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SPECTRAL SENSITIVITY OF CONES OF THE MONKEY *MACACA FASCICULARIS*

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SUMMARY

1. Spectral sensitivities of cones in the retina of cynomolgus monkeys were determined by recording photocurrents from single outer segments with a suction electrode.

2. The amplitude and shape of the response to a flash depended upon the number of photons absorbed but not the wave-length, so that the 'Principle of Univariance' was obeyed.

3. Spectra were obtained from five 'blue', twenty 'green', and sixteen 'red' cones. The wave-lengths of maximum sensitivity were approximately 430, 531 and 561 nm, respectively.

4. The spectra of the three types of cones had similar shapes when plotted on a log wave number scale, and were fitted by an empirical expression.

5. There was no evidence for the existence of subclasses of cones with different spectral sensitivities. Within a class, the positions of the individual spectra on the wave-length axis showed a standard deviation of less than 1·5 nm.

6. Psychophysical results on human colour matching (Stiles & Burch, 1955; Stiles & Burch, 1959) were well predicted from the spectral sensitivities of the monkey cones. After correction for pre-retinal absorption and pigment self-screening, the spectra of the red and green cones matched the respective π_5 and π_4 mechanisms of Stiles (1953, 1959).

INTRODUCTION

Human colour vision depends on the spectral sensitivities of three kinds of cone (Young, 1802; Maxwell, 1855). Psychophysicists have attempted to deduce these functions from the spectral sensitivity of colour-blind observers (Smith & Pokorny, 1972), as well as colour matching (Estevez, 1979) and chromatic adaptation (Stiles, 1953, 1959) experiments. Retinal densitometry on the intact eye has given estimates of the absorption spectra of the red and green cones (Rushton, 1963; Rushton, 1965). More recently, absorption in single cones has been measured by microspectrophotometry. Such experiments have been made on cones of humans (Dartnall, Bow-

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maker & Mollon, 1983) and cynomolgus monkeys (Bowmaker, Dartnall & Mollon, 1980), which are thought to have cones similar to those of man (DeValois, Morgan, Polson, Mead & Hull, 1974). Microspectrophotometric measurements are, however, limited to the spectral regions of strong absorption. By recording the photocurrent of single cones, we have determined the spectral sensitivities over a wider range of wave-lengths. Preliminary accounts of some of the work have been presented previously (Nunn, Schnapf & Baylor, 1984, 1985; Schnapf & Baylor, 1987; Baylor, 1987). A description of the response properties and electrical noise of the primate cones will be given in subsequent papers.

METHODS

Preparation

Photocurrents were recorded from single cones of the monkey, *Macaca fascicularis*, after drawing the outer segment into a suction electrode, using methods that are described in detail in Baylor, Nunn & Schnapf (1984). Briefly, enucleation was performed on an anaesthetized, dark-adapted male monkey. Anaesthesia was usually induced by ketamine (10 mg/kg, I.M.) and Nembutal (10–35 mg/kg, I.V.). In a few experiments inhalation anaesthesia (O_2–halothane, 1–3%, and N_2O, 40%) was used after intubating the trachea under ketamine (10 mg/kg, I.M.) and Surital (10 mg/kg, I.V.) sedation. Both procedures gave deep stage III surgical anaesthesia, evidenced by absence of reflexive movements and slow deep respirations at a regular rate. Under dim red light the posterior pole of the eye was removed. The retina was isolated under infra-red illumination and stored at 5 °C in L-15 tissue culture medium (Gibco) with 0·01% gentamicin. For making foveal preparations, the fovea was located prior to isolation of the retina by illuminating the interior of the posterior pole of the eye with 480 nm light (Bowmaker *et al.* 1980) of intensity about 10^7 photons $\mu m^{-2} s^{-1}$ for a few seconds; a 2 × 2 mm piece of retina containing this region was excised. A razor blade was used to chop sections of isolated retina into small pieces, from which electrical recordings were made.

Retinal pieces were placed in a recording chamber which was superfused with a bicarbonate-buffered Locke solution (Baylor *et al.* 1984). This solution was bubbled with 95% O_2–5% CO_2 and warmed to 37 °C. A calibrated thermistor was used to measure the temperature in the recording chamber.

Recording and light stimuli

For recording membrane current, a single cone outer segment was drawn into a silanized glass micropipette; usually the cell was pulled in until the distal portion of the inner segment entered the electrode tip. The inside diameter of the pipette was made to fit the inner segment and varied between 2 and 6 μm. The smallest electrodes were used for recordings from foveal cones. Signals were recorded on an FM tape-recorder (Ampex 2200) and later digitized and analysed with a laboratory computer (PDP 11/34 or 11/73).

The light was unpolarized and incident on the outer segment perpendicular to its long axis. Wave-length 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). Calibration of the filters and source was performed as described in Baylor *et al.* (1984).

It seems unlikely that the spectral sensitivity curves were significantly distorted by the presence of the suction electrode in the light path. The borosilicate glass wall absorbs negligibly at the wave-lengths studied. Because the refractive index of the glass is almost constant over the measured spectral band (Wyszecki & Stiles, 1982), the degree of light concentration by the suction electrode should have little wave-length dependence.

