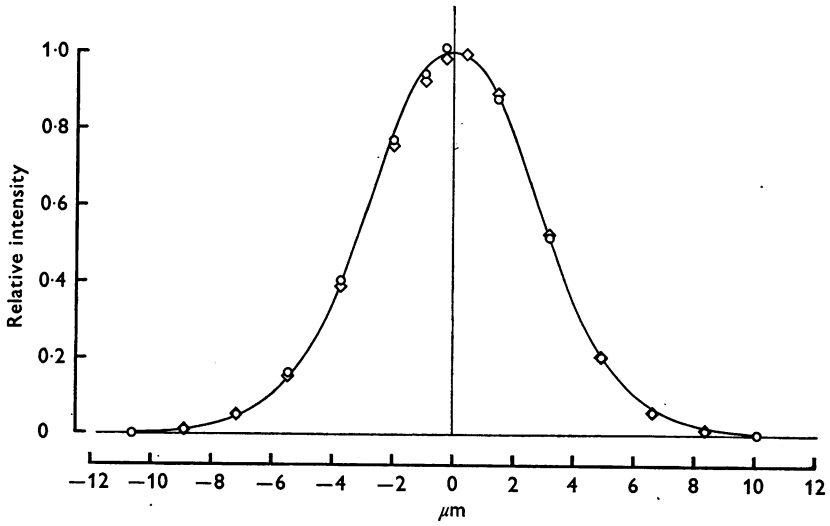

Fig. 2. Calibration of spatial resolution of optical stimulator. An electron-microscope aperture 9 μm in diameter was placed over a photomultiplier and the optical stimulator used to image a spot of light 7 μm in diameter (500 nm wave-length) at various positions near the hole. The relative intensity of light passing through the hole is plotted as a function of the position of the spot. The symmetrical continuous curve has been drawn by eye through the points.

RESULTS

General description of the electrical responses of different types of receptor

The retina of *Pseudemys* contains the following types of receptor (see Walls, 1963; Liebman & Granda, 1971; Liebman, 1972): (1) single cones with visual pigment 620_2 and red or orange oil droplets, (2) single cones with pigment 518_2 and an orange oil droplet, (3) single cones with a relatively thin inner segment, a colourless oil drop and pigment 450_2, (4) double cones consisting of a fat accessory member with no oil drop and probably containing pigment 518_2, and a thin principal member with unidentified oil drop and visual pigment, (5) rods which contain pigment 518_2 and lack an oil droplet and which represent 5–10 % of the receptor population. The oil droplets in the cones are spherical bodies 3–6 μm in diameter which act as filters cutting off the shorter wave-lengths (Strother, 1963; Liebman,

1972). *Pseudemys* has red, orange, yellow and colourless oil droplets but the visual pigment in the cones with yellow droplets has not been determined (Liebman, 1972 and personal communication).

The electrical responses most frequently encountered in the receptor layer are those of red-sensitive cones (red cones) of which the records in Fig. 3 are a fairly typical example. With weak flashes the hyperpolarization reached a maximum in 100 msec and returned to the base line in about 300 msec. As the flash was increased the peak moved progressively

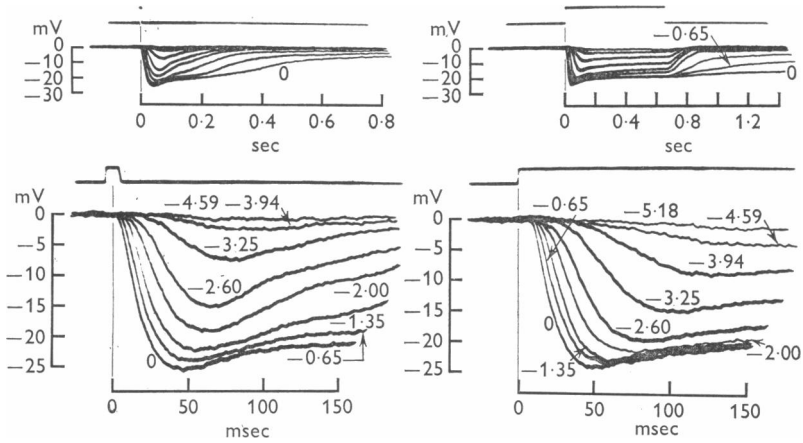

Fig. 3. General characteristics of the intracellular responses of a red-sensitive cone to 10 msec flashes (left) and steps (right). The records give the displacement of the internal potential from its resting value of − 39 mV, shown at low gain and slow time base, above, and expanded, below. The stimulus was a circular white spot of diameter 50 μm with a relative intensity shown in log units by the number against each trace. The unattenuated light of relative intensity 1 was equivalent to 67×10^6 photons μm^{-2} sec^{-1} at the optimum wave-length of 630 nm. The calculated flash sensitivity was 35 μV $photon^{-1}$ μm^2 and the calculated step sensitivity was 4·3 μV $photon^{-1}$ μm^2 sec. The temperature was 21·8° C.

earlier and was reached in 40–50 msec with flashes 10^4 stronger than those required to give a hyperpolarization of 1 mV. After the peak, which had a limiting value of about 25 mV in this experiment, the potential declined to a level of about 20 mV at which it often remained nearly constant for several hundred msec before declining more rapidly to its resting value. Strong flashes or steps of light brought in a slow component which decayed with a time constant of about 0·5 sec and which might hold the potential constant at the level of the plateau for several seconds (see the largest step response in Fig. 3). When the slow component was conspicuous the cell was desensitized for many seconds but a typical flash run such as shown on the

left in Fig. 3 could be carried out with an interval of only a few seconds between all but the strongest flashes. Records with decreasing intensities (which are not shown in Fig. 3) agreed closely with those on the ascending run.

Green-sensitive cones gave results which were very similar to those in Fig. 3, except that the response to weak flashes reached a maximum at 140–180 msec instead of 100–140 msec as in red cones (see Fig. 12, page 184).

Certain features of the responses of the five red-sensitive cones which gave the largest hyperpolarization are summarized in the upper part of Table 1. As will be shown later these cells gave maximum responses with circular light spots of diameter 50–100 μm and had fairly sharp spatial profiles. There is little doubt that they were cones since their characteristics were very similar to those identified in *Pseudemys* as cones with Procion Yellow by Baylor *et al.* (1971) or in other animals by Werblin & Dowling (1969) and Kaneko & Hashimoto (1967).

TABLE 1. Electrical characteristics of cells with large responses

	V_D (mV)	U_L max (mV)	t_{max} for I small (msec)	t_{max} for I large (msec)	$\dot{U}_{max}$ (V/sec)	Temp. (°C)
Red cones		32	138	48	1·3	20·8
	−40	28·1	115	50	1·3	21·6
	−42	26·0	125	40	1·6	22·1
	−43	25·5	128	49	1·2	20·9
	−39	25·2	120	49	1·6	21·8
Green cones	−39	24·5	180	47	1·2	20·8
	−34	22	162	53	1·0	20·3
	−35	23·2	150	45	1·4	21·9
Rods	—	32·5	355	100	0·9	20·4
	−44	20	300	140	0·3	20·8
	—	19·8	290	140	0·26	21·0

V_D is the resting potential in the dark; U_L is the maximum hyperpolarization after a flash of high intensity; t_{max} is the time to maximum hyperpolarization; $\dot{U}_{max}$ is the maximum rate of hyperpolarization after a strong flash. The flash duration was always close to 10 msec and t_{max} was measured from the middle not the beginning of the flash.

When the electrode was in the receptor layer it sometimes penetrated cells with electrical responses very different from those which have just been described. Fig. 4 is an example of such responses which we tentatively attribute to rods on the basis of the following evidence. (1) The spectral sensitivity agrees with that expected for a cell containing the 518_2 nm pigment without an oil droplet (page 181, Fig. 11). (2) The time scale of

the response is much slower than that of a cone, for the response to weak flashes reaches its maximum at 300–600 msec instead of 100–200 msec in cones (Fig. 12, page 184). (3) The desensitizing effect of flashes or steps of light was more pronounced and lasted longer than in cones. A further point about these rod-like cells is that the diameter of their receptive fields was about twice that in cones. (4) Schwartz (1973) has shown by dye-marking that rods are responsible for the slow responses in the receptor layer of the turtle *Chelhydra serpentina*.

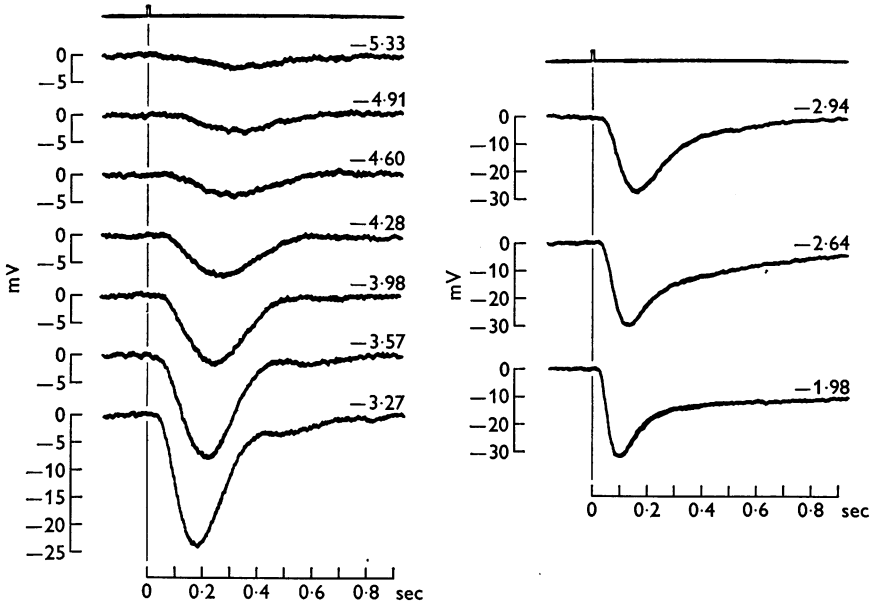

Fig. 4. General characteristics of the intracellular response of a rod-like receptor to 10 msec flashes of white light, shown as displacements of the internal potential from its resting value of about −40 mV. The stimulus was a circular white spot of diameter 300 μm of relative intensity shown in log units. The unattenuated light of relative intensity 1 was equivalent to 30×10^6 photons μm^{-2} sec^{-1} at the optimum wave-length of 520 nm. The calculated flash sensitivity of this rod was 1700 μV $photon^{-1}$ μm^2.

