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PHOTOCURRENTS OF CONE PHOTORECEPTORS OF THE GOLDEN-MANTLED GROUND SQUIRREL

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SUMMARY

1. Visual transduction in photoreceptors of the ground squirrel, *Citellus lateralis*, was studied by recording membrane current from individual cones in small pieces of retina.

2. Brief flashes of light produced transient reductions of the dark current; saturating response amplitudes were up to 67 pA. A flash strength of about 11000 photons μm^{-2} at λ_{max} was required to give a half-saturating response. The stimulus–response relation was well fitted by an exponential saturation curve. Responses below 20% of maximum behaved linearly.

3. The response to a dim flash in most cells had a time to peak of 20–30 ms and resembled the impulse response of a series of five low-pass filters.

4. The variance of the dim-flash response amplitude put an upper limit of 80 fA on the size of the single photon response. Estimates based on the effective collecting area suggest the single photon response to be of the order of 10 fA.

5. Flash responses of squirrel cones usually lacked the undershoot observed in primate cones, although in about $\frac{1}{3}$ of the cells a small undershoot developed during recording.

6. Background lights slightly shortened the time to peak of the flash response and reduced the integration time.

7. Spectral sensitivity measurements showed two classes of cones with peak sensitivities at about 520 and 435 nm. Rod sensitivity peaked near 500 nm. Spectral univariance was obeyed by all three classes of cells.

8. The shapes of the spectral sensitivity curves of the rod and both types of cones were similar to each other when plotted on a log wave number scale, but differed significantly from similar plots of monkey and human cone spectra.

9. The kinetics and sensitivity of flash responses of the blue- and green-sensitive cones were indistinguishable.

INTRODUCTION

The introduction of the suction electrode technique (Baylor, Lamb & Yau, 1979*a*) has allowed the study of relatively small photoreceptors including those from monkey and human retinas (Baylor, Nunn & Schnapf, 1984, 1987; Schnapf, Kraft &

Baylor, 1987). One of the difficulties in studying cone transduction in primates is that outside the fovea, which comprises less than 1 % of the retinal surface, the cones are hidden within dense forests of rods. An alternative preparation is provided by the retina of the diurnal ground squirrel in which about 90 % of photoreceptors are cones (Walls, 1942; West & Dowling, 1975; Jacobs, Fisher, Anderson, & Silverman, 1976; Long & Fisher, 1983). Ground squirrels are dichromatic and lack red cones, as demonstrated by behavioural (Crescitelli & Pollack, 1972; Jacobs, 1978) and physiological experiments (Tansley, Copenhaver & Gunkel, 1961; Michael, 1968; Gur & Purple, 1978; Raisanen & Dawis, 1983; Ahnelt, 1985; Jacobs, Neitz, & Crognale, 1985). Thus this retina offers a simple colour system and greater access to the blue-sensitive cones.

This paper describes the response properties and spectral sensitivities of cones from the retina of the ground squirrel. It is shown that these cells have different kinetics than those of primate cones. The spectral sensitivity curves are similar in shape to one another but have a somewhat different form than those of primate cones, thus not obeying the invariant spectral form described by Mansfield (1985).

METHODS

Animals

Golden-mantled ground squirrels are native to the mountain ranges of the western United States and southwestern Canada. Animals used in this study were captured during summer months in the Sierra mountains of northern California. Squirrels were housed individually in cages containing a nest box and cotton nesting material, and given *ad libitum* access to food and water.

Preparation

Animals were dark adapted for at least 1 h before being killed with an overdose of sodium pentobarbitone, or by carbon dioxide asphyxiation. The eyes were removed and hemisected under dim red light, and all further manipulations were performed under infra-red light with the aid of an infra-red-to-visible image converter (FJW Industries). The eyecup was divided into quarters and retinal pieces were isolated with a fine forceps into tissue culture medium (Liebowitz's L-15, Gibco) supplemented with vitamins and amino acids (BME mixture, Gibco). Pieces of isolated retina were chopped and placed in the recording chamber, where they were continuously perfused with an oxygenated Ringer solution warmed to 37 °C. The superfusate contained the following salts (in mM): NaCl, 120; KCl, 3·6; $NaHCO_3$, 20; $MgCl_2$, 1·2; $CaCl_2$, 2·4; buffered to pH 7·4 with 5 % CO_2 gas and 3 mM-HEPES buffer. The Ringer solution also contained vitamin and amino acid supplements and glucose (10 mM). One rod spectral sensitivity curve was determined in an experiment in which sulphate salts completely replaced chloride in the Ringer solution; sulphate did not effect the rod spectrum.

The outer-to-inner segment connection was quite fragile; often only a small number of cones retained their outer segments through the isolation and chopping procedure. Almost all cells with outer segments responded to light; however, the largest photocurrents were routinely recorded from retinal pieces in which almost all neighbouring cones had long (6–8 μm), straight outer segments.