Identification of cells

On the basis of their spectral sensitivities, cones fell into three classes denoted 'red', 'green' and 'blue', the names indicating that their respective peak sensitivities lie in the long-, middle-, and short-wave-length portions of the visible spectrum. When an outer segment was first drawn into the electrode, its type was determined from the relative sensitivity to flashes of 440, 500 and

659 nm. If the cell was more sensitive at 440 nm than 500 nm, it was identified as a blue cone. The ratio of sensitivities at 659 and 500 nm was about 0·1 for the red cones and 0·007 for the green cones. Rods had much slower response kinetics and greater quantal sensitivity; their ratio of sensitivities at 659 and 500 nm was 0·0002. By this method, a total of 300 red, 306 green and 7 blue cones were identified in foveal and peripheral preparations from eleven monkeys. In foveal preparations from five monkeys 192 red, 211 green and 7 blue cones were identified.

Determination of spectral sensitivity

Spectral sensitivity was determined relative to the sensitivity at a reference wave-length (500 nm) by the method of criterion response described in Baylor *et al.* (1984). The sensitivity at the reference wave-length was measured repeatedly throughout the experiment in order to avoid errors arising from slow changes in the quantal sensitivity of the cell.

The fraction of pigment molecules bleached during an entire spectral sensitivity determination was estimated from the total applied photon density and the effective photon capture cross-section of a pigment molecule. In deriving the cross-section, it was assumed that the molecular extinction coefficient of cone pigment is like that of rhodopsin, $1{\cdot}5 \times 10^{-16}$ cm^2 at the optimum wave-length (Dartnall, 1972). Assuming that the efficiency of isomerization is 0·67, the bleaching cross-section, a_c, is 1×10^{-16} cm^2. The total applied photon density during an experiment, i_t, expressed as the density at the optimum wave-length, was about 3×10^{14} cm^{-2}. The fraction bleached, f, obtained from the relation

$$f = 1 - e^{-i_t a_c}, \tag{1}$$

was about 0·03.

For an outer segment 2–4 μm in diameter with a specific pigment density of 0·016 μm^{-1}, there is negligible self-screening for the transverse illumination used in the experiments. Assuming that the quantum efficiency of excitation is not wave-length dependent over the range of wave-lengths used, the measured spectral sensitivity should be proportional to the molecular extinction coefficient, the probability that a photon will be absorbed as a function of its wave-length.

The average normalized spectra were plotted on double log coordinates (sensitivity *vs.* wave number) in order to obtain the coefficients of the polynomial expression of eqn. (6). The green and blue curves were shifted horizontally and vertically until the differences between the three curves were minimized by eye. These resulting points were then used to calculate the coefficients of the best-fitting sixth-order polynomial by least-squares criterion, using singular value decomposition (Press, Flannery, Teukolsky & Vetterling, 1986). The wave-length maximum, λ_m, of the red curve was obtained directly from the peak of the polynomial; the maxima of the green and blue curves were obtained similarly, allowing for the shifting on the ordinate.

Prediction of psychophysical colour matching

The average spectral sensitivity curves were used to predict results of the human colour matching experiments of Stiles & Burch (1955, 1959), tabulated in Wyszecki & Stiles (1982) and converted from an energy to a quantum basis. Stiles and Burch determined the light intensity at each of three primary wave-lengths λ_x, λ_y and λ_z which, when added together, matched a test wave-length λ_t of unit intensity.

From the spectral sensitivity of the monkey cones we calculated the primary intensities, i_x, i_y and i_z, which would in combination stimulate all three kinds of cone exactly as strongly as would λ_t of unit intensity. The calculations determined the simultaneous solution to the equations:

$$R(\lambda_t) = i_x R(\lambda_x) + i_y R(\lambda_y) + i_z R(\lambda_z), \tag{2a}$$

$$G(\lambda_t) = i_x G(\lambda_x) + i_y G(\lambda_y) + i_z G(\lambda_z), \tag{2b}$$

$$B(\lambda_t) = i_x B(\lambda_x) + i_y B(\lambda_y) + i_z B(\lambda_z), \tag{2c}$$

where $R(\lambda)$, $G(\lambda)$ and $B(\lambda)$ are the respective modified sensitivities of the red, green and blue monkey cones to light at wave-length λ. The modified cone sensitivities were obtained from the measured values in Table 1, allowing for light absorption by the human lens and macular pigment and photopigment self-screening. For example, $R(\lambda)$ was obtained from the measured sensitivity of the red cones, $S_R(\lambda)$, by:

$$R(\lambda) = A(\lambda)(1 - 10^{-a S_R(\lambda)}), \tag{3}$$

where

$$A(\lambda) = 10^{-[bL(\lambda) + cM(\lambda)]}. \tag{4}$$

Here a is the optical density of a red cone to axial illumination at the wave-length of maximum absorption, $L(\lambda)$ and $M(\lambda)$ give the forms of the densities of the lens and macular pigment as a function of wave-length (Wyszecki & Stiles, 1982), and b and c are scaling constants. An iterative computer program determined the values of a, b and c which minimized the squared differences summed over all wave-lengths between the colour matching functions of Stiles and Burch (expressed on a quantum basis) and the corresponding i_x, i_y and i_z values. For simplicity it was assumed that the constants were identical for the three classes of cones. Since different wave-lengths were used in the two sets of experiments, the cone sensitivities were estimated from the measured results by linear interpolation.