Linearity of cone-responses to weak flashes and steps of light

Measurements on a large number of cones indicated that for responses less than 1–2 mV the mean amplitude was directly proportional to light intensity and that the time course was identical if the responses were divided by the light intensity. In order to test this point more thoroughly we carried out the experiment shown in Fig. 5*A*. The records are responses to weak flashes of relative strength 2·74–55·6 averaged with a Biomac for 16 or 32 flashes. Measurement of the records indicated that the amplitude

was proportional to light intensity up to an intensity of 25·6 (records 1–7) but that the signal evoked by intensity 55·6 (record 8) was smaller than expected and reached a peak earlier than the other records. In order to determine whether responses 1–7 were strictly linear we calculated a mean response curve $\bar{U}(t)$ by summing all seven records and scaled it by multi-

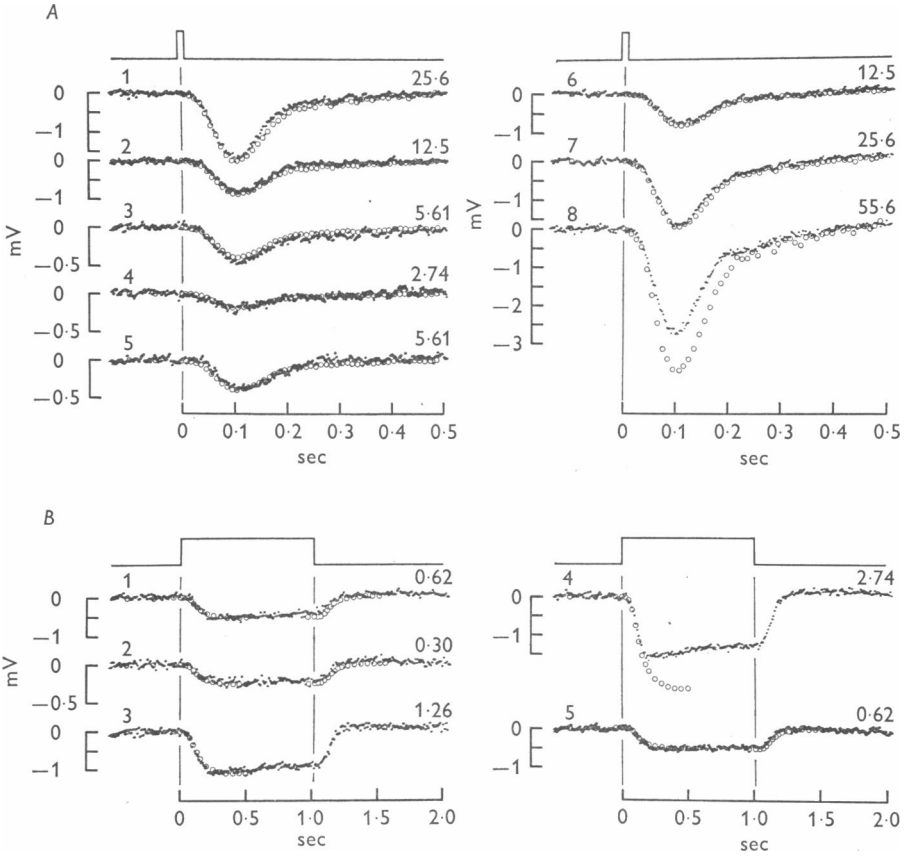

Fig. 5. Linearity of response of red-sensitive cone to weak flashes (*A*) and steps (*B*) of white light. The records show the averaged responses to 16 or 32 stimuli. *A* 3, 4, 5 and *B* 2 are 32 stimuli; the remainder are 16 stimuli. The calibration is for a single stimulus. Numbers on the right show the relative light intensities in units such that the unattenuated light had a strength of 10^6; 1 relative unit is equivalent to 67 photons μm^{-2} sec^{-1} at the optimum wave-length of 630 nm. The flash sensitivity of this cone at 630 nm was 94 μV $photon^{-1}$ μm^2 and the step sensitivity was 13 μV $photon^{-1}$ μm^2 sec.

The circles in *A* were calculated by multiplying the mean flash response in records 1–7 by a factor proportional to the light intensity (see text).

The circles in *B* were calculated from those in *A* by integration (see text).

Temperature 21° C; spot diameter 75 μm; resting potential −41 mV; maximum hyperpolarization 18 mV.

plying by $I'/\bar{I}$ where I' is the light intensity used in any particular run and $\bar{I}$ is the mean light intensity in all seven runs. The close agreement between the circles in Fig. 5, which were obtained by this method and the experimental results is strong evidence that responses of less than 1 mV are proportional to the intensity of the light at all times after the flash.

Average responses to steps of light are given in the lower part of Fig. 5. From the superposition principle one would expect that the response to a step of light would be the integral of the response to a flash, provided that the light intensity is sufficiently low for there to be no interaction between the effects of absorbed quanta. As the flashes were of finite duration it is simpler and more accurate to use numerical summation rather than graphical integration. If $\phi(t)$ defines the response to a step starting at $t = 0$ and $f(t)$ defines the response to a short rectangular pulse of the same intensity starting at $t = 0$ and ending at $t = \Delta t$ we can obtain $\phi(t)$ from $f(t)$ by

$$\phi(n\Delta t) = f(\Delta t)+f(2\Delta t)+\ldots+f(n\Delta t), \tag{2}$$

where Δt is the duration of the short rectangular pulse. The first term on the right-hand side of (2) gives the effect at time $n\Delta t$ of a pulse starting at $(n-1)\Delta t$ and ending at $n\Delta t$, the second the effect at $n\Delta t$ of a similar pulse starting at $(n-2)\Delta t$, and the last the effect of one starting at $t = 0$. The circles in Fig. 5*B* were calculated by this method from the flash responses ($f(t)$) in the upper part of the Figure and were scaled according to the light intensity. The agreement between calculated and observed values of the step response indicates that there is negligible interaction between the effects of absorbed photons for lights which hyperpolarize less than about 0·5 mV. The discrepancies which set in sharply with stronger lights after about 100 msec (records B 4 in Fig. 5) are attributed to a desensitizing mechanism which will be considered further in later papers.

From the experiment which has just been considered it is clear that the response $U_S(t)$ produced by a step of intensity I_S is related to the response $U_F(t)$ produced by a flash of intensity I_F and duration Δt by

$$\frac{U_S(t)}{I_S} = \frac{1}{I_F \Delta t}\int_0^t U_F(t')\,dt'. \tag{3}$$

On taking $t = \infty$, and defining the integration time t_1 of the flash response by

$$t_1 = \int_0^\infty U_F(t')\,dt'/U_{peak}, \tag{4}$$

where U_{peak} is the maximum value of $U_F(t)$, we obtain the relation

$$\frac{U_S(\infty)}{U_{peak}} = \frac{I_S t_1}{I_F \Delta t}. \tag{5}$$

In the experiment of Fig. 5 the integration time was 0·135 sec which is somewhat smaller than that observed in most other red-sensitive cones (Table 2). A relation equivalent to (5) will be used to calculate step sensitivities (which are difficult to determine without averaging many records) from flash sensitivities.

Spectral sensitivities

As a basis for examining the spectral characteristics of receptors, preliminary experiments were made to see whether they conformed to the principle of univariance (see Naka & Rushton, 1966), signalling the number of photons absorbed but not their wave-length. Univariance implies that a flash of intensity I' and wave-length λ_1, has effects which are identical in

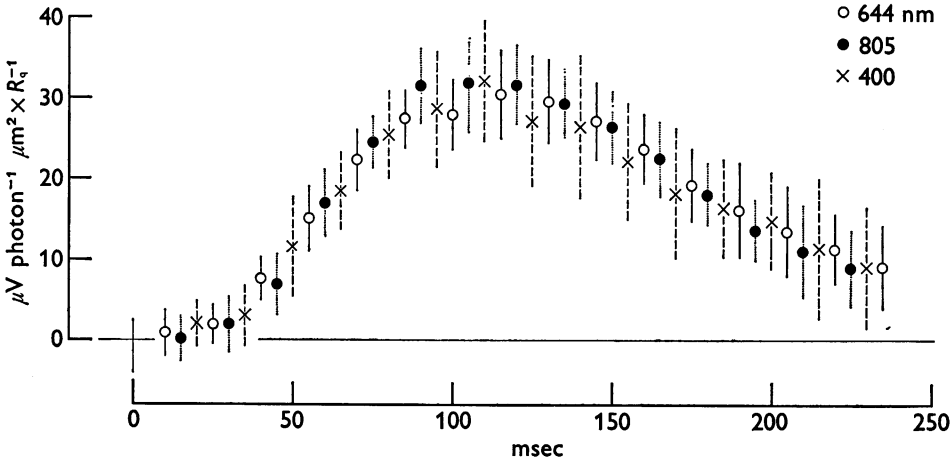

Fig. 6. Linear responses of a red-sensitive cone to flashes of wave-length 644 nm (○), 805 nm (●) and 400 nm (×). Points are the mean ± 1 S.E. of the mean for eleven responses at 644 nm, fourteen responses at 805 nm, and eleven responses at 400 nm. The ordinate is μV photon^{-1} μm^2 × R_q^{-1} where R_q, the relative quantum sensitivity, was taken as 1 for 644 nm, $7{\cdot}94 \times 10^{-4}$ for 805 nm, and 0·240 for 400 nm; these factors compare to 1, $9{\cdot}63 \times 10^{-4}$ and 0·186 respectively for the same factors determined from the routine large-signal spectral sensitivity run done on this cell. The abscissa gives the time from the 10 msec stimulating flash. The coincidence of the response shapes at different wave-lengths indicates that the excitation depends only on the number of photons absorbed, not their wave-length. 150 μm diameter stimulating spots. Resting potential in darkness −43 mV. Maximum response to light −23 mV. 22·2° C.

