J. Physiol. (1980), **298**, *pp.* 131–143
With 5 text-figures
Printed in Great Britain

MICROSPECTROPHOTOMETRIC DEMONSTRATION OF FOUR CLASSES OF PHOTORECEPTOR IN AN OLD WORLD PRIMATE, *MACACA FASCICULARIS*

BY J. K. BOWMAKER, H. J. A. DARTNALL AND J. D. MOLLON

From the Department of Zoology and Comparative Physiology, Queen Mary College, University of London, Mile End Road, London E1 4NS, the Centre for Research on Perception and Cognition, School of Biological Sciences, University of Sussex, Falmer, Brighton BN1 9QG and the Psychological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EB

(*Received 19 January 1979*)

SUMMARY

1. Microspectrophotometric measurements reveal four classes of photoreceptor in the retina of the cynomolgus monkey, *Macaca fascicularis*, which is known to possess colour vision similar to that of a normal human trichromat.

2. Although the eyes were removed in bright illumination, the densities of pigment were comparable to those we have measured in dark-adapted rhesus retinae.

3. The mean wave-lengths of peak sensitivity (λ_{max}) for the four classes of photoreceptor were 415, 500, 535 and 567 nm.

4. The band widths of the absorbance spectra decreased linearly as the wave-number of peak sensitivity decreased.

5. If, by assuming a reasonable value for the axial density of the rod outer segment and correcting for lens absorption, a spectral sensitivity for human vision is reconstructed from the P500 pigment, it is found to be systematically broader than the CIE scotopic sensitivity function.

6. Given explicit assumptions, it is possible from the P535 and P567 pigments to reconstruct human psychophysical sensitivities that resemble the π_4 and π_5 mechanisms of W. S. Stiles.

7. Although the P415 pigment has a λ_{max} much shorter than that of the psychophysically measured blue mechanisms, the two spectral-sensitivity functions are brought into proximity when the microspectrophotometric data are corrected for absorption by the optic media.

INTRODUCTION

Following a microspectrophotometric study of the photopigments of the rhesus monkey (Bowmaker, Lythgoe, Dartnall & Mollon, 1978) we had the opportunity of making measurements on a second macaque species, the cynomolgus or crab-eating macaque, *M. fascicularis*. We have been fortunate to record in this species the blue-sensitive cones that we were unable to find in the rhesus monkey, *M. mulatta*.

0022-3751/80/6080-0973 $07.50

The data for *M. fascicularis* have a second important interest: this species, and not *M. mulatta*, was used for psychophysical measurements by De Valois, Morgan, Polson, Mead & Hull (1974) in the most elaborate study so far made of primate colour vision. *M. fascicularis* was shown to resemble man in not having a spectral neutral point, in the positions of the maxima and minima of its wavelength-discrimination curve, in its Rayleigh match and in its saturation-discrimination function. Thus this species appears to provide a model for the colour vision of the normal human trichromat.

An additional interest of the present data derives from the conditions under which the eyes were obtained: since the animals were being sacrificed for other purposes, we had to enucleate the eyes under high illumination. It is traditional in microspectrophotometric work to enucleate under dim red light, but densitometric measurements on man (Rushton & Henry, 1968) suggest that there is little scientific ground for this practice: bleaching of the photopigments does not become significant until the retinal illuminance exceeds 10^3 td.

METHODS

Fresh eyes were obtained from a pharmaceutical company, where the animals were routinely killed in order to test for neuropathological conditions induced by vaccines. Male and female animals were used and they weighed from 2·5 to 3·5 kg. They had been imported from Malaysia (approximately 5 months before sacrifice) and were maintained for some months on a diet of BP Nutrition primate diet *ad libitum*, supplemented by fruit. Before sacrifice they were sedated with phencyclidine hydrochloride and anaesthetized with pentobarbitone sodium. Enucleation was performed under bright illumination provided by fluorescent lamps: the illumination measured at the working surface was $10^{2 \cdot 9}$ lm . m^{-2}. Eyes were wrapped in metal foil and placed immediately on ice, before being taken by road to Sussex University. Enucleation was done between 09.30 and 10.30 hr; measurements typically began at 13.00 hr.

Preparation of tissue. Eyes were maintained on ice in the dark until needed. Each eye was prepared under dim red light (Kodak safe-light No. 2). An equatorial section was made of the globe and the anterior half discarded. The vitreous was then carefully removed and the eye-cup placed in ice-cold mammalian Ringer solution pH 7·1. A small piece of retina (approximately 1 mm^2) was cut out, placed on a cover slip, and divided into smaller fragments with a few strokes of a razor blade. A drop of Ringer solution (to which 5 % Dextran had been added) was placed on the tissue and the preparation was then squashed under a second cover slip and sealed with paraffin wax. The first sample from each retina usually included the fovea, since cones are very sparsely distributed in samples of peripheral retina and valuable time is spent in searching them out.

