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PHOTOSENSITIVE AND PHOTOSTABLE PIGMENTS IN THE RETINAE OF OLD WORLD MONKEYS

BY J. K. BOWMAKER,

Institute of Ophthalmology, University of London, Judd Street, London WC1H 9QS, UK

SUSAN ASTELL, D. M. HUNT,

School of Biological Sciences, Queen Mary and Westfield College, University of London, Mile End Road, London E1 4NS, UK

AND J. D. MOLLON

Department of Experimental Psychology, University of Cambridge, Downing Street, Cambridge CB2 3EB, UK

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Summary

Microspectrophotometric measurements of retinal receptors are reported for eight species of Old World monkey. Although the animals vary greatly in size, colourings and habitat, they all appear to be trichromats and the peak sensitivities of their cones invariably lie near 430, 535 and 565 nm. This consistent pattern contrasts with the results reported earlier for New World monkeys and with the results reported here for *Tupaia glis*. The trichromacy of frugivorous catarrhine monkeys may have co-evolved with a particular class of coloured fruit.

Short-wave cones were rare in all species. The ratio of the numbers of middle-wave and long-wave cones varied between individual animals, but had an overall value close to unity.

In the case of all the species examined here, we have recorded a photostable pigment in the inner segments of rods and cones. The latter pigment has a peak sensitivity close to 420 nm and an absorbance spectrum that is narrower than that of a photosensitive visual pigment.

Introduction

The last decade has revealed that the retinal photopigments of New World monkeys vary greatly in their spectral positions, both between species and within a given species (e.g. Mollon *et al.* 1984; Jacobs, 1986; Travis *et al.* 1988). By contrast, it is commonly held that the visual pigments of Old World monkeys resemble those

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of the normal human trichromat and vary little, either within species or between species. Yet the direct microspectrophotometric and electrophysiological evidence for this view (e.g. Marks *et al.* 1964; Bowmaker *et al.* 1978; Hárosi, 1987; Baylor *et al.* 1987) is very slight, being drawn almost exclusively from two macaque species, the rhesus and cynomolgus monkeys. And these species are very closely related: they occupy overlapping regions of south Asia (Roonwal and Mohnot, 1977); hybrid offspring are fertile; and morphological intergradation of the two species in Thailand has been reported (Fooden, 1964).

In the present paper we set out to extend the microspectrophotometric data base to three other genera of Old World monkeys, *Papio*, *Erythrocebus* and *Cercopithecus*. We have been able to obtain fresh tissue from the baboon (*Papio papio*, Desmarest), the patas monkey (*Erythrocebus patas*, Schreber), the talapoin (*Cercopithecus talapoin*, Schreber) and several species of central African guenons. The sampled species are drawn from different geographical regions and different ecological niches. Thus, the baboon is found in West Africa from Senegal to Sierra Leone, often in savannah country; the talapoin is found in the swamp forests of the Atlantic coast of central Africa; the moustached guenon (*Cercopithecus cephus*) occurs in Gabon, Cameroon and the Congo basin, occupying during daylight hours the lower levels of dense tropical rain forests (Gautier-Hion *et al.* 1981), whereas the Diana monkey (*Cercopithecus diana*, Linnaeus) occupies the canopy and upper levels of the rain forest. In their body masses, the species range over more than an order of magnitude: the talapoin is the smallest of the catarrhine monkeys and the male typically weighs 1200 g, whereas the male baboon may exceed 22 000 g (Napier and Napier, 1967, p. 412).

The cercopithecine species had an especial interest for us. Many members of this genus are characterised by patches of slate-blue skin pigmentation that appear to be species-specific signals. In the case of the talapoin and the grivet (*Cercopithecus aethiops*, Linnaeus), the scrotum is strikingly blue. The moustached guenon exhibits a vivid blue face surrounded by a contrasting yellow ruff; stereotyped 'head flagging' movements, seen in the context of courtship and appeasement, confirm the social function of these facial markings (Kingdon, 1980, 1988). In seeking tissue from species that apparently employ blue coloration as social signals, we were motivated by the simple-minded expectation that short-wave cones might be found more readily in these monkeys than in other primate species. In macaques, in squirrel monkeys and in man, short-wave cones have proved to be consistently rare (Bowmaker *et al.* 1978, 1980; Mansfield *et al.* 1984; Hárosi, 1987; Mollon *et al.* 1984; Dartnall *et al.* 1983).

As a counterpoint to the data from catarrhine monkeys, we also report here microspectrophotometric measurements of retinal tissue from a tree shrew.

Materials and methods

Tissue was obtained from one female Rhesus macaque (*Macaca mulatta*), one female and three male baboons (*Papio papio*), one male patas mokey (*Erythro-*

cebus patas), one male talapoin (*Cercopithecus (Miopithecus) talapoin*), one female Diana monkey (*Cercopithecus diana*), one male grivet (*Cercopithecus aethiops*), one male spot-nosed monkey (*Cercopithecus petaurista*), one female moustached guenon (*Cercopithecus cephus*) and one male tree shrew (*Tupaia glis*). All animals were mature adults.