all respects to those produced by a flash of intensity kI' and wave-length λ_2 where k, the relative spectral sensitivity, is a function of λ_1, λ_2 but not of I'. If a cell is univariant with respect to wave-length its spectral properties can be described in terms of a sensitivity function which gives the

relative effectiveness of a photon as a function of its wave-length. One test of univariance is given in Fig. 6, which shows averaged linear responses of a red-sensitive cone to flashes of wave-length 644, 400 and 805 nm. The responses with each light have been scaled by the flash intensity (photons

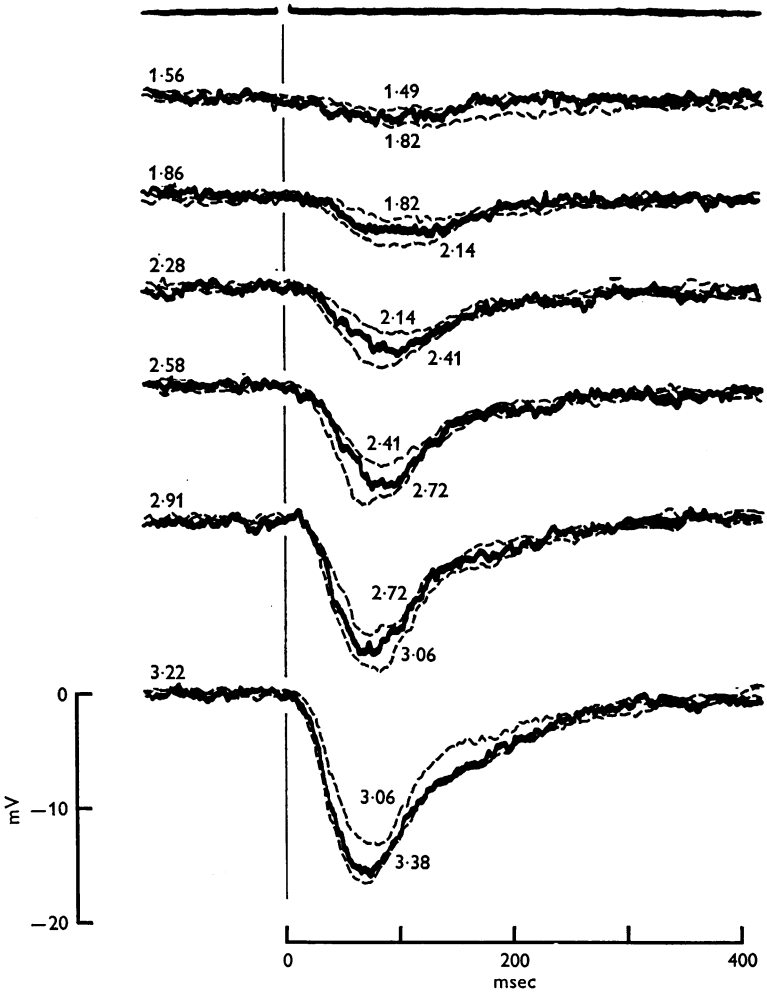

Fig. 7. Responses of a red-sensitive cone to red and green flashes out of the linear range. Numbers on the right give log I for the red light, where I is in photon μm^{-2} at 644 nm and applies to the dashed tracings of responses. Numbers on the left give log $I - 1{\cdot}27$, where I is in photons μm^{-2} at 539 nm and applies to the continuous records. The Figure shows that the response to 644 nm strength I' matches the response to 539 nm strength $10^{1{\cdot}27}\, I'$. Temperature, 21·9° C; 103 μm diameter spot; resting potential −43 mV.

μm^{-2}) multiplied by a constant which was chosen to bring the points into vertical coincidence. All three responses have the same shape, as would be expected if the effect of absorbed quanta on an individual receptor is independent of wave-length. The constants by which the intensities were multiplied are: 1 for light of 644 nm, 0·240 for light of 400 nm, and $7{\cdot}94 \times 10^{-4}$ for light of 805 nm. These represent the relative quantum sensitivities at the three wave-lengths, since univariance was obeyed.

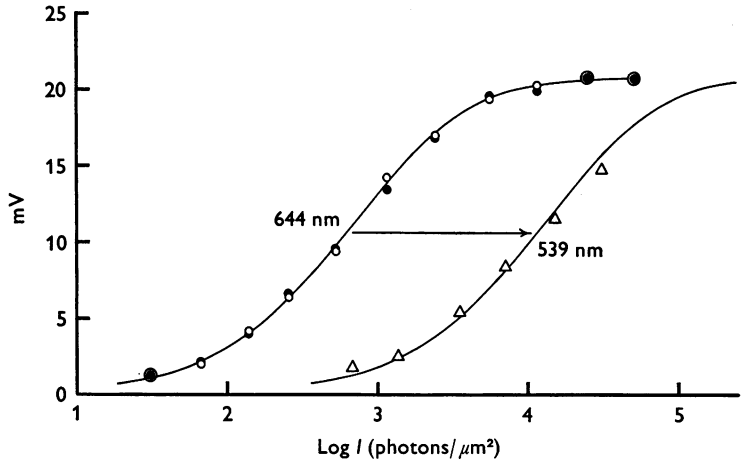

Fig. 8. Spectral univariance, and method of determining spectral sensitivity. The mean peak response of a red-sensitive cone is plotted as a function of log flash intensity (photons μm^{-2}) for lights of wave-length 644 and 539 nm. The curve through the points obtained with red light fits those with green light when displaced 1·27 log units to the right. The ratio of quantum sensitivities at 539 and 644 nm is thus taken as $10^{-1{\cdot}27} = 0{\cdot}054$. Results from the cell illustrated in Fig. 7. Sequence of runs ○, △, ●.

Results similar to this are observed for responses out of the linear range, as illustrated in Fig. 7. The solid traces are records of responses from a red-sensitive cone to flashes of green light (539 nm), while the dashed tracings are responses to red light (644 nm), which bracket the effective strengths of the green light. The numbers near the centre of the responses refer to the dashed responses and give the log strength of the red flashes in photons μm^{-2}. The numbers near the left end of the traces give the log strength $-1{\cdot}27$ of the green flashes in photons μm^{-2}. It is clear that responses of similar peak height are similar over their entire time course, and that adjustment of the stimulus intensity by a single fixed factor brings the entire family of responses into agreement. The interpretation from these and similar experiments is that for light spots less than 150 μm in diameter the receptors signal the number of photons absorbed, but not their wave-length (a possible exception is mentioned on p. 184).

Either of the kinds of measurement just mentioned would provide a way of determining spectral sensitivities, but both methods suffer disadvantages. The linear responses must be averaged to give reliable information, making it impractical to cover the entire spectrum in detail, and it takes too long to record a complete family at every wave-length. The principle of the method adopted is illustrated in Fig. 8 and is the same as that used

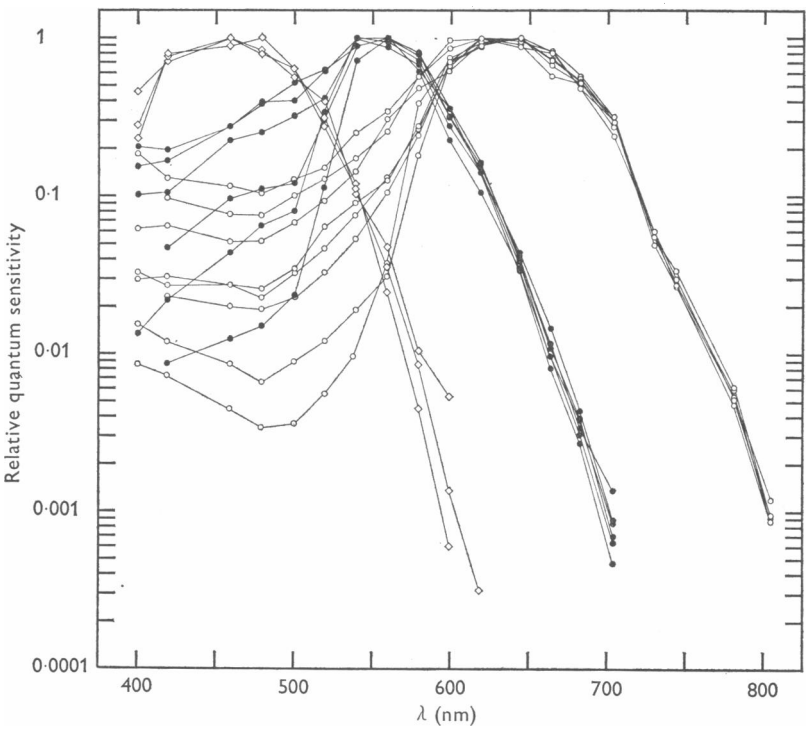

Fig. 9. Spectral sensitivities of cones. The ordinate is relative quantum sensitivity, the abscissa wave-length. Collected results from seventeen cells. Three cells have a peak sensitivity at about 460 nm, six have maxima at about 550 nm, and eight have maxima at about 630 nm.

by Naka & Rushton (1966). The ordinate is the peak hyperpolarization in mV of a red-sensitive cone to flashes at 644 and 539 nm. The smooth curve drawn through the points at 644 nm also fits those at 539 nm when shifted 1·27 log units to the right. Thus, the relative sensitivity to 539 nm photons is taken as $10^{-1\cdot27}$ of that to 644 nm photons. In using this method the intensity–amplitude relation was determined in detail near the wave-length of optimum sensitivity, and a smooth curve was drawn through the points. At all other wave-lengths, a pair of responses, about 0·25 and 0·6 of the maximal amplitude, were averaged in a few frames. These points were

plotted, and the displacement of each pair of points from the smooth curve was measured.