A method for localization of the fovea. It is customary to prepare retinae for microspectrophotometry in dim red light, but a clear disadvantage is that the fovea is then extremely difficult to localize, since the macular pigment does not absorb long wave-lengths. In some of the present series of measurements, we therefore illuminated the eye-cup for a few seconds with light of 480 nm. This wavelength was chosen because it is near one of the two main absorption peaks of the (human) macular pigment (Wyszecki & Stiles, 1967, Fig. 2.11), the longer of the two peak wave-lengths being preferred since we were concerned not to bleach short wave-length cones. When the retina is illuminated with blue light in this way the foveal depression appears as a dark, readily visible spot. Retinae briefly exposed to blue light yielded densities of pigment similar to those recorded from retinae prepared exclusively in red light.

Microspectrophotometry. The microspectrophotometer is of dual-beam design and is similar in most respects to that previously described by Leibman & Entine (1964) and by Liebman (1972). (A description of the Sussex version of the instrument is given by Knowles & Dartnall, 1977.) At the start of each session, the measurement and reference beams were usually set at 2×1 μm, but could be adjusted to suit the dimensions of a particular cell. The procedure of chopping and squashing the retina disperses the tissue; thus an area containing no cells can usually be found

for the reference beam. All measurements were made transversely. To increase the proportion of light absorbed by the outer segments, the primary measurements were made with the e-vector of the measuring beam perpendicular to the long axis of the outer segment, since the dichroic chromophores of the pigment molecules lie with their long axes in the plane of the disk membranes. Cells were lined-up in the measuring beam using an infra-red image converter. With experience, the experimenter was able with some reliability to distinguish outer segments from other retinal structures, and rods from cones; but we emphasize that the judgements 'rod-like' and 'cone-like', made in advance under infra-red inspection, were not absolutely correlated with the type of absorbance spectrum subsequently recorded. When, elsewhere in this paper, we refer to 'rods' and 'cones' we are making no histological claim but are basing our classification solely on the absorbance spectrum recorded.

The absorbance spectra were obtained by scanning from 700 to 400 nm and back to 700 nm. This procedure allowed us to check that significant bleaching did not occur during the recording. In the case of blue-sensitive outer segments, the scan was extended to 375 nm. The total time for the double scan was about 20 sec. Deliberate bleaching of receptors was carried out using white light from the tungsten source.

Diameters of the outer segments from which recordings were made were estimated by reference, to the dimensions of the measuring beam; the latter were determined using an eye-piece containing a graticule that had been calibrated with a micrometer slide.

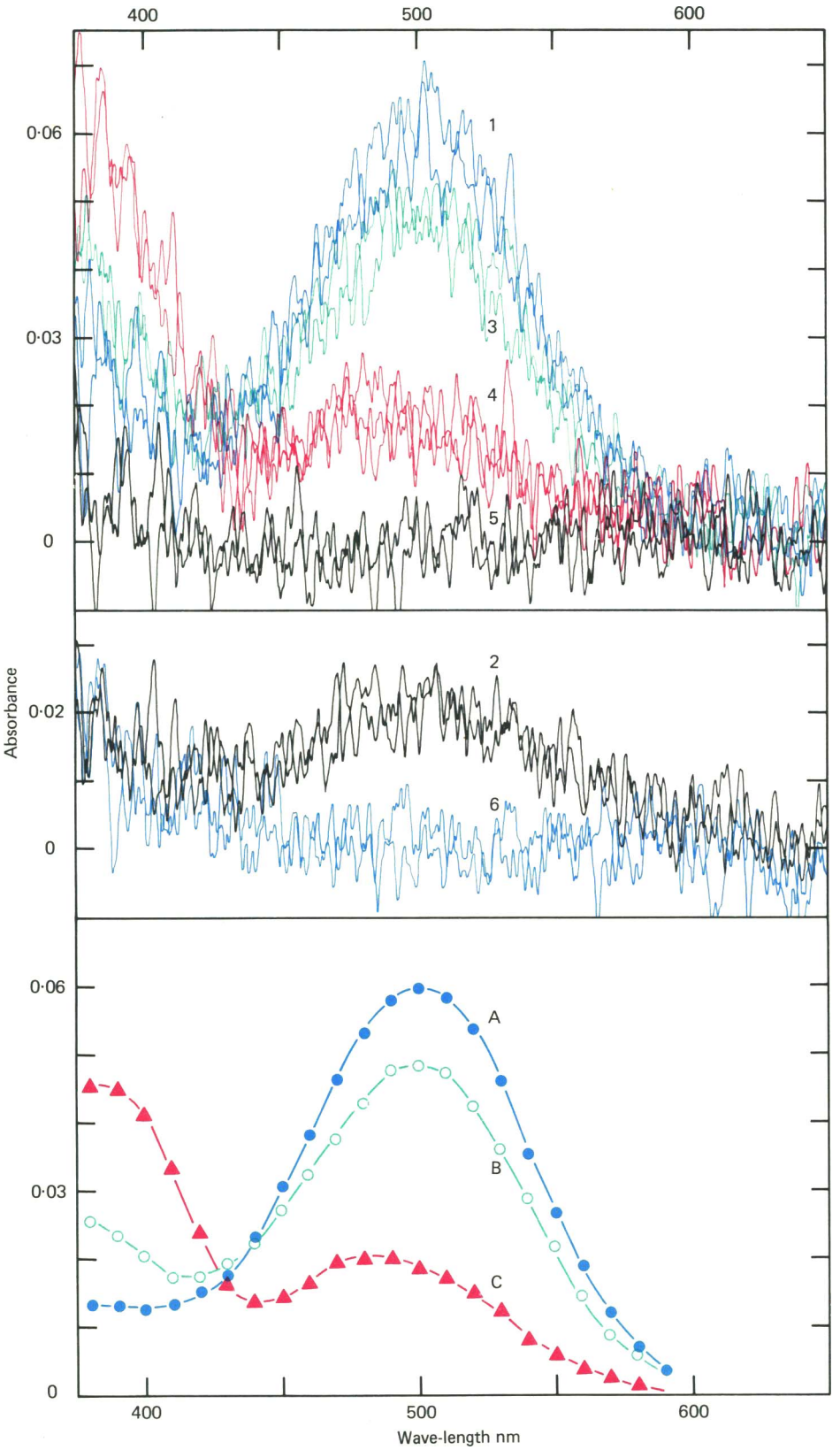
Wave-length calibration of the microspectrophotometer was performed with a mercury-cadmium lamp, as previously described (Bowmaker *et al.* 1975); weve-lengths given below are accurate to 1 nm.