RESULTS

Spectral univariance

A cone's response to a flash of light obeyed the 'Principle of Univariance' (Naka & Rushton, 1966), depending upon the number of photons absorbed but not the wave-length. Univariance was tested by applying flashes at three wave-lengths and several intensities in interleaved trials. The results of such an experiment on a red cone at wave-lengths of 500 and 659 nm are shown in Fig. 1. The two response families were virtually identical apart from a single scaling of the strength of the test flashes required to elicit the responses. This is illustrated by Fig. 2*A*, in which the amplitude of the response is plotted as a function of the flash strength. The smooth curves are drawn according to the exponential saturation function

$$r = r_m(1-e^{-ki}), \tag{5}$$

where r is the peak amplitude of the response, r_m the maximal amplitude, i the flash photon density, and k the proportionality constant that determines the position of the curve along the log i axis (Lamb, McNaughton & Yau, 1981; Baylor *et al.* 1984). The value for k was $2{\cdot}77 \times 10^{-4}$ photons^{-1} μm^2 at 500 nm and $3{\cdot}01 \times 10^{-5}$ photons^{-1} μm^2 at 659 nm. The ratio of these constants gives the relative sensitivity as about 9 times higher at 500 nm than at 659 nm. When the flash strength was adjusted to equalize the photon absorption at the two wave-lengths, the entire responses superimposed, as shown in Fig. 2*B*. Responses to 400 nm light (not illustrated) were also virtually identical after the absorption was matched. Similar results were obtained from the four other red cones and three green cones tested in this way. There was no evidence of a departure from univariance in any of the forty-one cones used to determine spectral sensitivity.

Spectral sensitivities of the three classes of cone

Fairly complete spectral sensitivity curves were determined from five blue, twenty green, and sixteen red cones from nine monkeys. All results were used for analysis without any pre-selection of cells. The method used to obtain spectral sensitivity is illustrated in Fig. 2*A*. The relation between the peak amplitude of the response to a flash and the flash strength was determined for a test wave-length and reference wave-length (500 nm). The difference in the positions of the two curves on the log abscissa gave the sensitivity at the test wave-length relative to that at 500 nm. Similar determinations were made with test wave-lengths in the range 381–830 nm. Final spectra were shifted vertically so that the mean sensitivities (sum of the log sensitivities across wave-length) were the same for all curves of a given type. After

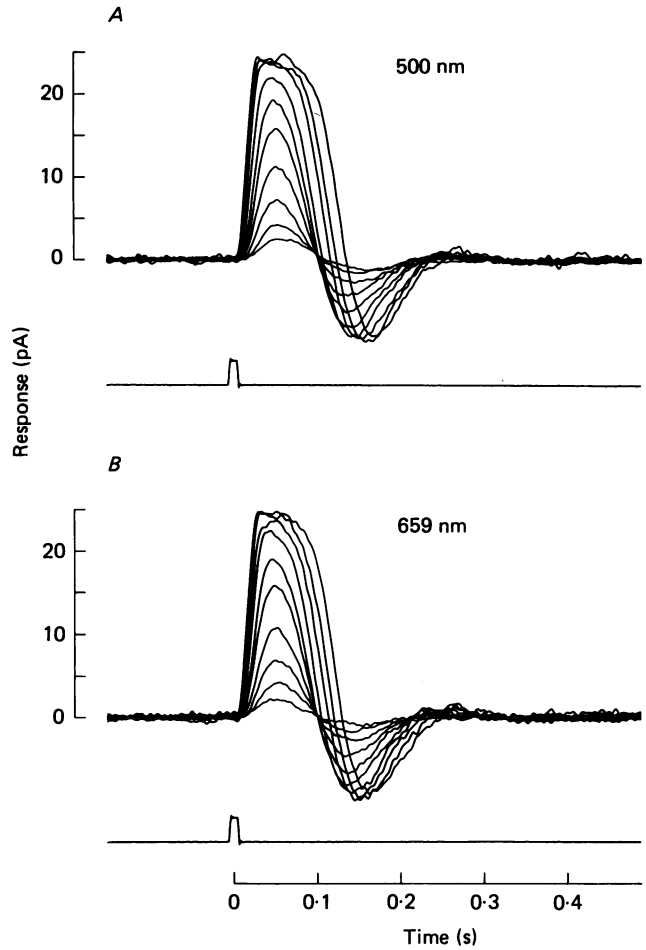

Fig. 1. Families of superimposed responses from a red cone to flashes of increasing strength; wave-length 500 nm (*A*) or 659 nm (*B*). Change in membrane current from the dark level plotted as a function of time after the flash. Each trace is an average of three to thirty-three responses. Flash strengths were increased by factors of approximately two. Flash monitor shown below current traces. Band width 0–100 Hz, temperature 37 °C.

normalization the average sensitivities and standard deviations were calculated at each wave-length.

Averaged normalized spectra for the red, green and blue cones are tabulated in Table 1 and plotted by the symbols in Fig. 3*A*. The smooth curves, based on the polynomial expression of eqn. (6), have maxima at 561, 531 and 430 nm, respectively (Table 2). The shapes of the spectra are different, the blue curve being broadest and the red narrowest. The band widths at half-height, estimated from the smooth curves, were 0·33, 0·35 and 0·43 μm^{-1} (red, green and blue) (Table 2). The width at half-height of the blue curve was determined by extrapolation at short wave-length.