Spectral sensitivities were determined in this way for seventeen cones and two rods. Collected results from the cones are shown in Fig. 9, in which relative quantum sensitivity is plotted as a function of wave-length. The curves from individual cones fall into three groups with peak sensitivities at about 460 nm (three cones), 550 nm (six cones) and 630 nm (eight cones). The curves from the blue-sensitive cones agree well with one another over their entire range in spite of the difficulty in making satisfactory recordings from these cells. The curves from the green- and red-sensitive cones show a different pattern; here agreement is good at long wave-lengths but there are striking variations in sensitivity at short wave-lengths. A possible explanation of the variations in short wave-length sensitivity is that the coloured oil droplets in red- and green-sensitive cones filter to varying extents.

Such variations in filtering action might be expected in the physiological preparations (as opposed to the intact eye) if there were variations in the angle at which the stimulating light fell on the cones. If the beam entered exactly along the long axis of the cone's inner segment, it would encounter the drop, and short wave-length sensitivity would be greatly attenuated. If, on the other hand, the beam entered a cone obliquely, light could reach the outer segment without being exposed to the filter. On this explanation, there should be a correlation between the extent of the decrease in sensitivity at short wave-lengths, and the absolute sensitivity at the spectral maximum, because the Stiles–Crawford (1933) effect should reduce the sensitivity of cells stimulated obliquely. A large Stiles–Crawford effect is expected in turtle cones because the diameter of the inner segment is about 5 times the diameter of the outer segment. In Fig. 10 the drop in log sensitivity at short wave-lengths is plotted against the log peak absolute sensitivity for the eight red cones and the six green cones from seven turtles. The lines are least-square fits to the points and have slopes of 0·960 (red) and 1·027 (green). The correlation coefficient was calculated as 0·75 for the red-sensitive cones and 0·85 for the green-sensitive cones, both values being different from zero at less than the 0·05 level of significance. Most of the uncorrelated variance arose from one animal, in which two red-sensitive cones and one green-sensitive cone were studied. If these results are excluded, the correlation coefficients become 0·99 (red) and 0·95 (green). The conclusion is that there is a correlation between the drop in short wave-length sensitivity and the peak absolute sensitivity, as would be expected on the directionality basis outlined above.

Spectral sensitivities from two cells thought to be rods are shown in Fig. 11, which also shows the curve from the green-sensitive cone with the

largest drop in sensitivity at short wave-length. As would be expected from both cells containing the same pigment, the curves agree at longer wave-length. The rods lack an oil droplet and their sensitivity falls gradually from the maximum around 515 nm, whereas the green-sensitive cone shows a large short wave-length cut-off effect, owing to the presence of the oil droplet.

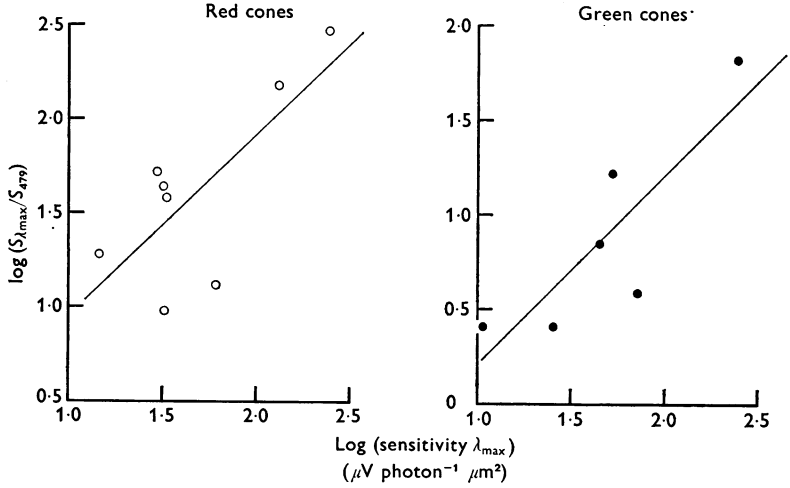

Fig. 10. Correlation between drop in sensitivity at short wave-length and peak absolute sensitivity. The log of the ratio of the sensitivities at the spectral maximum to that at 479 nm is plotted as a function of the log of the sensitivity at the spectral maximum (μV photon^{-1} μm^2) for red- and green-sensitive cones. Lines are least-square fits to the points.

Absolute sensitivities

Experiments with monochromatic light at the optimum wave-length give the ratio of the peak hyperpolarization to the intensity of the incident light measured in photons per square micron per second. It has also been shown that for weak flashes the response is directly proportional to light intensity and that the response to a step is the time-integral of the response to a flash. These results suggest that the sensitivity of a receptor to flashes and steps of light may be defined in the following way:

$$S_F = U_{peak}/I_F \Delta t, \tag{6}$$

where U_{peak} is the peak hyperpolarization in μV, $I_F \Delta t$ is the quantity of light per unit area expressed in photons μm^{-2} and S_F is the flash sensitivity in μV photon^{-1} μm^2. The step sensitivity S_S is defined in μV photon^{-1} μm^2 sec by

$$S_S = U_S(\infty)/I_S, \tag{7}$$

where $U_S(\infty)$ is the steady hyperpolarization produced by a step of intensity I_S.

The relation between the step and flash sensitivities, which can be obtained by combining (5) on p. 174 with the sensitivity definitions (6) and (7), is

$$S_S = S_F t_1, \tag{8}$$

where t_1 is the integration time defined by (4).

Table 2 gives values of flash sensitivity, integration times and calculated step sensitivities for cones and rods. From the results described on p. 180

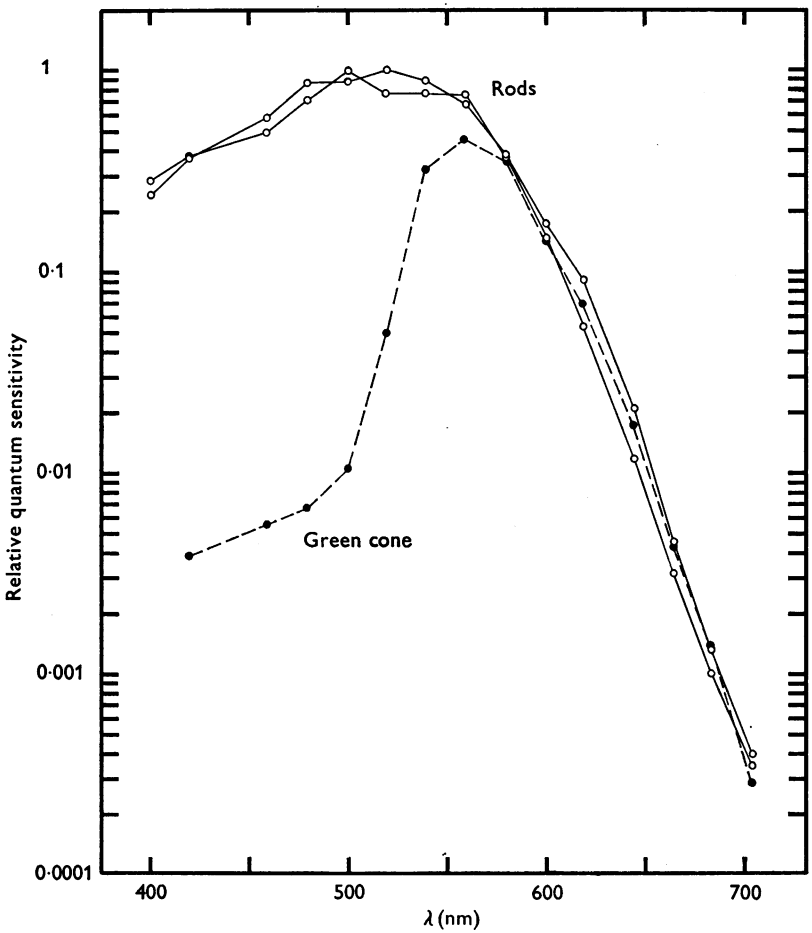

Fig. 11. Spectral sensitivity of two rods (○) compared with that of a green-sensitive cone with a large drop in short-wave-length sensitivity (●). The curves agree at long wave-length but diverge at shorter wave-length because of the presence of the oil droplet in the cone.

it seems that the variation in sensitivity among cones may be largely determined by differences in the effective collecting area of the receptor. If the effective collecting area of the most sensitive cones is taken as 10 μm^2 and proportionately less in less sensitive cones the flash sensitivities in Table 1 would be 25 μV per photon absorbed. The basis for estimating the collecting area, which includes a factor for the fraction of light absorbed, is given on p. 195.