Analysis of results. Records were selected for detailed analysis if they showed a maximal transverse absorbance greater than 0·015. For each record selected, a smooth curve was drawn by eye through the trace obtained from the cell and also through the baseline trace obtained by passing the measuring beam through clear space in the preparation (see Fig. 1). The difference between these two curves, sampled at 10 nm intervals, was used to compute a normalized point-curve. From this curve the λ_{max} was then estimated by the method developed by Dartnall, Lander & Munz (1961) and Bridges (1967): a suitable nomogram was assumed for the pigment (see below) and at each 10 nm interval the per cent absorbance obtained empirically was used to define the position of the nomogram on a wave number abscissa. The mean estimate of the λ_{max} for a given cell is typically based on ten such estimates. It is now clear that the absorbance spectra of visual pigments are not constant in band width when plotted against wavenumber but become narrower as the λ_{max} increases (e.g. Liebman & Entine, 1968; Harosi, 1975; Bowmaker, 1977*a*, *b*; and see below. For reviews see Ebrey & Honig, 1977; Knowles & Dartnall, 1977). Therefore, in analysing the red-sensitive cones of the present sample we used our own mean absorbance spectrum obtained for the red cones of the rhesus monkey (Bowmaker *et al.* 1978). This spectrum is very close to that of Wald's iodopsin (Wald, Brown & Smith, 1955). To estimate the λ_{max} for the green cones and the rods, we used the nomogram of Dartnall (Wyszecki & Stiles, 1967, Table 7.9). In the case of the blue cones (see below), estimates were obtained by means of the frog 'green-rod' nomogram of Bowmaker (Knowles & Dartnall, 1977, p. 76).

In the Results mean values for λ_{max} and bandwidth are shown as ± 1 S.D.

Deterioration of retinae. The rapid deterioration of fresh retinae is seldom mentioned explicitly in reports of microspectrophotometry. Our own measurements were sometimes continued on the morning following the day of enucleation, but retinae often had to be discarded because cells were disintegrating. As time passes, the outer segments of cones lose their cylindrical form and become spherical; photolabile pigment can still be measured but no dichroism survives. Even in the freshest retinae, complete photoreceptors are seldom found: the outer segments have usually broken away from the inner segments.

We have found that retinae deteriorate at very different rates: we do not yet understand why. However, we do have some statistical evidence that the measured λ_{max} is not correlated with the recorded appearance of the outer segment or with the time since enucleation; systematic data for the rhesus retina will be published elsewhere. We emphasize that the retinae used here were probably fresher than those used in any previous microspectrophotometric study of a primate retina.


400
500
600
0·06
0·03
0
1
3
4
5
2
6
Absorbance
0·02
0
0·06
0·03
0
A
B
C
400
500
600
Wave-length nm

RESULTS

Rods

Absorbance spectra were analysed for a total of twelve rod outer segments from six different eyes. Records from a large rod with a diameter of about 3 μm are shown in Fig. 1. The upper panel shows the raw traces obtained with light polarized perpendicular to the long axis of the outer segment; the middle panel shows traces obtained with light polarized parallel to the long axis; and the lower panel shows the smoothed spectra derived from the perpendicular records according to the procedure described in the Methods.

Trace 1, which was recorded first, is an absorbance spectrum obtained with perpendicularly polarized light. The absorbance was then measured with parallel polarized light (trace 2) and thirdly a further measurement was made with perpendicularly polarized light (trace 3). The decrease of about 0·01 in absorbance at 500 nm between traces 1 and 3 is due to bleaching during the sequence of measurements; the increase in absorbance below 430 nm in trace 3 reflects the presence of photoproducts of the bleached rhodopsin.

The outer segment was next deliberately bleached for 15 sec with white light and trace 4 was then recorded. Trace 5 is the instrumental base line. The bleaching caused a decrease in absorbance at 500 nm of about 0·025; there was a corresponding increase in absorbance below 430 nm, suggestive of a peak at about 380–390 nm. The λ_{max} of the α-peak was displaced from 500 nm to about 480–490 nm owing, presumably, to the formation of metarhodopsin III.