The descending slopes at low wave number were determined by fitting a linear regression line to the individual points from each cell at values of $\log_{10} S$ of less than

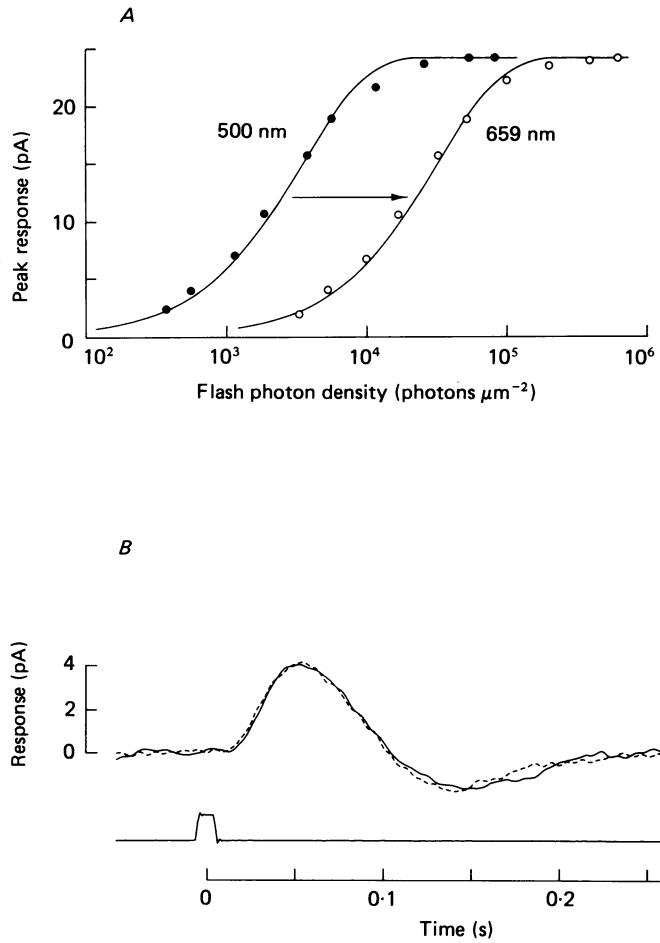

Fig. 2. *A*, relation between peak response amplitude and flash photon density for the families illustrated in Fig. 1. ●, 500 nm; ○, 659 nm. Smooth curves drawn according to eqn. (5) with $k = 2{\cdot}77 \times 10^{-4}$ photons^{-1} μm^2 at 500 nm, and $3{\cdot}01 \times 10^{-5}$ photons^{-1} μm^2 at 659 nm. Light was unpolarized. *B*, responses at 500 nm (continuous curve) and 659 nm (dashed curve) after adjusting flash strength to equate photon capture. Flash strength in photons μm^{-2} (and number of responses comprising the average) was 574 (17), and 5360 (13) at 500 and 659 nm, respectively.

$-2{\cdot}5$. The slopes (and standard errors of the mean slopes) in $\log_{10}$ units μm for the red, green and blue cones were 17·2 (0·2), 15·9 (0·2) and 12·7 (0·3) (Table 2). The slope for the red cones is very similar to that for the sensitivity of human foveal vision (17·4 $\log_{10}$ units μm) determined by Goodeve (1936) and Griffin, Hubbard & Wald (1947).

Spectral form

Mansfield (1985) found that the absorption spectra of macaque photoreceptors assume a common shape when plotted on a normalized wave number axis (wave number divided by wave number of maximum sensitivity). We have found that the