TABLE 2. Flash sensitivities, integration times, and calculated step sensitivities

	U_L (mV)	λ'_{max} (nm)	S_F (μV photon^{-1} μm^2)	t_i (msec)	S_S (μV photon^{-1} μm^2 sec)
Red-sensitive	20·6	644	29·7	180	5
cones	23·5	618	61·4	170	10
	23·7	618	32·3	170	5
	28·1	644	31·5	175	6
	15·2	644	31·8	175	6
	17·2	644	14·6	170	2·5
	17·9	618	132	175	23
	14·7	618	246	190	47
Green-sensitive	14·6	559	247	190	47
cones	11·8	559	44·8	210	9
	20·7	559	72·1	240	17
	17·9	559	25·3	210	5
	13·6	559	10·7	260	2·8
	12·7	539	52·8	170	9
Blue-sensitive	10	458	9·45	175	1·7
cones	6	458	3·86	160	0·6
	11·3	479	117	190	22
Rods	15·3	519	1290	790	1000
	11·6	500	71·4	310	22

U_L is the peak hyperpolarization to a saturating flash; λ'_{max} is the wave-length of the filter nearest the wave-length of peak quantum sensitivity; S_F, flash sensitivity measured at λ'_{max}; t_i, integration time; S_S step sensitivity calculated from eqn. (8) in the text. These experiments were made at 19·8–22·7° C; the integration times have been corrected to 20° C, assuming a Q_{10} of 1·8 as found in other experiments.

Rods and blue-sensitive cones were encountered so rarely that it is difficult to make any definite statement about their absolute sensitivity. We think that the great variability in the sensitivity of rods, of which the two measurements in Table 2 are examples may be partly caused by variations in the extent to which the rods have recovered from previous exposure to bright lights.

In many experiments interference filters were not used so it was desirable to determine calibration factors which allow one to express the

intensity of the unfiltered source in equivalent photons at the optimum wave-length. Table 3 compares experimentally determined values of these calibration factors with those calculated by the equation

$$R = \frac{\int f_1(\lambda) f_2(\lambda) f_3(\lambda) \mathrm{d}\lambda}{\int f_1(\lambda) f_3(\lambda) f_4(\lambda) \mathrm{d}\lambda}, \tag{9}$$

where R is the factor by which the linear response was reduced when the Chance glass heat-filter was exchanged for a narrow-band interference filter transmitting a wave-length near the optimum wave-length of the receptor; $f_1(\lambda)$ is the receptor sensitivity as a function of wave-length; $f_2(\lambda)$ is the transmission characteristic of the interference filter; $f_3(\lambda)$ is the photon emission of the source as a function of wave-length, and $f_4(\lambda)$ is the transmission characteristic of the Chance glass.

TABLE 3. Comparison of effectiveness of white and monochromatic light

	Log R observed	Log R calculated
Red-sensitive cones (644 nm)	−1·100	—
	−1·110	—
	−1·120	—
	−1·010	—
	−1·060	−1·053
	−1·040	−1·065
	−1·065	—
	−1·055	—
Mean	−1·070	
Green-sensitive cones (559 nm)	−1·045	—
	−1·065	−1·146
	−1·055	−1·087
Rod (519 nm)	−1·075	−1·177

R is the factor by which the linear response was reduced when the Chance glass heat filter was exchanged for a filter passing the wave-length shown in brackets in the left-hand column. A white light of intensity RI' has the same effect as monochromatic light of intensity I'. The right-hand column was calculated by eqn. (9).

Differences between the linear responses of red and green cones and rods

Although the wave-length of an absorbed photon has no effect at all on the time course of the response of individual receptors there are striking differences between the temporal characteristics of rods and cones and smaller differences between red- and green-sensitive cones. These are illustrated by the linear responses collected in Fig. 12. In red-sensitive cones the time to maximum was 100–140 msec, in green-sensitive cones 140–180 msec and in rods 300–600 msec. The great variability of the time

course of responses from rods as well as the variability of their sensitivity may be caused by differences in the extent to which rods have recovered from previous exposure to bright light. It is interesting that there should be such striking differences between the responses of green-sensitive cones and rods in view of the fact that both cells contain the same 518_2 visual pigment (Liebman & Granda, 1971).

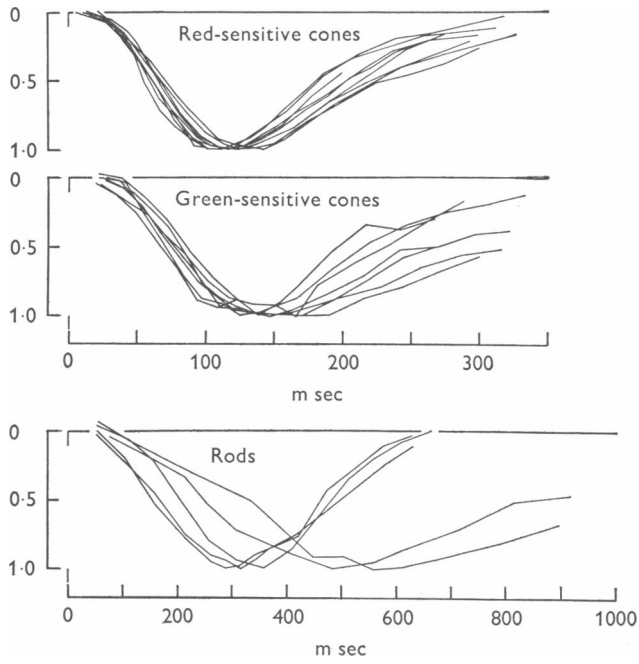

Fig. 12. Linear responses of nine red-sensitive cones, seven green-sensitive cones and five rods obtained by averaging several responses of about 1 mV amplitude to weak flashes. The experiments were carried out at 19–23 °C and have been corrected to 20° C assuming a Q_{10} of 1·8 as found in other experiments.

Possible exceptions to spectral univariance in cones: large fields

All our quantitative measurements on the spectral sensitivity of cones were made with spots of diameter less than 150 μm (usually 100 μm). From the recent work of Fuortes, Schwartz & Simon (1973) it is clear that spectral univariance would not have been found if large fields had been employed. In agreement with these authors we noticed that one cone which hyperpolarized to green flashes gave late depolarizing waves sometimes turning into spike-like deflexions when stimulated with a red annulus. This effect was probably mediated through the luminosity horizontal cells as Fuortes *et al.* suggest. However, even with a 100 μm spot

the response to a red flash was shorter than the response to a green flash. This is the only case in which spectral univariance clearly did not apply in experiments with small spots of light.

Spatial characteristics of cones

Baylor *et al.* (1971) showed that injection of current into one cone might cause electrical changes in a neighbouring cone within a distance of about 40 μm. Not all cones within 40 μm showed the interaction and it was never detected at distances greater than 50 μm. Except for one pair of cones the polarity and reciprocal nature of the effect was consistent with the presence of an electrical connexion between certain cones.

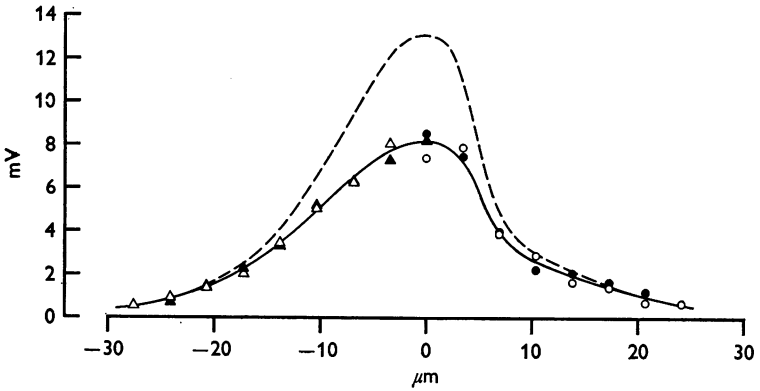

Fig. 13. Profile of spatial sensitivity for an isolated red-sensitive cone. Points are averaged response amplitudes plotted as a function of the position at which a spot of nominal diameter 7 μm was applied on the retina. The continuous curve was drawn through these points by eye; the dashed curve was obtained from it by the 'linearization' procedure described in the text. The stimulus was a white flash of intensity 10^{-2}. The resting potential was −35 mV and the maximum hyperpolarization was 22 mV.

The experiments described in the following sections indicate that most but not all cones are coupled and that only cones of the same spectral sensitivity are connected together. Although cones which behaved like isolated units were encountered rarely their behaviour will be considered first since it is simpler than that of coupled cones.