The difference between the 'perpendicular' (1 and 3) and 'parallel' (2) traces provides clear evidence of dichroism in this primate rod. If the mean of traces 1 and 3 is taken as the absorbance for perpendicularly polarized light, then the dichroic ratio of the outer segment (i.e. the ratio of the absorbance for perpendicular polarization to that for parallel polarization) was 3·22, a value comparable to the dichroic ratio obtained for other vertebrate rods (e.g. Harosi, 1975).

The individual values of the λ_{max} for the twelve rods ranged from 497 to 502 nm with a mean of 500·1 $\pm$ 1·6 nm. The distribution of the individual values is shown in Fig. 2*C*. The mean absorbance curve is shown in Fig. 2*A* and is slightly broader than the Dartnall nomogram: its band width at 50 % absorbance is 4290 cm^{-1} compared with 4155 cm^{-1} for the nomogram. The transverse absorbance of the rods ranged from 0·016 in outer segments with a diameter of about 1 μm, up to 0·05–0·06 in large rods (as in Fig. 1). In the case of rods exposed to blue light during location of the fovea, transverse absorbances were found to be within the normal range.

Fig. 1. Absorbance spectra of a rod outer segment from *M. fascicularis*. *Upper panel:* original traces obtained with perpendicularly polarized light. Trace 1 is the initial absorbance spectrum; trace 3 was obtained after the parallel measurements and trace 4 after deliberate bleaching for 15 sec. Trace 5 is the instrumental base line. *Middle panel:* original traces obtained for the same receptor with light polarized parallel to the long axis of the outer segment. Trace 2 is the absorbance spectrum obtained after the initial perpendicular measurement. Trace 6 is the base line obtained with parallel light. *Lower panel:* curves *A*, *B*, and *C* correspond to the subtraction of the base line (trace 5) from traces 1, 3 and 4 respectively. The presence of photoproducts is clearly evident at short wavelengths in traces *B* and *C*.

Cones

The remaining outer segments fall into three major groups according to the values of their λ_{max} (Fig. 2*C*). It is on this basis alone that we classify them. We follow the convention of referring to 'red' 'green' and 'blue' 'cones' when we mean structures that absorb maximally in the yellow-green, green and violet regions of the spectrum.

Red cones. Fourteen of the outer segments analysed had values of λ_{max} in the range 554–575 nm (Fig. 2*C*), with a mean of $567{\cdot}0 \pm 6{\cdot}1$ nm. The mean absorbance spectrum of these 'red cones' is shown in Fig. 2*A* and has a band width at 50 % absorbance of 3625 cm^{-1}. It might be thought that the averaging of individual records ranging over 21 nm in their λ_{max} would lead to a gross broadening of the band width. However, effects of this kind are smaller than might be supposed (see Knowles & Dartnall, 1977, p. 84). We have independently calculated the mean of the band widths from the individual records for the red cones and obtain a value of 3634 ± 264 cm^{-1}.

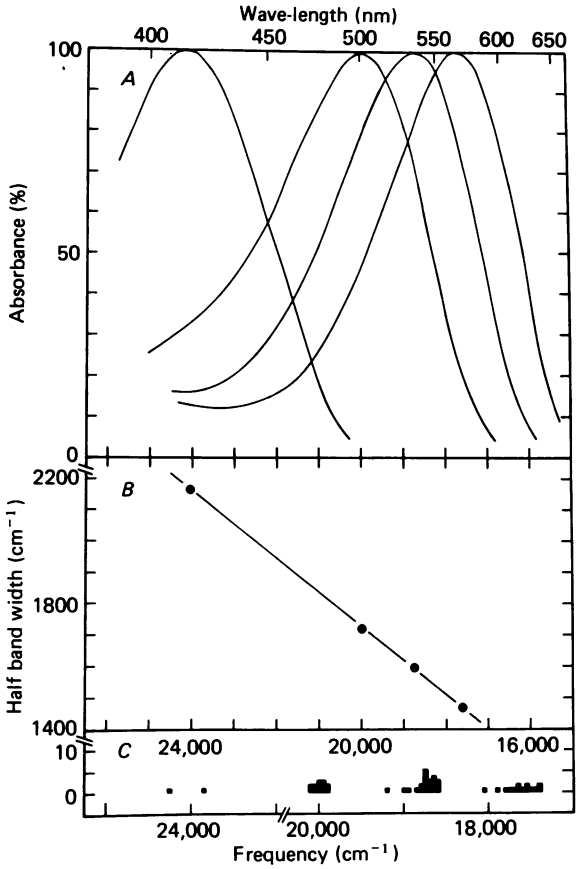


Fig. 2. *A*, mean absorbance spectra for four classes of receptor cell. *B*, half band-widths of the mean absorbance spectra (measured on the long wave-length limb) as a function of wave number of maximum absorbance. *C*, distribution of values of wave-number of peak sensitivity for individual receptors. Note that the abscissa of this panel is on a scale twice that of panels *A* and *B*.

The 21 nm range of values of λ_{max} for red cones (Fig. 2*C*) is considerably greater than might be expected from experimental error: on a number of occasions three separate measurements were made on a single receptor with light polarized in two directions (cf. Fig. 1) and the three estimates of λ_{max} always fell within a range of 4 nm. The variability within the class of red cones is not wholly attributable to differences between animals, since we have found a range of values of λ_{max} within a retina.