Electrical recording and light stimuli

The optical and recording apparatus have already been described (Baylor *et al.* 1979*a*, 1984). The addition of a pressure transducer (Omega Engineering Inc.) in parallel with the suction electrode proved useful in stabilizing a cell once the outer segment had been drawn into the pipette. The cell's photocurrent and the stimulus were digitally recorded on video tape (Neuro Data Instruments) during the experiments and later analysed with a PDP 11/73 computer (Indec). The light monitor output and cell photocurrent were filtered identically to avoid introducing spurious response delays.

Wavelength was varied by interference filters with half-widths of about 10 nm (Ditric, 3 cavity). The light was attenuated by inconel neutral density filters (Bausch and Lomb). The power output of the stimulating lamp through each of the interference filters was measured daily with a radiometer (United Detector Technologies). Details of the calibration of the interference and neutral density filters were as described by Baylor *et al.* (1984). Because of the relatively high sensitivity found at short wavelengths three of the interference filters (381, 402 and 421 nm) were recalibrated at the end of the experiments, and found to be unchanged from previous calibrations. All stimuli consisted of unpolarized light.

The shutter was driven by a powerful stepping motor (Tormax 20-010, IMC Magnetics Corp.) controlled by an interval generator (WPI model 830). Photomultiplier measurements showed the maximum variation in the flash duration to be about 50 μs, or less than 0·5%.

Flash and step responses

A simple test of linearity is to observe the scaling of the cell's flash response amplitude with the flash strength. A more severe test that reveals slowly developing non-linearities was described by Baylor & Hodgkin (1973); assuming linearity, the response to a step of light delivered at $t = 0$, $R_s(t)$, is predicted by the scaled integral of the response to a flash, $R_f(t)$

$$\frac{R_s(t)}{I_s} = \frac{1}{I_f \Delta t} \int_0^\infty R_f(t)\,dt, \tag{1}$$

where I_s and I_f are the step and flash intensities respectively and Δt is the duration of the flash.

Collecting area

The effective collecting area (Baylor & Hodgkin, 1973) of a cone can be calculated from the measured dimensions of the outer segment, assuming values for the quantum efficiency of isomerization, Q_{isom}, and the specific pigment density, α. From Baylor, Lamb & Yau (1979*b*)

$$A = V_{os} Q_{isom} f 2{\cdot}303\alpha, \tag{2}$$

where V_{os} is the volume of the outer segment, and f, is a factor that allows for the polarization of the stimulating light. The cone outer segment dimensions observed with the infra-red viewing system, 6–9 μm length, 2 μm tip diameter and 3 μm base diameter, agreed with previous anatomic studies (Jacobs *et al.* 1976). Using typical values for outer segment dimensions V_{os} is about 41 μm^3. Q_{isom} was assumed to have a value similar to rhodopsin, 0·67 (Dartnall, 1972). Microspectrophotometric measurements of pigment density in rods and cones reveal α to be near 0·016 μm^{-1} (Rodieck, 1973; Hárosi, 1975; Bowmaker, Dartnall & Mollon, 1980). Assuming a dichroic ratio of 4, f is 0·63 for unpolarized light, and the resulting estimate for the collecting area is 0·64 μm^2.

Spectral sensitivity

Spectral sensitivity was measured relative to the sensitivity at 500 nm, using diffuse illumination (300 μm spot) incident transverse to the long axis of the outer segment. With a path length of 2–3 μm and assuming a specific pigment density of 0·016 μm^{-1} pigment self-screening can be ignored and the measured sensitivity should be proportional to the probability of photon absorption. Measurements at the standard wavelength were repeated frequently to avoid errors associated with changes in the physiological state of the cell. Spectra from individual cells were normalized by shifting them on the logarithmic ordinate scale so that the mean log sensitivities were equated. Other details of the method have already been published (Baylor *et al.* 1984, 1987).

Polynomial expressions for pigment nomograms (Dawis, 1981) were used to estimate the wavelength of maximum sensitivity (λ_{max}) for the green-sensitive cones and the rods. The best fit was found by minimizing the sum of the squared errors. The blue-sensitive cone data were fitted by a cubic spline routine (Mathsoft Inc.) and λ_{max} estimated by interpolation.