An apparently isolated red-sensitive cone. Fig. 13 gives the spatial profile of an isolated red-sensitive cone. The experiment consisted in moving a circular spot of light with a nominal diameter of 7 μm across the retina in a straight line which passed through the position of maximum sensitivity. The average amplitude of the response to a 10 msec flash of relative intensity 10^{-2} was determined at intervals of 3·4 μm. These results gave the experimental points and continuous curve in Fig. 13. The dashed curve

was determined by a linearization procedure analogous to that used in determining spectral sensitivity. In this experiment the intensity-amplitude relation which is illustrated in Fig. 14 was a rectangular hyperbola, i.e.

$$U = \frac{U_L I^*}{1+I^*}, \tag{10}$$

where U_L is the maximum hyperpolarization and $I^* = I/I_{\frac{1}{2}}$, $I_{\frac{1}{2}}$ being the value for $U = U_L/2$. Linearization then involves calculating the variable

$$y = \frac{U_L U}{U_L - U}, \tag{11}$$

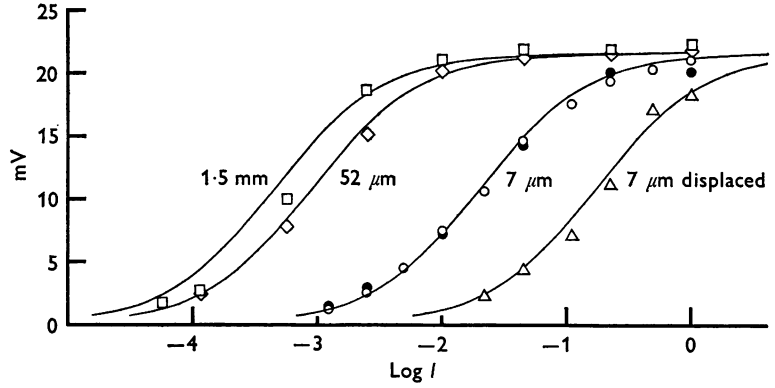

Fig. 14. Intensity amplitude relations from the isolated red-sensitive cone of Fig. 13. Peak response in mV is plotted as a function of log relative stimulus intensity for white flashes 7, 52 and 1500 μm in diameter, and for a 7 μm flash applied 17 μm from the centre. A single curve, laterally displaced, fits each set of points. This cell signals the strength of the light on it but not that on other cones.

which should be proportional to the quantity of light reaching the receptor. It can be seen from Fig. 13 that there are no bumps on the descending limb of the curve and that the spatial profile is sharp, though not as sharp as the calibration curve in Fig. 2. The result is consistent with the idea that light scattering in the retina blurs the image of the 7 μm spot and increases its total width to about 16 μm at half maximum and about 40 μm at one-tenth maximum.

If the spatial profile in Fig. 13 depended only on straightforward physical factors such as the collecting area of the cone and light scattering in the retina one would expect the cone to be univariant with respect to the spatial characteristics of the light stimulus. This means that the effect of displacing the spot or changing its area will be to shift the relation between response amplitude and log light intensity horizontally but not to alter its shape. Fig. 14 verifies this prediction for three very different

stimuli, i.e. 52 and 7 μm diameter spots and for 7 μm displaced 17 μm. Even with the 1500 μm diameter spot there is no very serious discrepancy.

Fig. 15 provides a more complete test of spatial univariance. Here records in a given horizontal row should all be nearly identical if univariance holds. This has been tested by superposing the tracings from the 52 μm centred spot on records obtained under other conditions. If can be seen that the three right-hand sets of records are virtually identical, but that with strong flashes a 1500 μm spot gave a record with a conspicuous notch after the maximum. This is attributed to feed-back from the horizontal cells.

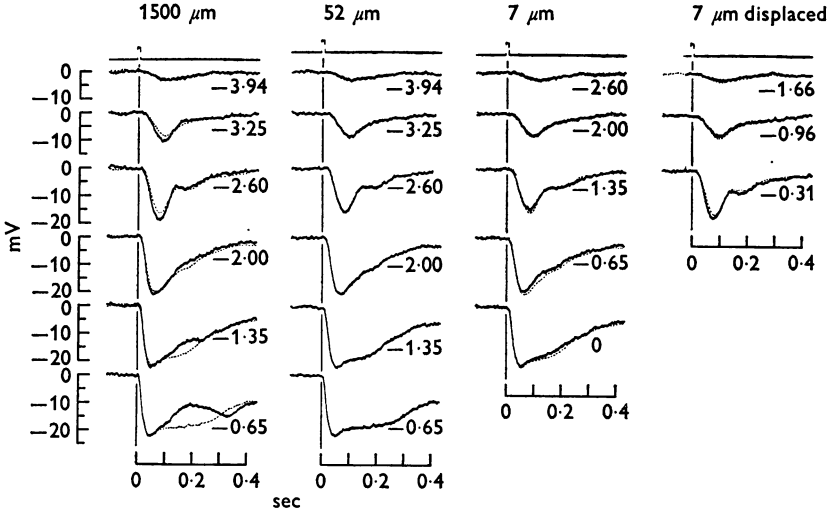

Fig. 15. Responses to light-spots of varying diameter. Records from the isolated cone of Fig. 13. Flashes of increasing intensity were applied with spots of diameter 7, 52 and 1500 μm centred on the cell, and with a spot of 7 μm at -17 μm from the centre (7 μm displaced). The frames obtained with the 52 μm spot have been dotted in on the other records in each horizontal row. Numbers at the right end of the traces give the relative strengths of the white flashes in log units. The relative vertical position of the two right-hand columns was arranged so that the logarithm of the light intensity in each row differed from the 52 μm column by about 1·3 units for the third column and 2·3 units for the fourth.

Coupled cones. The two spatial profiles in Fig. 16 illustrate the properties of a green-sensitive cone (*A*) and a red-sensitive cone (*B*) which behaved as though they were coupled to other cones. Both curves are broader than for the isolated cone and there is a bump on the falling phase of curve *B* at 20–30 μm from the origin. Stronger evidence for coupling is that the electrical responses were not univariant with respect to the spatial

characteristics of the light stimulus and that the intensity amplitude relations changed with the area illuminated in a manner expected if the cone were coupled to others. From Fig. 17 which applies to the green-sensitive cone it can be seen that the intensity amplitude relations for the 7 and 103 μm spots are completely different and that records cannot be matched by scaling the light intensity. There is also a strong suggestion

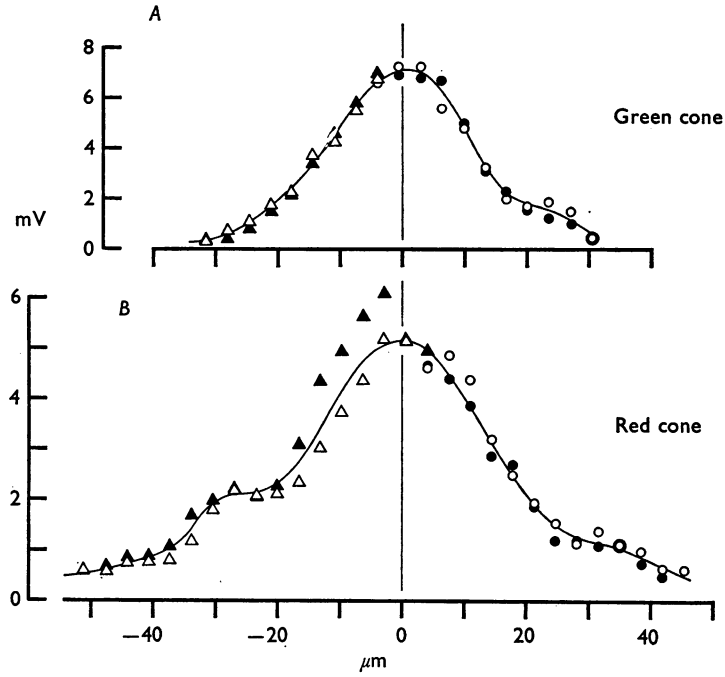

Fig. 16, *A*, profile of spatial sensitivity for a coupled green-sensitive cone. Response amplitude plotted *vs.* the position of flashes with a spot 7 μm in diameter. There is a suggestion of a bump in the profile at +25 μm. 559 nm light, resting potential −37 mV, maximum response to light 15 mV.

B, profile of spatial sensitivity in a coupled red-sensitive cone. Peak responses to flashes plotted as a function of the position of a white spot 7 μm in diameter. There is a bump in the profile at −25 μm, seen in this and other runs. Resting potential −35 mV; maximum response to light −21 mV.

that with a 7 μm spot the response reached an upper limiting value of about 7 mV as compared with 14 mV for the 103 μm spot. These rather complicated effects are explained by supposing that in this particular case the cones to which the central cone was coupled were separated from it by distances of not less than about 25 μm. To simplify the argument we shall consider a hypothetical situation in which the central cone is coupled to

four other cones all located at 25 μm from the central cone and coupled to it in such a way that the recorded response is given by

$$U_r = \tfrac{1}{2}U_1 + \tfrac{1}{8}U_2 + \tfrac{1}{8}U_3 + \tfrac{1}{8}U_4 + \tfrac{1}{8}U_5, \tag{12}$$

where U_1 is from the central cone which contributes 4 times as much as any of the others acting alone, this being roughly consistent with the relative size of the bump and main peak shown in Fig. 16*A*. With a spot centred on the impaled cone we can assume $U_2 = U_3 = U_4 = U_5$ so

$$U_r = \tfrac{1}{2}U_1 + \tfrac{1}{2}U_2. \tag{13}$$

The situation is then equivalent to that expected for two tightly coupled cones. When the impaled cone is activated with a 7 μm spot very little light should reach the surrounding cones so the response saturates at 7 mV instead of the 14 mV observed with a 103 μm spot. If the spot diameter is increased from 7 to 34 μm the amount of scattered light which reaches the surrounding cones is much larger and this gives the secondary rise on the middle curve in Fig. 17*A*.

The pattern shown in Fig. 17 was seen only occasionally; more often the intensity amplitude relation observed with both red- and green-sensitive cones was as in Fig. 18. Here the 103 μm diameter spot gives the usual type of intensity-amplitude relation, whereas the curve from the 7 μm spot rises much more slowly but fairly continuously, though there are suggestions of steps which are more obvious with the 2·4 μm spot. What is required to give this type of behaviour is a group of cones in which the distances to the impaled cone's neighbours are something like 10, 15, 20 μm etc. Records from cones which gave an intensity amplitude relation of this kind with small spots were similar to those described by Baylor *et al.* (1971, Fig. 4*a*).