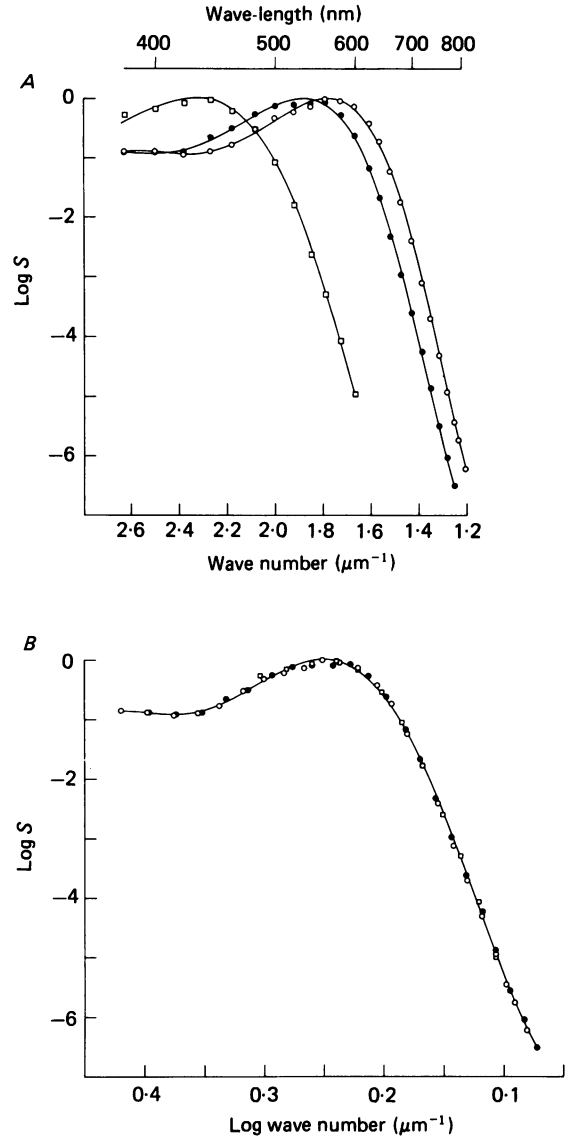

Fig. 3. Average normalized spectral sensitivities of five blue (□), twenty green (●) and sixteen red (○) cones from nine monkeys. Smooth curves are sixth-order polynomials (eqn. (6)) with $\lambda_m = 561$ nm (red), 531 nm (green) and 430 nm (blue). The coefficients ($a_0 - a_6$) are −5·2734, −87·403, 1228·4, −3346·3, −5070·3, 30881 and −31607. *A*, log sensitivity is plotted as a function of wave number; wave-length scale above. Results tabulated in Table 1. *B*, log sensitivity is plotted as a function of log wave number. The blue and green cone spectra have been shifted on the abscissa (see text).

cone spectral sensitivities behave similarly, as shown by the average spectra plotted in Fig. 3*B* on double logarithmic coordinates. The green and blue spectra have been shifted along both axes so that the points were brought into coincidence as judged by eye.

TABLE 1. Spectral sensitivities

	Red			Green			Blue		
λ (nm)	log *S*	S.D.	*n*	log *S*	S.D.	*n*	log *S*	S.D.	*n*
381	−0·873	0·066	14	−0·818	0·076	19	−0·240	0·017	4
400	−0·890	0·074	14	−0·845	0·048	19	−0·137	0·023	5
420	−0·951	0·042	14	−0·826	0·057	19	−0·039	0·025	5
440	−0·898	0·060	15	−0·596	0·043	19	0·000	0·049	5
459	−0·780	0·043	14	−0·439	0·041	19	−0·172	0·037	5
480	−0·512	0·040	16	−0·192	0·048	19	−0·508	0·034	4
500	−0·326	0·026	16	−0·053	0·024	20	−1·032	0·018	5
520	−0·221	0·041	16	−0·037	0·054	19	−1·764	0·032	4
541	−0·137	0·053	16	−0·034	0·043	20	−2·576	0·054	4
559	0·000	0·045	16	0·000	0·047	20	−3·271	0·031	4
579	−0·039	0·051	16	−0·214	0·081	20	−4·040	0·046	3
600	−0·134	0·061	16	−0·565	0·049	20	−4·934	0·013	2
622	−0·424	0·041	16	−1·114	0·056	20			
640	−0·735	0·052	16	−1·613	0·072	20			
659	−1·238	0·041	16	−2·256	0·043	20			
679	−1·758	0·062	16	−2·910	0·047	20			
700	−2·409	0·074	16	−3·556	0·039	20			
722	−3·116	0·041	16	−4·203	0·117	19			
740	−3·713	0·092	15	−4·819	0·099	16			
760	−4·309	0·077	15	−5·440	0·103	6			
781	−4·945	0·089	9	−5·976	0·142	3			
800	−5·453	0·123	7	−6·447	—	1			
811	−5·755	—	1						
830	−6·234	—	1						

λ is wave-length; log *S*, the average value of $\log_{10}$ relative sensitivity; S.D., the standard deviation; and *n*, the number of cells comprising the average. Results plotted in Fig. 3.

The smooth curve near the points is a sixth-order polynomial, which can be written in the following form to take into account wave number normalization for the green and blue spectra:

$$\log S = \sum_{n=0}^{6} a_n \left[\log\left(\frac{1}{\lambda}\frac{\lambda_m}{\lambda_r}\right)\right]^n, \tag{6}$$

where λ_m is the wave-length of maximum sensitivity in nm, $\lambda_r = 561$ nm, and the wave number, $1/\lambda$, is in units of μm^{-1}. The values of the coefficients of the polynomial are given in the legend of Fig. 3. λ_m is 561, 531 and 430 nm for the red, green and blue cones. The polynomial has no theoretical significance but provides an empirical expression for interpolation.

It is difficult to accurately estimate the true wave-length maxima because of experimental error and the relative flatness of the tops of the spectra. The λ_m values obtained from the polynomial fitting procedure were derived by assuming a particular shape for the top. The true shape, however, may differ slightly, so that the

actual maxima may differ from the polynomial estimates by as much as 10 nm. Similar problems are associated with estimation of the spectral band width.

Variation in the positions of spectral sensitivity curves

Results from both microspectrophotometric (Bowmaker *et al.* 1980; Dartnall *et al.* 1983; MacNichol, Levine, Mansfield, Lipetz & Collins, 1983) and psychophysical

TABLE 2. Properties of cone spectra

Cone	λ_{max}(nm)	$W_{\frac{1}{2}}(\mu m^{-1})$	$m(\log_{10}$ units $\mu m)$	σ_λ(nm)
Red	561	0·33	17·2	1·0
Green	531	0·35	15·9	1·3
Blue	430	0·43	12·7	1·4

λ_{max} is the wave-length of maximum sensitivity; $W_{\frac{1}{2}}$, the band width at half-height; m, the final slope at low wave number; and σ_λ, the standard deviation of the position of the spectra on the wave number axis, converted to nanometres.