RESULTS

Flash responses

Figure 1 shows a family of superimposed photocurrents evoked by flashes of increasing strength in a green-sensitive cone; the inset is a schematic diagram of the recording procedure. The ordinate is the change of outer segment current from the level in darkness, and the abscissa is time from the centre of the stimuli. Brief flashes of dim light produced photocurrents that scaled linearly with flash strength (see

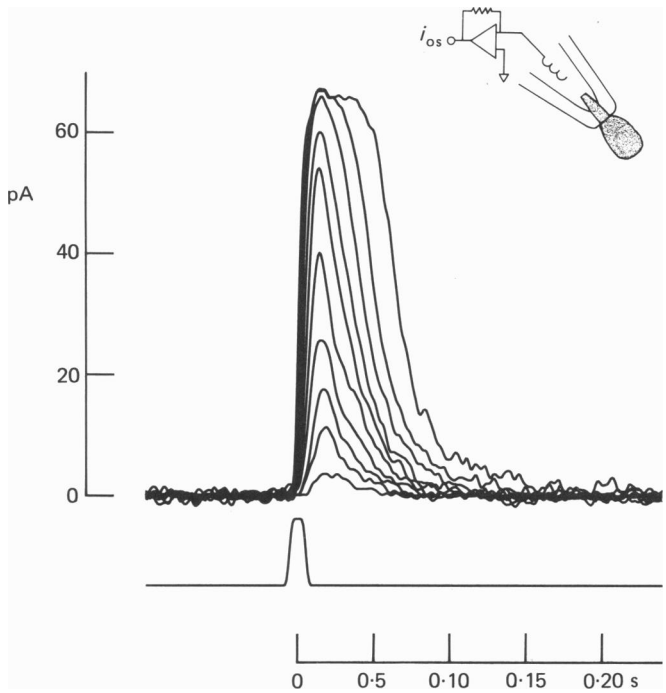

Fig. 1. Family of superimposed responses from a green-sensitive cone to 11 ms flashes of increasing strength. Changes in dark current plotted as a function of time after the flash. Linear range responses rose to a peak in roughly 20 ms; saturating response was 67 pA. Each trace is the average of two to twenty-three sweeps. The upper trace is a single sweep. Flash intensities increased in nominal factors of two between 978 and $5{\cdot}07 \times 10^5$ photons μm^{-2}. Flash monitor shown below current traces. Bandwidth 0–100 Hz, temperature 36 °C. i_{os}, outer segment current.

below). Stronger flashes produced larger responses; the two brightest flashes shut off the dark current entirely, producing a saturating photocurrent. Responses below about 15 pA reached a peak in about 20 ms in the records of Fig. 1, and the maximum response amplitude was 67 pA. The average saturating photocurrent was about 30 pA ($n = 10$) in the better recordings.

The photocurrent grew with flash strength according to the exponential saturation function described by Lamb, McNaughton & Yau (1981) as shown in Fig. 2. The normalized peak photocurrent amplitude is plotted against the scaled flash strength. On the logarithmic abscissa the flash strengths have been multiplied by the constant

k, the reciprocal of the flash strength that gave a response 0·63 of maximum. The smooth curve was drawn according to the expression

$$r/r_{max} = 1 - e^{-ki}, \tag{3}$$

where r is the peak response amplitude, r_{max} is the maximum response, k is the proportionality constant characteristic of the cell, and i is the stimulus strength. The proportionality constant, k, is related to the half-saturating stimulus strength, $i_{\frac{1}{2}}$, by $k = (\ln 2)/i_{\frac{1}{2}}$.

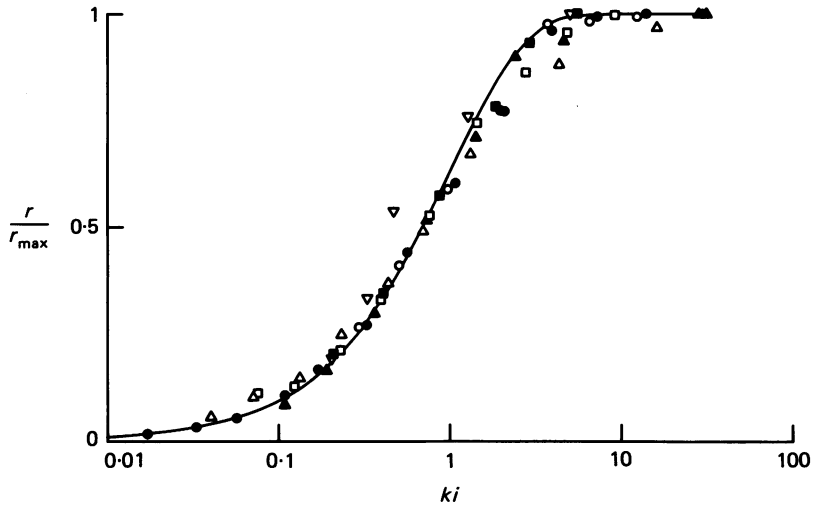

Fig. 2. Intensity–response relations for seven cells. Peak response amplitude relative to maximum value is plotted against the logarithm of the normalized photon density. Smooth curve drawn according to the exponential saturation function (eqn (3)).