Green cones. Twenty-two outer segments yielded values of λ_{max} in the range 521–538 nm (Fig. 2*C*) with a mean λ_{max} of 533·3 ± 3·9 nm. The mean transverse absorbance was 0·024 ± 0·006. The mean absorbance spectrum for the sixteen best records is shown in Fig. 2*A*: the λ_{max} lies at 535 nm and the band width is 3985 cm^{-1}. The mean of the individual band widths (see above) was 3971 ± 262 cm^{-1}. Like that for the red cones, the range of the values of λ_{max} for the green cones is greater than would be expected from experimental error and probably indicates a real variability in the pigment absorbance of different receptors.

Blue cones. Absorbance spectra with values of λ_{max} between 410 and 420 nm were recorded from two cells from different animals. One cell had a λ_{max} of 420 nm and a transverse absorbance of 0·015. The other cell had a λ_{max} of 412 nm and a transverse absorbance of 0·021. The mean of the two absorbance spectra is shown in Fig. 2*A* and has a λ_{max} of 415 nm, with a half band width at 50 % absorbance on the longwave limb of 2166 cm^{-1}. The individual normalized spectra are shown separately in Fig. 3. Both of these violet-sensitive structures were found to be bleached by a 30 sec

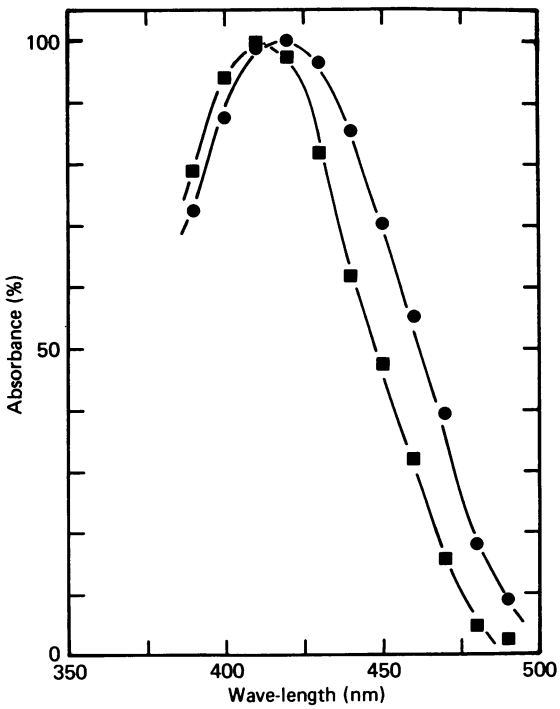


Fig. 3. Normalized absorbance spectra for the two blue cones.

exposure to white light; the pigment is thus explicitly shown to be photosensitive.

The two violet-sensitive structures did not differ strikingly in form or size from structures in which we characteristically found the P535 and P567 pigments, but we emphasize that only gross differences would have been manifest to us under infra-red viewing.

Band widths

The band widths at 50 % absorbance of the P500, P535 and P567 pigments show a decreasing band width with decreasing wavenumber; this relationship is plotted in Fig. 2*B*. In order to include the P415 pigment (the relative absorbance of which is greater than 50 % at 380 nm, the limit of our measurement) it is the half-band widths on the longwave limbs of the spectra that are plotted.

DISCUSSION

Pigment densities

We have been able to obtain satisfactory records from light-adapted eyes. The densities of rod pigment (cf. Fig. 1) are as high as any we have obtained from dark-adapted rhesus retinae. Thus the present results suggest that the traditional practice of enucleating eyes in dim red light is not necessary for microspectrophotometry of primate pigments. Freedom from this constraint should in particular facilitate human microspectrophotometry, in that fresh human eyes are more readily obtained in a light-adapted state; few surgeons are prepared to operate under low red illumination.

Band widths

It has been apparent for some time that the absorbance spectra of visual pigments are not constant in form when plotted on a frequency abscissa but become narrower as the wavenumber of peak sensitivity decreases. This variation was explicitly shown by Liebman & Entine (1968) and the accumulated evidence has been summarized by Ebrey & Honig (1977). Fig. 2*B* now demonstrates that the relationship between band width and the wavenumber of peak sensitivity holds within a single primate species.

Reconstruction of psychophysical sensitivities

Assumptions. M. fascicularis is of interest as a model for normal human colour vision. In Figs. 4 and 5 we attempt to reconstruct human psychophysical sensitivities from the microspectrophotometric measurements for *M. fascicularis*, but we emphasize the assumptions on which this reconstruction depends. To calculate the *absorptance* of a given class of receptor (i.e. the ratio of absorbed to incident light for a beam passing axially through the receptor) it is necessary to assume (*a*) the length of the outer segment over which effective absorptions occur, and (*b*) the specific absorbance of the pigment (i.e. absorbance per unit length). We take the effective length of the absorbing cylinder to be 35 μm for foveal cones and 25 μm for extra-foveal rods, following the values given by Polyak (1941) for the total length of the outer segments (for the case of the blue cones, see below).

On the basis of our transverse measurements, we estimate the specific absorbance

at the λ_{max} to be approximately 0·018 μm^{-1} for rods and 0·015 μm^{-1} for cones; these values are of the same order as those that have been tabulated by Knowles & Dartnall (1978) for receptors from a variety of species whose visual pigments are based on vitamin A_1.