(Alpern, 1979) studies on humans and macaques have led to the idea that in individuals with normal colour vision, there may be substantial variability in the position on the wave-length axis of cone spectra within a given class. Dartnall *et al.* (1983) have reported differences of as much as 21 nm in the wave-length of peak absorption and suggest the existence of subpopulations of pigments. Dartnall *et al.* (1983) found such variability among cones from different individuals and among cones from a single eye.

To investigate the variability of cone spectra, straight lines were fitted to the descending limb of individual spectra, each normalized to the sensitivity at 500 nm, and the positions at which the lines intersected $\log S = 0$ were used. This proved to be a very sensitive index of the positions of spectra owing to the steepness of the curves at low wave number. The slope of the line used to analyse individual spectra for a given class of cone was taken as the value determined by linear regression on the average spectrum. A line with this slope was fitted to each cone spectrum by least-squares criterion. Three cells were omitted from the analysis because their spectra were incomplete at long wave-length. Analysis was performed using only those wave-lengths at which the sensitivities had been determined for all of the remaining cones within each class. The standard deviations of the spectral position in nanometres were 1·0, 1·3 and 1·4 for the red, green and blue cones, respectively (Table 2). These results were derived from fifteen red cones from six monkeys, nineteen green cones from seven monkeys, and four blue cones from three monkeys. The greatest difference in the spectral positions within a single class was 4·3 nm (green). It seems likely that the variability between spectra is due to experimental error, and thus there is no evidence for subpopulations of pigments in this sample.

Comparison with human psychophysics

The smooth curves in Fig. 4 *A* and *B* show human colour matching results obtained with 2 deg (Stiles & Burch, 1955) and 10 deg (Stiles & Burch, 1959) fields. The curves give the number of photons at each of the three primary wave-lengths (645, 526 and

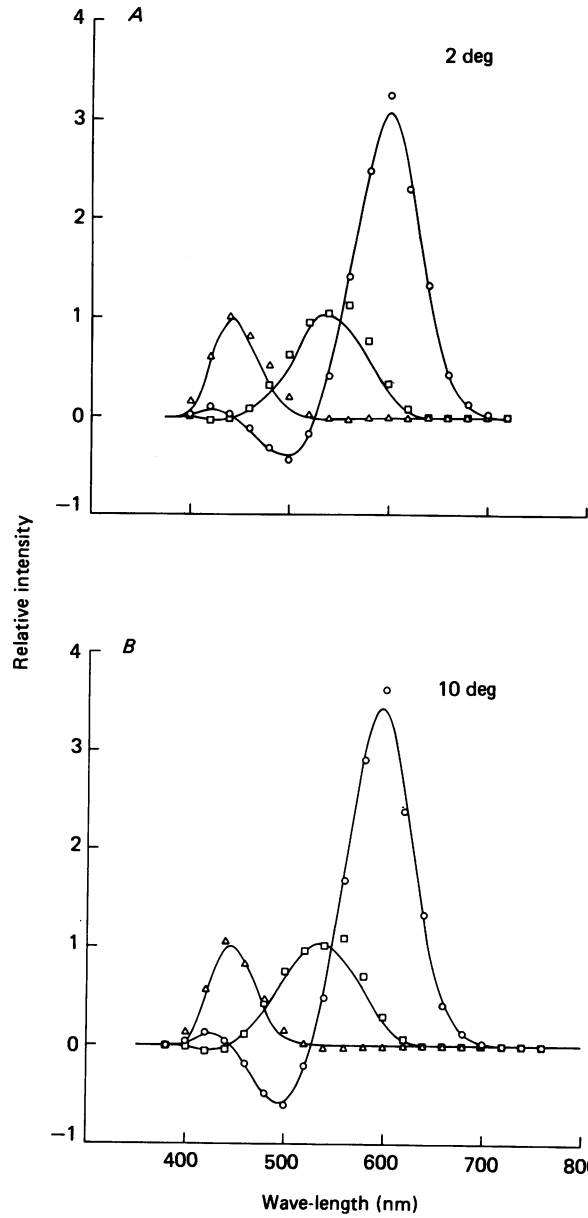

Fig. 4. Predicted and actual results from human colour matching experiments. Smooth curves plot the number of photons at each of the three primary wave-lengths required to match a single photon at the wave-length indicated on the abscissa; results obtained from human psychophysical experiments with 2 deg illumination of the retina (Stiles & Burch, 1955) in *A*, and 10 deg illumination (Stiles & Burch, 1959) in *B*. Tabulated matching function values in Wyszecki & Stiles (1982) were recalculated here for plotting on a quantum basis. Symbols plot the colour matching functions of the long (○), middle (□) and short (△) wave-length primaries produced from the spectral sensitivities in Fig. 3 after correction for screening by the lens, macular pigment and photopigment (see text).