The integration time of a cell is given by (Baylor & Hodgkin, 1973)

$$t_i = \int_0^\infty R_f(t)\mathrm{d}t/R_{peak}, \tag{4}$$

where $R_f(t)$ is the response to a brief flash of light at $t = 0$ and R_{peak} is the peak amplitude of the flash response. The average integration time, (t_i), for eleven cones was 30 ms. The time to peak and integration time for a squirrel rod were 150 and 209 ms respectively.

The time to peak for blue-sensitive cone responses, 30 ± 9 ms (mean $\pm$S.D., $n = 7$), was similar to that measured for green-sensitive cones, 27 ± 8 ms ($n = 22$). These populations were not significantly different based on a Wilcoxon rank-sum statistic (Brown & Hollander, 1977). For both cell types the fastest linear responses in darkness reached a peak in 20–22 ms (flash duration 11 ms), which is equivalent to an impulse response with a time to peak of 14 ms (see below).

At the respective peak wavelengths (λ_{max}), the flash sensitivity in darkness, S_F^D, and flash strength required for half saturating response, $i_{\frac{1}{2}}$, were also indistinguishable for the two types of cones. The greatest sensitivity to brief flashes in the dark was $3{\cdot}8 \times 10^{-3}$ pA photon^{-1} μm^2, as measured in cells with the largest saturating

responses. The average sensitivity was $1{\cdot}7 \pm 0{\cdot}9 \times 10^{-3}$ pA photon^{-1} μm^2 ($n = 16$). The half-saturating intensity, 10900 ± 2200 photons μm^{-2} ($n = 11$), was less variable from cell to cell. This finding is consistent with the idea that in cells with a reduced dark current a portion of the outer segment was damaged or missing, the remaining outer segment being normal.

Impulse response

A simple quantitative description of the response to a brief flash is given by the Baylor, Hodgkin & Lamb (1974) filter model. Their equation for the impulse response to a series of n low pass filters with equal time constants written as in Baylor *et al.* (1984) is,

$$r^*(t) = iS_F^D\{t^* e^{(1-t^*)}\}^{n-1}, \quad (5)$$

where $r^*(t)$ is a linear range response, i is the flash strength, and t^* is time after the flash normalized by the time to the peak of the response ($t^* = t/t_p$). Figure 3*A* shows the averaged response to a dim flash of light (continuous line), and the impulse response predicted by eqn (5) with $n = 5$, and $t_p = 22$ ms (dotted curve).

Because the time to the peak of the response was short relative to the stimulus duration (22 *vs.* 11 ms), the stimulus was not truly impulse-like. To determine the impulse response the stimulus and response were deconvoluted using fast Fourier analysis. The cell response in Fig. 3*A* was modelled by eqn (5), and the stimulus represented by a square wave with duration 11·2 ms. The deconvolution is shown in Fig. 3*B* (continuous line). The time to the peak of the predicted impulse response was 16 ms. The shape of the curve was reasonably fitted by eqn (5) with $n = 3$ (Fig. 3*B*, dotted curve), except that the falling phase of the derived impulse response was faster than that predicted by eqn (5), this may indicate the existence of additional delays.

After correcting for the stimulus duration the average linear range cone responses reached a peak in 21 ms; the fastest corrected time to peak was 14 ms.

Differentiation of the response to a step of light was also used to obtain an estimate of the impulse response of the cone (see Methods). This technique, applied to responses from two additional cells also predicted impulse responses with a time to peak of about 16 ms.

Linearity

Figure 4 demonstrates the linearity of flash responses that were below 20% of the maximum response amplitude.

The current traces show a cell's response to a brief flash (upper) and a step of light. The dotted curve shows the step response predicted from the integral of the flash response (eqn (1), see Methods). These responses were from a relatively slow cell where the brief flash was a good approximation to an impulse. A slight undershoot is evident in the flash response and a more dramatic dip is present in the response to the 800 ms stimulus. Linearity was observed in similar tests of superposition on nine other cells.