Information about the spatial characteristics of cones is collected in Table 4. The two red-sensitive cones marked with an asterisk, which had narrow sensitivity profiles, gave small depolarizations with the spot displaced more than about 20 μm. None of the other cones showed any indications of this type of behaviour.

Response–area relations. The circles in Fig. 19 give the experimentally determined relation between the relative sensitivity of a red-sensitive cone and the area (or radius) of the circular spot of light centred on the impaled cone. The relative sensitivity is the amplitude of a linear response divided by the intensity of the light in arbitrary units. The red-sensitive cone from which these results were obtained was the same as that in Fig. 16*A*. The smooth curve in the area-sensitivity graph (Fig. 19), which is a good fit to the points, was calculated from the spatial profile in Fig. 16*A* by the following method.

If the response dU to an infinitesimal area dA of light intensity I applied at a distance r from the centre of the impaled cone is given by

$$dU = If(r)dA, \tag{14}$$

the response to a circular spot of radius a centred on the impaled cone should be

$$U_0(a) = 2\pi I \int_0^a rf(r)dr. \tag{15}$$

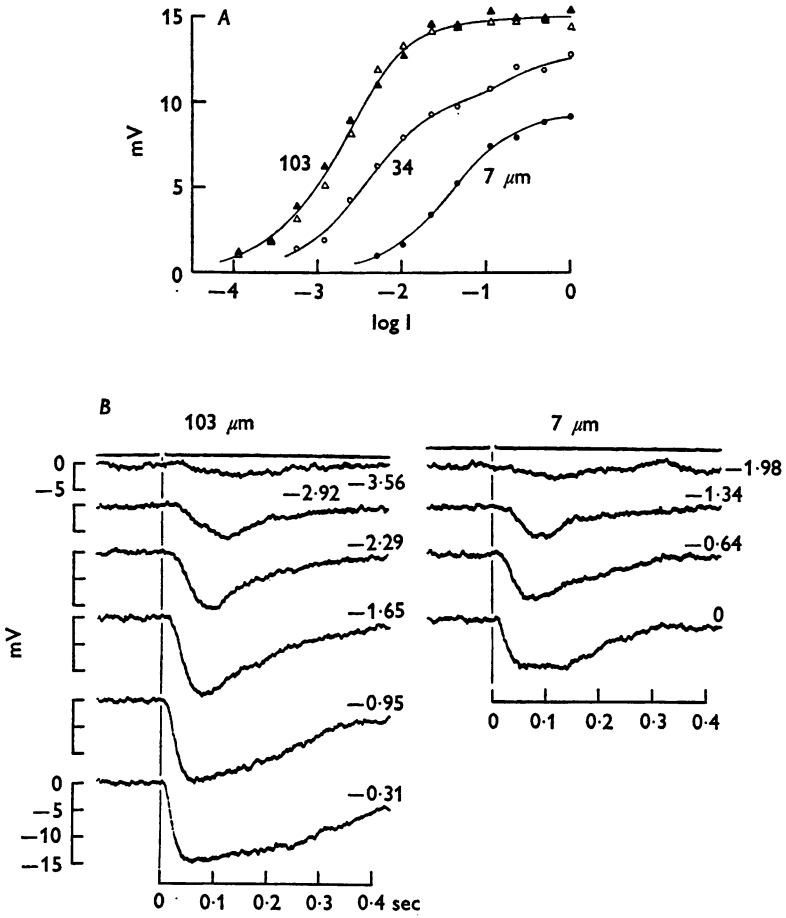

Fig. 17. Responses of the green-sensitive cone of Fig. 16 to flashes of varying diameter. Records below show responses to flashes of increasing relative intensity (log units) with spots 7 and 103 μm in diameter. With the smaller spot, the responses saturate in amplitude at a lower level than with the larger spot. Above is a plot of peak response in mV versus log relative flash intensity for spots of diameter 7, 34 and 103 μm; sequence of runs (△, ●, ○, ▲); 559 nm light.

To apply this formula we assume that $f(r)$ is proportional to the curve $U(r)$ obtained by linearizing the spatial profile determined with a 3·5 μm radius spot. If the light intensity I is taken as unity

$$f(r) = \frac{U(r)}{\pi(3{\cdot}5)^2} \tag{16}$$

and

$$U_0(a) = \frac{2}{(3{\cdot}5)^2}\int_0^a rU(r)\,\mathrm{d}r. \tag{17}$$

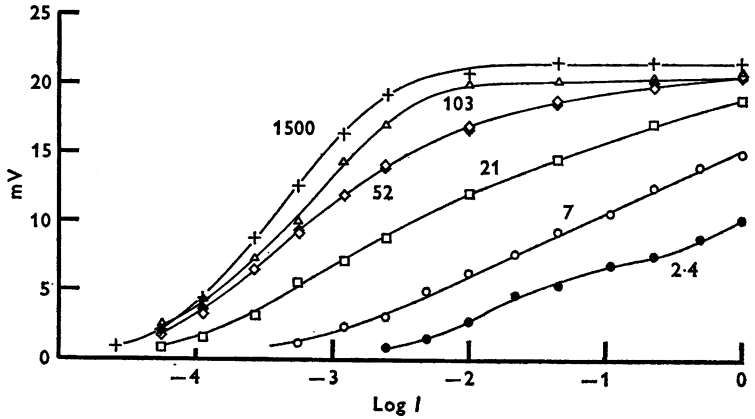

Fig. 18. Intensity amplitude relations with various spot diameters for the red-sensitive cone of Fig. 16*B*. Peak hyperpolarization in mV is plotted *vs.* the relative flash intensity (log units). Spot diameters in μm are indicated next to the curves. Sequence of runs △ + ◇ ○ ● ◆ □. White light.

TABLE 4. Spatial properties of cones

		$W_{1/2}$	$W_{1/10}$	$W'_{1/2}$	$W'_{1/10}$	D_N	$D_{1/2}$
Red-sensitive cones	1*	15	24	13	21	3.5	—
	2	68	110	48	85	3·5	—
	3	26	66	19	56	3·5	41
	4	13	39	9	35	2·4	—
	5	9	35	8	28	2·4	—
	6*	18	27	16	26	7	—
	7	19	46	16	40	7	—
	8	35	97	20	53	7	40
	9	40	107	23	56	7	57
	10	28	60	15	34	2·4	—
Green-sensitive cones	1	40	70	36	66	3·5	—
	2	27	54	16	38	7	50

$W_{1/2}$ is the total width in μm of the profile at 1/2 maximum, $W_{1/10}$ the total width in μm at 1/10 maximum. $W'_{1/2}$ and $W'_{1/10}$ are the corresponding quantities for the linearized profiles. D_N gives the nominal spot diameter (μm) used in determining the profile. $D_{1/2}$ gives the spot diameter in μm which would yield a sensitivity of 1/2 maximum. The two cones marked with an asterisk gave depolarizing responses when the spot was displaced more than 20 μm.

Since $U(r)$ was not symmetrical the integration was carried out in both directions and the formula used was

$$U_0(a) = \frac{1}{3 \cdot 5^2} \int_0^a rU_+(r)\,dr + \int_0^a rU_-(r)\,dr, \tag{18}$$

where $U_+(r)$ is the curve for positive displacements and $U_-(r)$ for negative ones.

Equation (18) was tested on three other cones with broadly similar results although the agreement was not as good as in Fig. 19. Discrepancies at large areas may arise from neglect of horizontal cell feed-back and from

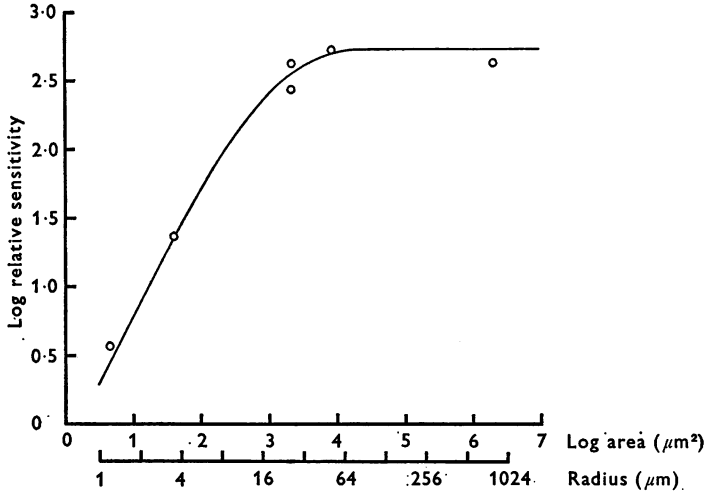

Fig. 19. Sensitivity-area relation for the red-sensitive cone of Figs. 16*B* and 18. The log relative sensitivity to white test flashes is plotted against the log of the area of the stimulating spot. The continuous curve was calculated from the profile of spatial sensitivity shown in Fig. 16*B*, using the method described in the text. The sensitivity was tested with responses about 1/10 maximal amplitude.

the difficulty of determining the outer part of the spatial profile with sufficient accuracy. The linearization procedure may also introduce errors since it is not strictly applicable to coupled ones. In the isolated cone of Fig. 13 where the error should not be present the sensitivity difference between the 7 and 52 μm spots was 1·32 log units, whereas the shift calculated from the sensitivity-profile was 1·08. The difference would be explained if there had been somewhat more light scattering in the co-ordinate at right angles to that used in measuring the profile.

Evidence that coupling is only between cones of similar spectral sensitivity

When taken with the findings of Baylor *et al.* (1971) the present experiments provide evidence that a green-sensitive cone such as that in Fig. 16*A* is coupled to other cones. The spectral sensitivity of this cone, which has already been illustrated in Fig. 11, showed no trace of any red component on the long wave-length side of the maximum. From that curve it can be shown that the coupling coefficient to red-sensitive cones must have been less than about 1/200. From this and similar results in other experiments we conclude that green cones are coupled to other green cones but not to red cones. Further evidence is provided by the observation that the spectral sensitivity curve of a green cone determined with a 10 μm spot was identical to that determined with a 100 μm spot.