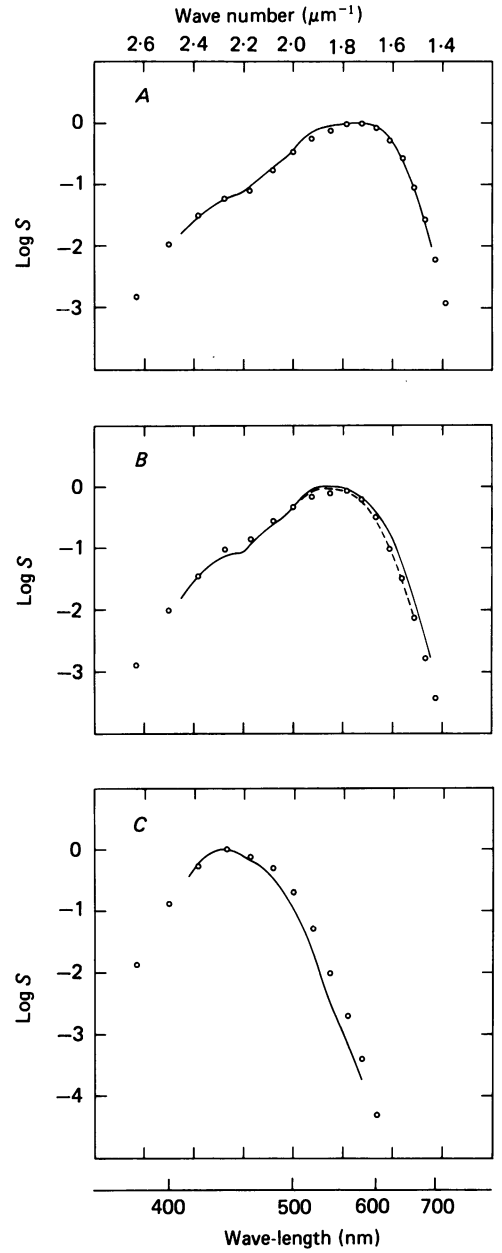

Fig. 5. Comparison of monkey cone spectral sensitivities and π mechanisms. Points give the spectral sensitivity of monkey cones modified by the pre-retinal absorption and self-screening expected in the human eye. Corrections for pigment self-screening and absorption by the lens and macula as in Fig. 4*A*. (see text). Smooth curves show the π mechanisms of Stiles (1953, 1959) obtained from tabulations in Wyszecki & Stiles (1982). *A*, red cones and π_5; *B*, green cones, π_4 (continuous curve) and π_4' (dashed curve); *C*, blue cones and π_3.

444 nm) required to match a single photon of the test wave-length plotted on the abscissa. The symbols give the results predicted from the monkey cone spectral sensitivities (Table 1) after adjustments for pre-retinal and pigment screening as described in the Methods. The values of a, b and c in eqns. (3) and (4) that minimized the differences between the points and the psychophysical curves were: 0·27, 0·59, 1·02 for the 2 deg field, and 0·17, 0·26, 0·94 for the 10 deg field. Corresponding density values were: peak axial pigment density 0·27 (2 deg field) or 0·17 (10 deg); macular pigment density at 460 nm, 0·29 (2 deg) or 0·13 (10 deg); lens density at 400 nm, 1·22 (2 deg) or 1·13 (10 deg). The differences in the values for the densities of macular pigment and visual pigment derived from the two sets of psychophysical experiments are roughly consistent with regional differences in the macular pigment density and the length of cone outer segments (Polyak, 1941).

The corrected monkey cone spectral sensitivities give an estimate of the spectral sensitivity of the cones to light incident at the cornea of the human eye. Those results are plotted by the points in Fig. 5, using the correction factors obtained from the fit to the 2 deg colour matching experiments. The smooth curves plot the foveal π mechanisms of Stiles (1953, 1959) obtained from tabulations in Wyszecki & Stiles (1982). The π mechanisms, derived from chromatic adaptation experiments in humans, are thought to reflect spectral mechanisms of the retina within a 1 deg foveal test spot. Our estimate of the red and green cone sensitivities to corneal illumination are close to the π_5 and π_4' mechanisms. None of the π mechanisms fitted the blue cone spectrum over its entirety.

DISCUSSION

Univariance applied to the electrical signals observed in these experiments, so that wave-length determined only the effective stimulus intensity. Univariance would not necessarily apply to intracellular voltage signals, which might be influenced by signals arising from other types of cone.

Comparison of spectral sensitivities to pigment absorption spectra

The spectral sensitivities presented here may be compared to cone absorption spectra whose tabular values have been published and used in interpreting colour vision. The absorption of chicken iodopsin (Wald, Brown & Smith, 1955) at wave-lengths longer than the peak resembles the red cone spectrum, but the iodopsin curve lies significantly above the red spectrum at wave-lengths below 480 nm. In analysing psychophysical experiments, Smith & Pokorny (1975) assumed that the human red and green cone spectra resemble the iodopsin spectrum slid along the wave number scale so that the peak absorption of iodopsin (λ_{max}, 565 nm) is moved to 555 nm for the red cones and 532 nm for the green cones. The iodopsin spectrum fits the monkey red cone spectrum considerably better when it is not shifted and fits the green best when shifted to a peak wave-length of about 538 nm.

The spectra of human cones measured by microspectrophotometry (Dartnall *et al.* 1983) show peaks at wave-lengths similar to those found here, but the absorption spectra are somewhat broader. A possible explanation for this difference is that the probability that an absorbed photon will elicit an isomerization is wave-length

dependent. Contrary to this notion, however, absorption and action spectra from rod outer segments of the tiger salamander agree well over the wave-length range 450–700 nm (Cornwall, MacNichol & Fein, 1984).