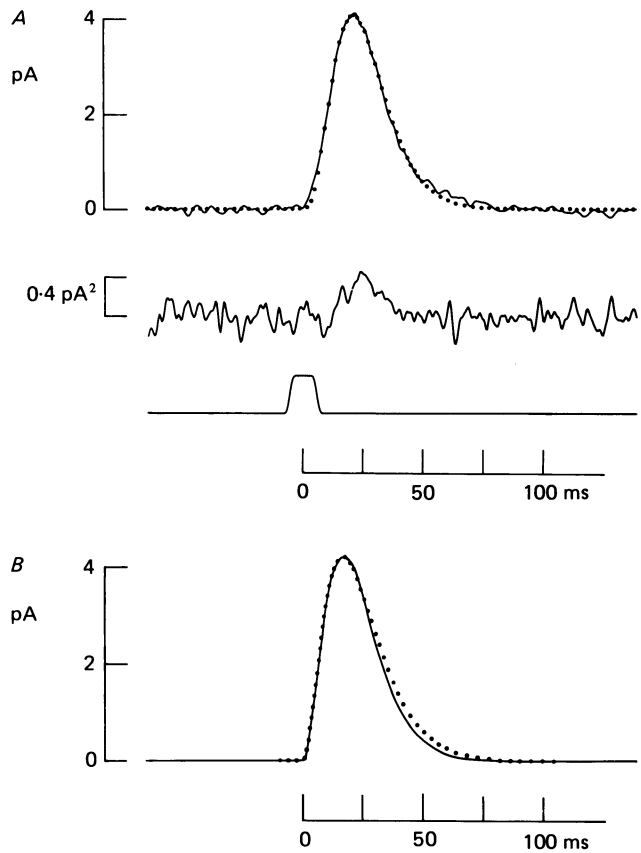

Fig. 3. *A*, averaged responses to dim flashes of diffuse light (continuous curve, $n = 185$). Dotted curve drawn according to the five-stage filter model of eqn (5), $\tau = 5{\cdot}5$ ms. Stimuli were 11·2 ms flashes of 560 nm light, 1540 photons μm^{-2}. Variance measurements from the same series of flash responses are below the photocurrent tracing. Flash monitor shown below current traces. *B*, impulse response of the cell predicted from the deconvolution (see text); the dotted curve shows the impulse response for a three-stage filter model of eqn (5). Bandwidth 0–150 Hz, temperature 35 °C.

Single photon response

Assuming a Poisson distribution of events consisting of single photon absorptions giving responses of constant amplitude, the ensemble variance of the response amplitudes (σ^2) to a series of dim flashes should be related to the mean response amplitude, μ, by

$$\sigma^2 = \mu a, \tag{6}$$

where a is the amplitude of the single photon response. The ratio of the variance to the mean provides an upper limit for the amplitude of the single photon response because several factors can increase the measured variance: (1) desensitization during recording, (2) changes in the seal resistance or length of outer segment in the suction electrode, (3) drift in the dark current of amplifier and (4) real variation in

the amplitude of the single photon response. Many trials were required to obtain reliable estimates of the variance. The lower trace of Fig. 3*A* shows the current variance. The variance measured at the peak of the photocurrent was elevated by 0·35 pA^2, roughly a 40% increase. Thus for the data shown in Fig. 3*A* the single photon response amplitude was calculated to be 85 fA. Similar results from three other cones gave values of about 80 fA.

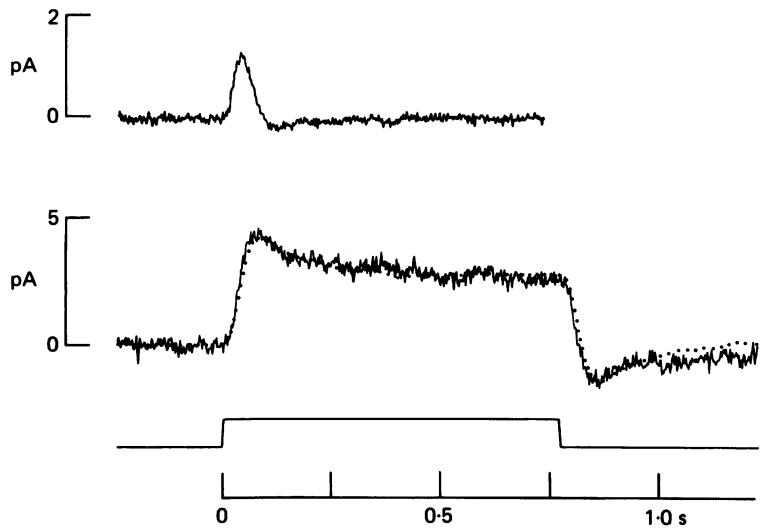

Fig. 4. Demonstration of linearity for small responses in a blue-sensitive cone. The response to an 11 ms flash (upper trace) was used to predict the cell's response to an 800 ms step (lower trace). The prediction is given by the dotted curve (see text for explanation). Bandwidth 0–150 Hz, temperature 36 °C.

A second method of calculating the single photon response amplitude makes use of the relation,

$$a = \mu/Ai, \tag{7}$$

where μ is the mean amplitude of the response to a brief flash, A is the collecting area of the cell (see Methods) and i is the flash strength in photons μm^{-2}. Results from three cells where the specific dimensions of the outer segment were known gave values for a of about 7 fA.