A similar argument applies to red cones since none of the spectral sensitivity curves in Fig. 9 are of the kind expected if there was an appreciable green component in the red response.

TABLE 5. Absorption maxima and spectral sensitivity of receptors in *Pseudemys scripta elegans*

Receptor	Wave-length (nm) for: Maximum sensitivity	Wave-length (nm) for: Absorption maximum of pigment	Type of oil droplet
Red-sensitive cones	630	620 ± 5	Red, orange
Green-sensitive cones	550	518	Orange (or absent)
Blue-sensitive cones	460	450 ± 5	Colourless
'Rods'	520	518 ± 2	Absent

The figures in the third column are from Liebman & Granda (1971). The fourth column which is from Liebman (1972 and personal communication) is necessarily based on a very small sample as outer segments usually become detached when the cone is isolated. The visual pigment associated with yellow droplets is unknown.

DISCUSSION

Spectral sensitivity

The receptors studied here can be classified into four distinct groups on the basis of their spectral sensitivity and electrical characteristics. Table 5 summarizes these results and compares them with the measurements of Liebman & Granda (1971) and Liebman (1972) of visual pigments and coloured oil droplets in *Pseudemys*. In 'rods' and blue-sensitive cones where the oil droplets are absent or colourless the two sets of measurements are in close agreement. In red- or green-sensitive cones the sensitivity is

maximal at a longer wave-length than that for peak absorption by the visual pigment, as would be expected from absorption of short wave-lengths by the coloured oil droplets. The shifts in maximum wave-length are probably consistent with the idea that the red-sensitive cones usually contain red oil drops and that the green-sensitive cones contain orange or yellow ones.

Our results support the idea that coloured oil-droplets improve spectral resolution by reducing the short-wave-length tail of the absorption curve. Thus in Fig. 11 the reduction of sensitivity attributed to a coloured oil drop in a green-sensitive cone amounts to 2 log units at 450 nm. This should help to avoid confusion between green and blue lights and reduce the necessity for the central sharpening mechanisms which have been elaborated in primates.

The finding that 'rods' and green-sensitive cones, which probably contain the same 518_2 pigment, should differ so strikingly in the time scale of their electrical responses and rate of dark adaptation is of some general interest. It suggests that other factors besides pigment chemistry are important in the transduction mechanism, and reinforces the conclusion of Penn & Hagins (1972) that 'the kinetics of the photocurrent bear no simple relation to the formation or decay of any of the spectroscopic intermediates so far detected during the photolysis of rhodopsin'.

The shapes of the spectral sensitivity curves appear to differ systematically from the monogram for a standard pigment having retinal_2 as the prosthetic group (Bridges, 1967; Munz & Schwanzara, 1967). The blue-sensitive mechanism observed here is considerably broader than the nomogram for a 450_2 pigment and the discrepancy appears much too large to be due to self-screening. The red-sensitive mechanism, allowing for the effect of the oil droplet and self-screening is narrower than the nomogram for a 620_2 pigment. The green-sensitive mechanism roughly corresponds to a nomogram for a 518_2 pigment. These discrepancies are similar to those observed by Liebman & Granda (1971) using microspectrophotometry.

From Liebman's (1972) information about the association of different oil drops with different pigments it may seem surprising that the spectral sensitivity of cones should fall into three such well defined groups. If there were many 620_2 cones with orange drops or many 518_2 cones without drops one would expect to find receptors corresponding to these types. However, it should be remembered that our sample may not be complete and that variation in the angle of incidence of the light (which determines the amount of absorption in the oil drop) may obscure subdivisions within our three main groups. For example, among green-sensitive cones those with little fall in sensitivity at short wave-lengths might be droplet-containing cells hit obliquely by the light, or they might be accessory members of a double cone without an oil droplet. Further study, in which the angle of the light beam is varied systematically, could clarify this and also test the directionality hypothesis outlined on page 180.

Absolute sensitivity

Our measurements give the absolute flash sensitivity of receptors in units of microvolts per (photon per square micron). The values for the most sensitive cells which we consider should approximate most closely to those in the living animal with normal optics are: red- and green-sensitive cones about 250 μV photon^{-1} μm^2, blue-sensitive cones 120 μV photon^{-1} μm^2, 'rods' 1300 μV photon^{-1} μm^2. It is clearly of interest to know the size of the response produced by photoisomerization of one chromophore. To do this we need to estimate the effective collecting area of the receptor. By effective collecting area we mean the geometrical collecting area multiplied by factors which allow for the fraction of light effectively absorbed in the outer segment. The geometrical collecting area will be taken as 50 μm^2 on the basis that the cone inner segments are about 8 μm in diameter over most of their length. From the statement of Liebman & Granda (1971) that the specific axial density of pigment in the outer segment is 0·013/μm and that the length of the outer segment is 35 μm it follows that about 0·6 of the light entering the outer segment should be absorbed. In a red-sensitive cone with a red oil drop, absorption in the drop might introduce another factor of 0·9 so that if the quantum efficiency is taken as 0·5 the effective collecting area is found to be 13·5 μm^2. This estimate is likely to be too high because it has made no allowance for light scattering in the retina or for imperfections in the concentrating mechanism. We shall therefore take 10 μm^2 as a tentative estimate of the effective collecting area of a cone. On the assumption that this figure applies to the most sensitive cones and rods, and is proportionately less in the less sensitive ones, our flash sensitivities imply that the mean peak hyperpolarization produced by one photoisomerization is 25 μV in red- and green-sensitive cones, 12 μV in blue-sensitive cones and 130 μV in rods. The effective duration of these hyperpolarizations may be taken from the integration times as 0·17 sec in red-sensitive cones, 0·2 sec in green-sensitive cones and 0·5 sec in rods, all at 20° C.

It should be possible to check the magnitude of the quantal voltage change in other ways. If a 2·5 mV response is the sum of 100 photoisomerizations each giving 25 μV it should have a standard deviation of 250 μV. Preliminary analysis of the variance of responses gave figures of 20–30 μV for the quantal unit in one red-sensitive cone. The difficulty in interpreting this figure is that we do not know how to allow for the effect of coupling to other cones. However, it is clear that the variance of receptor responses is a subject which should repay further investigation and which might yield more definite information about the magnitude of the quantal voltage change. A different approach might be to measure the

bleaching of chromophores in a cell in which flash sensitivity could also be determined.

Unless our values of the magnitude of the quantal voltage change are wrong by several orders of magnitude it is clear that there is a large amplification factor in the transduction mechanism. If the electrical resistance between the inside and outside of a cone in darkness is taken as 50 MΩ (D. A. Baylor & A. L. Hodgkin, in preparation) a hyperpolarization of 25 μV lasting 0·17 sec would correspond to a suppression of the movement of about 5×10^5 monovalent ions through the light sensitive channel. This happens to agree with the estimates from current measurements made by Penn & Hagins (1972) for rat rods at 33° C whose responses are similar in time scale to those of turtle cones at 20° C. From osmotic measurements Korenbrot & Cone (1972) calculate that one photon suppresses the movement of about 10^7 Na ions in frog rods at room temperature. Our figures for turtle rods would be roughly consistent with this statement if the electrical resistance of a rod is taken as 50 MΩ.

Coupling between cones

The measurements of the spatial resolution of cones provide indirect support for the conclusion of Baylor *et al.* (1971) that cones within a radius of 50 μm may be electrically coupled to one another. They also indicate that cones are coupled to cones of the same spectral sensitivity, i.e. red to red and green to green, but not red to green or vice versa. Unless this were so it is very difficult to see why a green-sensitive cone which appeared to be coupled to other cones should show less than 1 % of a red component when illuminated with a circular patch 100 μm in diameter at a wavelength where the red response should be maximal. The conclusion seems reasonable as it would be an odd design in which information about colour was thrown away at the outset by averaging the response of cells containing different pigments.

At first it seems curious that cones should be coupled at all as this must decrease acuity to some extent. In considering possible explanations it is important to remember that all our measurements were made in the periphery of the retina. In this region and perhaps throughout the retina there is no evidence for the presence of midget bipolars and a single bipolar collects from many cones (Lasansky, 1971). The usual teleological explanation for this is that there would not be room in the optic nerve to have one nerve fibre from each receptor. At all events, if bipolars do collect from many cones, electrical coupling between cones would not greatly impair spatial resolution and it might provide a convenient method of averaging the responses of several cones without introducing synaptic noise. A similar argument has been developed by Falk & Fatt (1972) who conclude

that there must be some form of coupling, which is finer grained than the usual synaptic mechanism, between rods in the mammalian retina.

One of the reasons for studying the spatial resolution of cones was to decide on the optimal size of light spot for analysing electrical responses to light. Our tentative conclusion is that a spot with a diameter of 50–150 μm is about right. At this size the central cone should be at about the same potential as its neighbours which receive approximately the same illumination. With a smaller spot one has the complicated situation that the performance of the central cone is modified by flow of current from other cones which are less strongly illuminated. Spots of diameter greater than 200 μm should be avoided since the response of the cone is likely to be affected by feed-back from the horizontal cells as shown by Baylor *et al.* (1971). Confirmatory evidence that a 100 μm diameter spot minimizes the complications introduced by cone coupling is that the responses seen under these conditions are similar to those recorded from an uncoupled cone illuminated with a spot of any diameter up to about 300 μm.

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