Further assumptions concern absorption by pre-receptoral factors. As previously (Bowmaker *et al.* 1978) we have used tabulated data from Wyszecki & Stiles (1967, p. 216) to correct for absorption in the human ocular media at wavelengths below 500 nm, but have assumed no change in absorption at wavelengths longer than 500 nm. In the case of the red and green cones an additional correction has to be made for absorption by the macular pigment: again we take values tabulated by Wyszecki & Stiles (1967, p. 219).

An additional source of uncertainty lies in the choice of psychophysical estimates of cone sensitivities: we have here used the π mechanisms of W. S. Stiles (Stiles, 1978, p. 18), since these sensitivities are expressible as linear transformations of small-field colour-matching functions (Estévez & Cavonius, 1977; Pugh & Sigel, 1978), a crucial requirement if they are to be taken as estimates of the human pigments. For the rods we take the CIE scotopic luminosity function, V_λ', converted to a quantum basis.

Finally, in so far as these and earlier results suggest that the values of λ_{max} are distributed within a class of photoreceptor, we make the temporary assumption that the psychophysical sensitivity for a class of photoreceptor reflects the unweighted mean of the untransformed pigment sensitivities.

Rods. The reconstructed sensitivity for scotopic vision is shown in Fig. 4 (continuous line) and there compared with $\log V_\lambda'$. The maxima of these two functions are coincident but the reconstructed sensitivity is systematically broader. A better fit is provided by the absorbance spectrum (corrected only for lens absorption), which is shown as an interrupted line. However, to prefer the latter comparison requires us to adopt the improbable assumption that the axial density of the pigment is infinitely small (or, effectively, $< 0{\cdot}1$). This discrepancy is also present in reconstructions from both rhesus and human microspectrophotometric measurements and is discussed by Bowmaker & Dartnall (1979).

Red and green cones. Sensitivities reconstructed from the P535 and P567 pigments are shown in Fig. 5 (continuous lines) and compared with Stiles' green and red mechanisms, π_4 and π_5. π_4 provides an excellent fit to the reconstructed sensitivity for the green cones. The fit of π_5 to the reconstruction from the P567 pigment is a little poorer in that π_5 appears to be systematically displaced by about 3 nm to shorter wavelengths.

Blue cones. The reconstruction of psychophysical sensitivity from the blue-sensitive pigment is in one way more straightforward than for the other cones and in another way, less so. We need not assume a correction for macular pigment: Stiles (1953, Fig. 15) provides precise measurements for π_1, the blue-sensitive mechanism, at an eccentricity of 8°, where absorption by the macular pigment should be slight (indeed, it was by subtracting the relative spectral sensitivity for π_1 for central targets from sensitivity at 8° that Stiles derived an estimate of the relative density of the macular pigment); and these measurements have been used to reconstruct the blue mechanism in Fig. 5. On the other hand, the absorption due to the lens is increasing rapidly in the region of the λ_{max} of the blue pigment and moreover varies

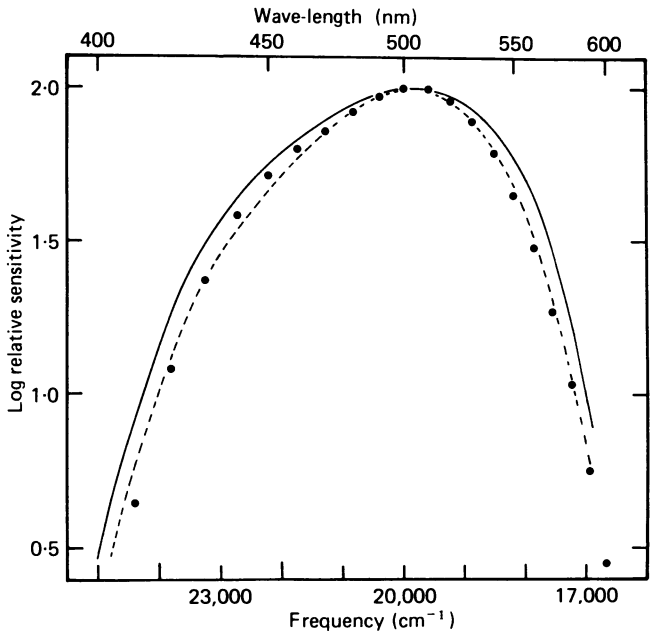


Fig. 4. Comparison of human scotopic sensitivity (V_λ'), plotted on a quantum basis, with the sensitivity (continuous line) reconstructed from the results for *M. fascicularis* on the assumption of an axial density of 0·45. The interrupted line shows the sensitivity that would be expected if the axial density of the receptors were very low. For discussion see text.

considerably between observers (Wyszecki & Stiles, 1967, p. 216); we have used the average values tabulated by Wyszecki & Stiles but the limitations of this correction should be appreciated. As before, a specific absorbance and a length must be assumed for the outer segments. We assume the same specific absorbance as for the red and green cones (0·015 μm^{-1}); but since we are attempting to reconstruct sensitivity in the parafovea, where outer segments are shorter, we take the length to be 25 μm on the basis of the measurements of Polyak (1941).