Background light

The effects of background light on flash responses are illustrated in Fig. 5. In Fig. 5 *A* flash responses recorded in the dark and with three background lights have been superimposed after scaling the amplitudes by the stimulus strengths. The uppermost curve was obtained in the dark. Background light speeded the recovery after a flash, reduced the integration time, and made the response more symmetrical. Parameters of the three responses in background light shown in Fig. 5*A* are given by the first three filled circles of Fig. 5*B* and *C*. The time to the peak of the flash response (t_p)

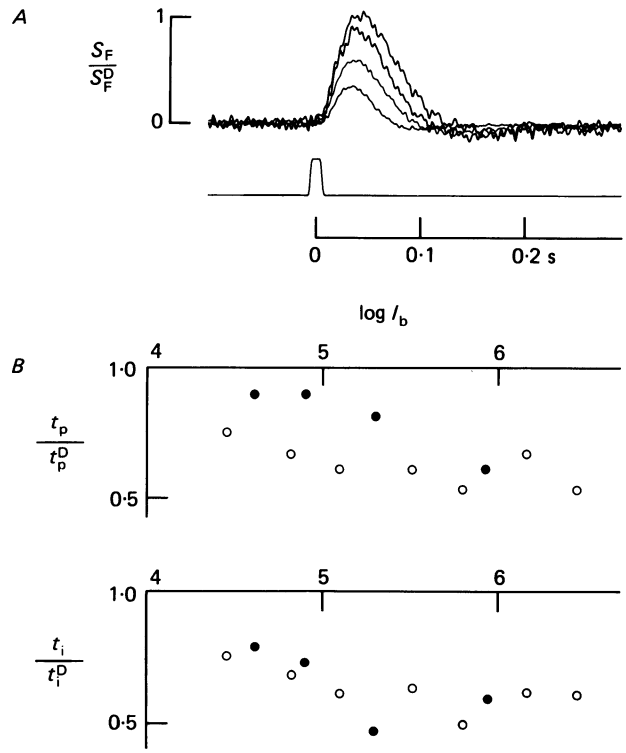

Fig. 5. Background light effects. *A*, superimposed responses to brief flashes in darkness (uppermost trace) and with background light present. Responses are scaled by their stimulus strengths, 3400 and 6900 photons μm^{-2} at 420 nm for the two upper and two lower traces, respectively. *B* and *C*, the time to the peak amplitude of the flash response (t_p) and integration time (t_i) relative to their values in darkness are plotted for two cells against the log of the background intensity (I_b). Bandwidth 0–150 Hz, temperature 36 °C. S_F and S_F^D are, respectively, the flash sensitivity in background light and in darkness.

and the cells' integration time (t_i) were slightly reduced by background light. Time to peak and integration times have been corrected for the duration of the flash. The flash sensitivity was reduced by background light, but because there was incomplete and inconsistent recovery following background illumination no further conclusions were drawn.

During recording, about one-third of the cells developed a small undershoot in the recovery phase of the response (e.g. Fig. 4). Background light did not produce or enhance the undershoot.

Spectral sensitivities

Spectral sensitivity functions were measured in fourteen green-sensitive cones, six blue-sensitive cones, and three rods from six animals. Action spectra were measured by determining the flash sensitivity at the test wavelength relative to that at the reference wavelength (500 nm). Averaged data for the cones are plotted in Fig. 6.

Nomogram fits to the spectral sensitivity functions (Dawis, 1981) were used to

estimate the wavelength of maximum sensitivity for the green-sensitive cone and rod. Data from green-sensitive cones were well fitted by a Dartnall nomogram with the wavelength of maximum sensitivity (λ_{max}) at 517 nm. The rod data were fitted by a Dartnall nomogram with λ_{max} at 501 nm. The fit of the blue-sensitive cone spectrum by the short-wavelength nomogram suggested by Liebman & Entine (1968) was quite poor, underestimating the cone sensitivity below 430 nm. A cubic spline fit to the data produced a curve quite like the one drawn by eye in Fig. 6; interpolation of that curve provided an estimate of the maximum sensitivity at