Another explanation, suggested by Mansfield, Levine, Lipetz, Collins, Raymond & MacNichol (1984), is that microspectrophotometric absorption spectra are broadened and shifted to shorter wave-lengths because of light scatter. These authors demonstrated an effect of this kind by measuring the absorption spectrum of the blue cones in the macaque monkey by two methods. The first method was similar to that of Dartnall *et al.* (1983); the second was a bleaching difference technique expected to produce artifacts in the opposite direction. The bleaching difference spectrum matched the blue curve in Fig. 3 at wave-lengths near the peak and longer. At shorter wave-lengths, however, the difference spectrum fell off much more rapidly.

It seems unlikely that the differences between the human microspectrophotometric spectra and the present results are due to true interspecies differences. Tabulations of the microspectrophotometric results from the macaque (Bowmaker *et al.* 1980) have not been published, but the spectra appear to have the same general features as the human spectra obtained by the same group (Dartnall *et al.* 1983). Furthermore, the spectral sensitivities of red and green cones from a human retina were found by electrical recording to be indistinguishable from those of the macaque (Schnapf, Kraft & Baylor, 1987).

Spectral variation

Nathans, Thomas & Hogness (1986) described a redundancy in the genes that code the green pigment and the extent of redundancy varied among human males whose Rayleigh colour matches were normal. Likewise, the large spectral variation obtained from microspectrophotometry on human and macaque cones has been interpreted to indicate subpopulations of pigments (Bowmaker *et al.* 1980; Dartnall *et al.* 1983; MacNichol *et al.* 1983). Our results show no evidence for such variability within a class of cones, and our failure to observe it does not appear to be due to limited sample size. The results from microspectrophotometry suggest approximately equal numbers of cones in each of the two subpopulations within a class in a single eye (Dartnall *et al.* 1983). Assuming the members of the subtypes to be roughly equally numerous, the probability that we would have missed seeing them is negligible. Perhaps the appearance of subpopulations may arise from technical problems in the microspectrophotometry, as suggested by Levine & MacNichol (1985).

A sensitive colour matching test given to a large number of human subjects with normal colour vision revealed small but significant differences in the perceived colour of wave-length mixtures (Neitz & Jacobs, 1986). The results suggested two types of red pigments with wave-length maxima differing by 3 nm. Our technique would probably be capable of revealing such differences if the sample size were large enough.

From intersubject variability in colour matching, MacLeod & Webster (1983) have calculated that the standard deviation in the positions of the cone spectra is less than 1·3 nm. This value is consistent with the present results. It is not yet clear how the uniformity of the spectra reported here relates to the multiple green genes seen by Nathans *et al.* (1986).

Cone spectra and psychophysics

The macaque cone sensitivities successfully predicted the colour matching data of Stiles & Burch (1955, 1959). It should be pointed out, however, that other estimates of the spectral sensitivities of human cones can also predict colour matching when the screening parameters are varied to optimize the fit. For example, the microspectrophotometric spectra of Dartnall *et al.* (1983) and the estimated sensitivities obtained by Estevez (1979) from the Stiles and Burch data, differ somewhat from ours and yet agreed well with the colour matching results when the values of the screening parameters were optimized as described in the Methods.

Form of spectral sensitivity curves

Greenberg, Honig & Ebrey (1975) noted that the band width of visual pigments decreases as the wave-length of maximum sensitivity increases. The present results confirm that the relation is inversely proportional (Bowmaker *et al.* 1980) and show that the entire spectra of the three cones are similar when plotted on a log wave number axis.

Mansfield (1985) reported that the spectra of macaque rods and cones were similar on a normalized wave number axis but found systematic differences in the shapes of the curves at short wave-length. While we were unable to measure the sensitivity of the blue cones at sufficiently short wave-length to determine if the entire *cis* peak (secondary peak at short wave-length) scales in the same way, we did not find systematic differences within the range of wave-lengths explored by Mansfield (1985). Furthermore, when the macaque rod spectrum (Baylor *et al.* 1984) was plotted on a log wave number scale it was slightly broader than the cone curves.

When measured from the polynomial of eqn. (6), the band width of the spectra at half-height ($W_{\frac{1}{2}}$) followed the relation

$$W_{\frac{1}{2}} = 0{\cdot}185/\lambda_{\mathrm{m}}. \tag{7}$$

The proportionality constant here is somewhat smaller than the value of 0·217 found by Mansfield (1985).

While eqn. (6) provides a reasonable approximation to the experimental spectra, the expression should not be used to extrapolate beyond the measured values. From psychophysical studies of human foveal vision, for example, the fall-off of log sensitivity at low wave number was found to be linear to at least 1 μm^{-1} (1000 nm) (Griffin *et al.* 1947). When plotted on a linear wave number scale, however, the polynomial expression for the red cones deviates strongly from linearity beyond 830 nm.

Honig, Greenberg, Dinur & Ebrey (1976) suggest that narrowing of the absorption band with increasing λ_{m} is a rather general phenomenon expected of several possible mechanisms by which the retinal chromophore's spectral absorption might be regulated. We do not know the physical significance of the simple scaling principle, nor why it breaks down for the rods.

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