The psychophysical sensitivity thus reconstructed is shown in Fig. 5 (continuous line) and there compared with Stiles' measurements of the test sensitivity of π_1 at an eccentricity of 8°. Notice that the effect of the correction for lenticular absorption is to shift the λ_{max} of the pigment to 440 nm and thus bring it into close coincidence with that of π_1. The agreement between the two functions is pleasingly good, except at the shorter wave-lengths; in the latter region we could not reasonably hope for a better fit since lenticular absorption varies between individuals by as much as 1 log unit at 400 nm (Wyszecki & Stiles, p. 216) and since our microspectrophotometric measurements suffer from an increase in variance at short wave-lengths owing to the low intensities available from the tungsten lamp.

In summary, although the λ_{max} of the blue cones lies in the extreme violet, at a wave-length much shorter than traditionally supposed, nevertheless the absorptance spectrum of the pigment is brought into close coincidence with the psychophysical

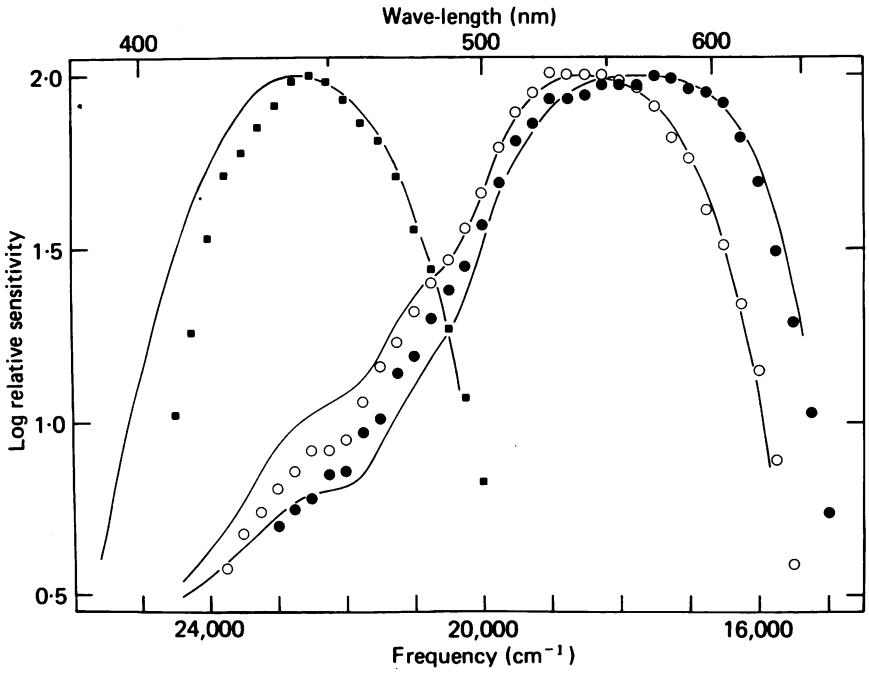


Fig. 5. Comparison of π mechanisms of W. S. Stiles (symbols) with sensitivities reconstructed from microspectrophotometric measurements for *M. fascicularis* (continuous curves). ■, π_1 measured at 8° eccentricity; ○, π_4 measured at the fovea; ● π_5 measured at the fovea. An axial density of 0·525 has been assumed for red and green cones and 0·375 for blue cones. For assumptions see text.

sensitivity when reasonable assumptions are made about pre-receptoral absorption. We note that the P415 pigment here demonstrated has the lowest λ_{max} of any vertebrate pigment so far recorded.

Rarity of blue cones

The paucity of blue cones in the present sample is consistent with the small number (three out of thirty-seven) found by Bowmaker & Dartnall (1980) in their sample of cones from a human retina and with the total absence of such receptors in a sample of eighty-two rhesus cones (Bowmaker, Dartnall, Lythgoe & Mollon, 1978). It remains possible that these results reflect a sampling bias: for example, the blue cones may be disproportionately vulnerable to anaesthetics or their outer segments may tend to adhere to the pigment epithelium when the retina is teased away, or they may deteriorate more quickly post mortem (in this context we note that one of our blue cones was found on the second day of recording). However, a true difference in numerosity would be consistent with two other sources of evidence. Identifying cones according to light-stimulated reduction of nitroblue tetrazolium chloride, Marc & Sperling (1977) suggest that in the baboon retina the blue cones have a maximum frequency of 20 % in an annulus that is concentric with the foveola and has a radius of 1°; in the parafovea the frequency falls to 12–14 % and in the centre of the fovea it

is only 3–4 %. Secondly, extensive psychophysical evidence has shown that the blue-sensitive mechanism, isolated by Stiles' two-colour method, is absolutely less sensitive than the red- and green-sensitive mechanisms (Stiles, 1946; Barlow, 1958).

The reader should not suppose that the blue cones are rare in our records simply because our samples are primarily taken from the fovea. 'Foveal tritanopia' characterizes a region corresponding to less than 20′ of visual angle in man (Willmer & Wright, 1945; Wald, 1967). The psychophysical measurements of Brindley (1954), as well as the results of Marc & Sperling for the baboon retina, suggest that blue cones are at their *most* numerous in a foveal annulus of 1° radius; and this region would certainly be included in our samples, since the complete fovea, which has a diameter of ~1 mm, corresponds to ~5° of visual angle (Polyak, 1941).