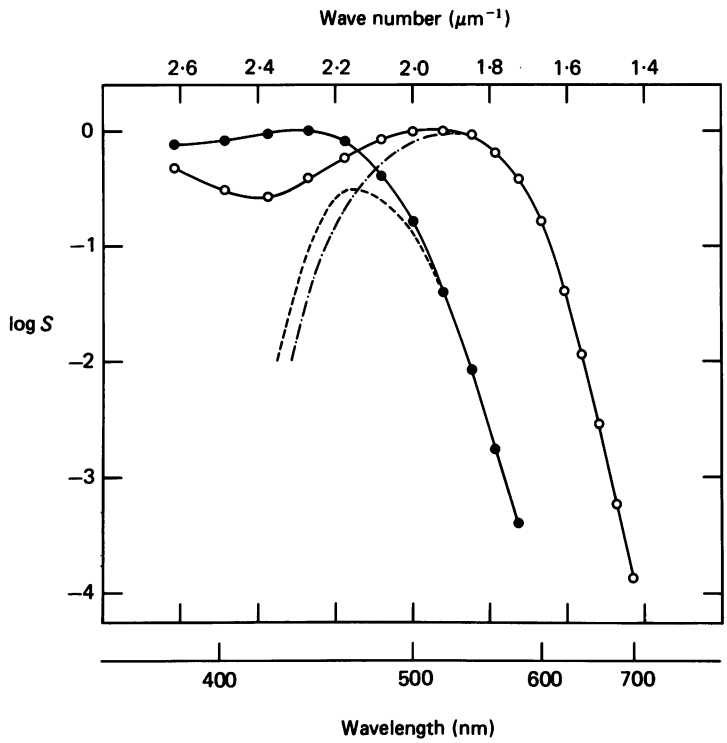

Fig. 6. Average normalized spectral sensitivity data for six blue-sensitive (●) and fourteen green-sensitive (○) cones from five golden-mantled ground squirrels. Continuous curves drawn by eye. Dashed curves are corrected for strong short-wavelength absorption by the lens (see text). *S*, relative sensitivity.

437 nm. The spectral bandwidth at half-height ($W_{\frac{1}{2}}$) for the green-sensitive cone was 0·46 μm^{-1}, and could not be measured for the blue-sensitive cone because of the high sensitivity (absorbance) at short wavelengths.

The lenses of many ground squirrels contain a yellow pigment with extraordinarily strong absorption at short wavelengths (Walls, 1942; Yolton, Yolton, Renz & Jacobs, 1974), the optical density being as high as 20 at 380 nm (Cooper & Robson, 1969). The absorption of the lens should shift the effective sensitivity maxima of the blue- and green-sensitive cones in the intact eye to about 463 and 528 nm respectively (dashed curves Fig. 6).

Spectral form

Trends in the shapes of spectral sensitivity curves from many species have led to speculations on relations between the shape and peak wavelength (Greenberg, Honig & Ebrey, 1975; Ebrey & Honig, 1977; Bowmaker *et al.* 1980; Mansfield, 1985). Figure 7 plots the normalized log sensitivity of the squirrel photoreceptors as a function of log wave number; the symbols for the cones are the same as those in Fig. 6. A portion of the rod spectrum is shown by the open triangles. The spectra of the rod and blue-sensitive cone have been shifted along the log wave number axis to minimize differences with the green-sensitive cone spectrum. The continuous curve is the sixth-

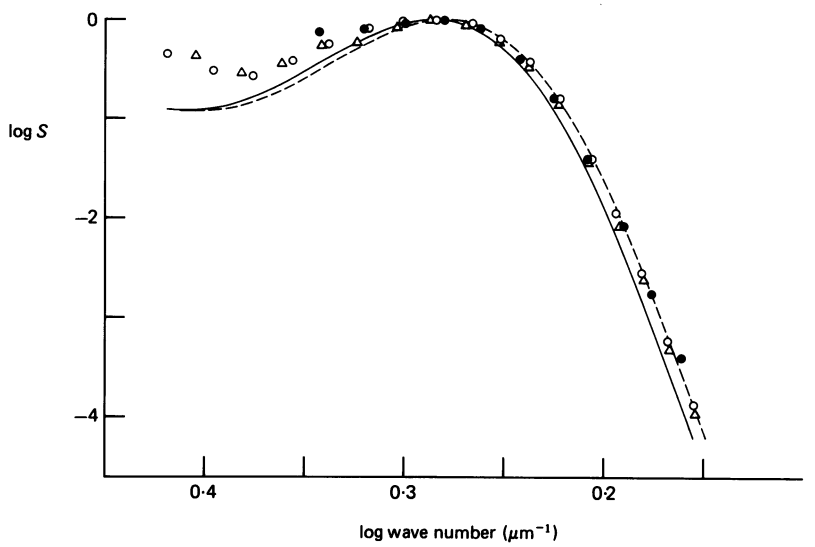

Fig. 7. Spectral sensitivity curves plotted on a log wave number axis. Data for the blue-sensitive cones (●) and rods (△) have been shifted laterally for a best fit, by eye, to the curve for the green-sensitive cones (○). Continuous curve is the sixth-order polynomial from Baylor *et al.* (1987); the maximum has been shifted to coincide with that of the squirrel cones. The dashed curve is the same polynomial shifted to fit the descending limb of the spectral sensitivity curve. *S*, relative sensitivity.

order polynomial fitted by Baylor *et al.* (1987) to macaque cone spectra, shifted laterally to match the peak sensitivities for the squirrel cones. The shape of the squirrel photoreceptor spectra are similar to one another but do not fit the general shape of the monkey cone spectra. The dashed curve is the Baylor *et al.* polynomial shifted for a best fit by eye to the long-wavelength end of the squirrel spectra. The descending limb of both monkey and squirrel spectra coincide nicely at the long-wavelength end, but at the expense of a poor fit for the short-wavelength end of the spectra.

Spectral univariance

The waveform of the response for all photoreceptors did not depend on the wavelength of the stimulating light, as predicted by the univariance principle (Naka

& Rushton, 1966). As an additional test of univariance, wavelength sensitivity data from a green-sensitive cone were analysed at two times after the flash (25 and 130 ms). The two spectral sensitivity curves derived were indistinguishable.

DISCUSSION

Photocurrent kinetics and sensitivity

One of the most striking characteristics of the squirrel cone responses was their speed, most cells' responses had a corrected time to peak of about 20 ms. Salamander and turtle cone photocurrents (Schnapf & McBurney, 1980) reach peak in 100–200 ms at room temperature. Assuming a Q_{10} of 2·7 (Baylor, Mathews & Yau, 1983), these times would decrease to 23–46 ms on raising the temperature from 21 to 37 °C. Primate cone responses typically reach peak at 50–100 ms (Baylor *et al.* 1987; Schnapf *et al.* 1987).

Squirrel cone responses also lacked the undershoot characteristic of primate cone responses. The ionic mechanisms responsible for the undershoot are unclear. Altering the internal calcium or calcium buffering capacity have produced undershoots in rods (Lamb, Matthews & Torre, 1985; Sather, Rispoli & Detwiler, 1988), presumably due to calcium's ability to regulate guanylate cyclase activity (Pepe, Panfoli & Cugnoli, 1986; Koch & Stryer, 1988; Rispoli, Sather & Detwiler, 1988).

The counterpart to the increased speed of the squirrel cone photocurrent is a low sensitivity relative to other cones studied. The flash sensitivity in darkness in squirrel cones was five to ten times less than that of monkey and human cones (Baylor *et al.* 1987; Schnapf *et al.* 1987). The flash strength required for half-maximal response ($i_{\frac{1}{2}}$) in squirrel cones was correspondingly greater than that in primate cones.

Blue- and green-sensitive cones had indistinguishable kinetics and sensitivities. This finding is in agreement with similar data obtained from monkey cones (Schnapf, Nunn & Baylor, 1985), and supported by electroretinogram recordings from squirrel retinas which isolated the blue- and green-sensitive cone mechanisms (Crognale & Jacobs, 1988).

Single photon response

The portion of channels closed by a single photoisomerization in turtle cones has been estimated as 0·16% (Baylor *et al.* 1974) and 0·07% (Schnapf & McBurney, 1980). For a conservative estimate in the squirrel cone consider a 50 pA dark current and a single photon response of 10 fA. This figure represents a reduction of only 0·02% of the dark current. Assuming a uniform current density through an outer segment with about 200 discs (Long & Fisher, 1983), this current reduction represents the closure of channels over only about $\frac{1}{25}$ of a disc. Based on these geometrical considerations the spread of excitation for a single activated photopigment would cover only a 0·5 μm diameter circular area on one side of a disc.

Colour vision in squirrels

Behavioural observations on the golden-mantled ground squirrel indicated cone sensitivity peaks of 440 and 525 nm (Jacobs, 1978). Ganglion cell recordings from intact eyes of Mexican and thirteen-lined ground squirrels (Michael, 1968; Gur &

Purple, 1978) gave estimated spectral peaks at 460 and 525 nm, very near those obtained here after correction for lens absorption (463 and 528 nm). Raisanen & Dawis (1983) reported a peak of 516 nm for the green-sensitive cone from measurements of the mass receptor potential (P_{III}). Jacobs *et al.* (1985) confirmed that peak, and estimated the blue-sensitive cone peak at 437 nm. These recent studies of cone spectral sensitivity correspond well with the action spectra reported here.

The proportion of cones identified as blue sensitive in this study, seven of thirty-eight (18%), was somewhat higher than previous estimates of 7–10% in ground squirrels (Long & Fisher, 1983; Ahnelt, 1985). The sample size is relatively small, but to date thirteen blue-sensitive cones have been identified in a population of ninety-four cells from thirteen animals.

Spectral form

The shapes of the two squirrel cone spectra are similar to one another when plotted on a log wave number axis, in agreement with the finding that pigment bandwidths are inversely related to their peak wavelength (Ebrey & Honig, 1977; Mansfield, 1985). The shapes of the squirrel spectra do not, however, conform to the idea of an invariant spectral form for all vertebrate pigments (Mansfield, 1985; MacNichol, 1986). The Baylor *et al.* (1987) template provides a very good fit to squirrel spectra at long wavelengths (dashed curve in Fig. 7) but falls below the measured sensitivities at wavelengths shorter than the peak. A possible explanation for the discrepancy at short wavelengths could be an unusually prominent cis-band in squirrel pigments. In any case these results suggest that the form of the spectral sensitivity curve is not general across all mammalian species.

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