This work was supported by M.R.C. grant no. G977/536/N and was carried out at the former M.R.C. Vision Unit, Centre for Research in Perception and Cognition, University of Sussex.

REFERENCES

BARLOW, H. B. (1957). *Visual Problems of Colour*. National Physical Laboratory Symposium, pp. 615–639. London: H.M.S.O.

BOWMAKER, J. K. (1977*a*). Long-lived photoproducts of the green-rod pigment of the frog, *Rana temporaria*. *Vision Res.* **17**, 17–23.

BOWMAKER, J. K. (1977*b*). The visual pigments, oil droplets and spectral sensitivity of the pigeon. *Vision Res.* **17**, 1129–1138.

BOWMAKER, J. K. & DARTNALL, H. J. A. (1980). Visual pigments of rods and cones in a human retina. *J. Physiol.* (in the Press).

BOWMAKER, J. K., DARTNALL, H. J. A., LYTHGOE, J. N. & MOLLON, J. D. (1978). The visual pigments of rods and cones in the rhesus monkey, *Macaca mulatta*. *J. Physiol.* **274**, 329–348.

BOWMAKER, J. K., LOEW, E. R. & LIEBMAN, P. A. (1975). Variation in the λ_{max} of rhodopsin from individual frogs. *Vision Res.* **15**, 997–1003.

BRIDGES, C. D. B. (1967). Spectroscopic properties of porphyropsins. *Vision Res.* **7**, 349–369.

BRINDLEY, G. S. (1954). The summation areas of human colour-receptive mechanisms at increment threshold. *J. Physiol.* **124**, 400–408.

DARTNALL, H. J. A., LANDER, M. R. & MUNZ, F. W. (1961). Periodic changes in the visual pigment of a fish. In *Progress in Photobiology*, ed. CHRISTENSEN, B. CHR. & BUCHMANN, P. Amsterdam: Elsevier.

DE VALOIS, R. L., MORGAN, H. C., POLSON, M. C., MEAD, W. R. & HULL, E. M. (1974). Psychophysical studies of monkey vision. I. Macaque luminosity and color vision tests. *Vision Res.* **14**, 53–67.

EBREY, G. T. & HONIG, B. (1977). New wavelength dependent visual pigment nomograms. *Vision Res.* **17**, 147–151.

ESTÉVEZ, D. & CAVONIUS, C. R. (1977). Human color perception and Stiles' π mechanisms. *Vision Res.* **17**, 417–422.

HAROSI, F. (1975). Absorption spectra and linear dichroism of some amphibians photoreceptors. *J. gen. Physiol.* **66**, 357–382.

KNOWLES, A. & DARTNALL, H. J. A. (1977). *The Photobiology of Vision*, vol. 2B of *The Eye*, ed. DAVSON, H. London and New York: Academic.

LIEBMAN, P. A. & ENTINE, G. (1964). Sensitive low-light-level microspectrophotometer detection of photosensitive pigments of retinal cones. *J. opt. Soc. Am.* **54**, 1451–1459.

LIEBMAN, P. A. (1972). Microspectrophotometry of photoreceptors. In *Handbook of Sensory Physiology*, vol. VII/1, ed. DARTNALL, H. J. A., pp. 481–528. Berlin: Springer.

LIEBMAN, P. A. & ENTINE, G. (1968). Visual pigments of frog and tadpole. *Vision Res.* **8**, 761–775.

MARC, R. E. & SPERLING, H. G. (1977). Chromatic organisation of primate cones. *Science, N.Y.* **196**, 454–456.

POLYAK, S. L. (1941). *The Retina*. Chicago: University of Chicago Press.

PUGH, E. N. & SIGEL, C. (1978). Evaluation of the candidacy of the π-mechanisms of Stiles for color-matching fundamentals. *Vision Res.* **18**, 317–330.

RUSHTON, W. A. H. & HENRY, G. H. (1968). Bleaching and regeneration of cone pigments in man. *Vision Res.* **8**, 617–631.

STILES, W. S. (1946). A modified Helmholtz line-element in brightness-colour space. *Proc. phys. Soc.* **58**, 41–65 (reprinted in STILES (1978)).

STILES, W. S. (1953). Further studies of visual mechanisms by the two-colour threshold technique. *Coloquio Sobre Problemas Opticos de la Vision*, chap. I, pp. 65–103. *Gen. Assembly int. Un. pure appl. Phys. Madrid* (reprinted in STILES (1978)).

STILES, W. S. (1978). *Mechanisms of Colour Vision*. London: Academic.

WALD, G. (1967). Blue-blindness in the normal fovea. *J. opt. Soc. Am.* **57**, 1289–1301.

WALD, G., BROWN, P. K. & SMITH, P. H. (1955). Iodopsin. *J. gen. Physiol.* **38**, 623–681.

WILLMER, E. N. & WRIGHT, W. D. (1945). Colour sensitivity of the fovea centralis. *Nature, Lond.* **156**, 119–121.

WYSZECKI, G. W. & STILES, W. S. (1967). *Color Science*. New York: Wiley.