The details of the experimental procedures and methods of analysis used in this study have been given previously (Mollon *et al.* 1984; Bowmaker *et al.* 1985). Animals were anaesthetized with ketamine hydrochloride and then given a lethal dose of sodium pentabarbitone. Eyes were enucleated under low levels of illumination and a hemisection of the globe was made under dim red light. Normally, the first sample of retinal tissue (approximately 1 mm^2) was taken from the foveal region, and subsequent samples were taken from neighbouring areas. The tissue was dispersed on a cover-slip with a few strokes of a razor blade.

Absorbance spectra of individual outer segments were measured by means of a modified Liebman microspectrophotometer (Liebman and Entine, 1964; Knowles and Dartnall, 1977). Writing about the modified instrument at Queen Mary College, Mansfield (1985) has claimed that 'They [Bowmaker and colleagues] used a large aperture condenser that scattered more light outside the aperture of the objective'. We take the opportunity to state that the numerical apertures of the condenser and objective of the modified Liebman instrument are 0.4 and 0.9, respectively.

The measuring beam, typically 2 μm$\times$2 μm in cross-section, was passed through the outer segment of an isolated photoreceptor, while a reference beam of the same dimensions passed through an adjacent clear space in the preparation. The absorbance was sampled at 2-nm intervals from 700 to 390 nm and, in a return scan, at the interleaved wavelengths from 391 to 699 nm. A baseline was obtained by placing both beams in a clear space and measuring absorbance as before. Only one double scan was normally made of each outer segment, but, in the case of all species except the macaque and the baboon, two separate baselines were obtained for a given cell and were each subtracted from the record obtained when the beam passed through the cell. The final absorbance spectrum for the cell was obtained by averaging the two resulting records. The latter operation reduces the noise present in the estimated spectrum without increasing the degree to which the visual pigment is bleached.

In the case of cells absorbing at short wavelengths, three separate absorbance spectra and three corresponding baselines were obtained (the measuring beam being alternately positioned within and outside the cell); the low output of our tungsten source makes it possible, and desirable, to repeat measurements in this way when measuring putative short-wave cones. After absorbance measurements of such structures were complete, we always exposed the cell to white light for 5 min and repeated the measurements. This last operation ensured that the pigment was indeed photolabile and allowed us to distinguish short-wave cones from other retinal structures (e.g. inner segments) that absorb at short wavelengths.

In some preparations we deliberately obtained records from inner segments. In these cases, since the measured pigment appears to be photostable, several spectral scans were made with the measuring beam within the cell.

Analysis of data

The wavelength of maximal absorption (λ_{max}) of the absorbance spectrum from a given outer segment was estimated by a computer program that fitted a template curve to a selected portion of the absorbance spectrum. The template used was the Dartnall absorbance spectrum for frog rhodopsin with $\lambda_{max}=502$ nm (Knowles and Dartnall, 1977, p. 76). In previous studies, we have expressed the template and the absorbance spectrum in terms of $\lambda^{1/4}$, since absorbance spectra of different λ_{max} assume a similar shape when expressed on this abscissa (Barlow, 1982). However, Mansfield (1985) and MacNichol (1986) have suggested that spectra are more exactly similar when expressed in terms of F/F_{max}, where F_{max} is the frequency of maximum absorbance (this is equivalent to an abscissal scale of log frequency). We agree, in that our long-wave absorbance spectra are slightly, but consistently, broader than predicted from a template translated on a $\lambda^{1/4}$ abscissa (see Fig. 2). For the purpose of the present analysis we have therefore expressed our template in terms of log frequency. However, the two methods of translating the template produce only small differences in the estimated λ_{max}. What we do emphasize is that the template is merely a tool, which allows us to apply a consistent and automated analysis to all records without artificially creating discrete categories of visual pigment (see Mollon *et al.* 1984).

In order to estimate the λ_{max} of a given absorbance spectrum, the absorbance values at pairs of adjacent wavelengths were first averaged to obtain a mean curve from the outward and return scans, and the resulting values were then re-expressed as percentages of the maximum absorbance. Two separate estimates of λ_{max} were made, using different sections of the curve. One estimate of λ_{max} was made from the right-hand limb of the mean curve (as in previous studies; Mollon *et al.* 1984): each of 20 percentage absorbance values on the long-wavelength limb of the curve (corresponding to a 40-nm segment of the trace and to absorbances in the range approximately 45–90 % of the maximum for that cell) was referred to the template curve to find the position of λ_{max} that would give the percentage absorbance value under consideration. The 20 individual positions were then averaged to give a mean estimate of the λ_{max} of the cell. The second estimate of λ_{max} was obtained by applying analogous operations to a set of 51 points centred on the peak of the absorbance spectrum and corresponding to a range of 100 nm.

Although both these estimates were used in the selection of cells (see below, criterion iii), the values of λ_{max} actually quoted and plotted in the present paper are those obtained from the right-hand limb. These recommend themselves because (a) the right-hand limb includes the steepest portion of the absorbance spectrum and thus small changes in wavelength correspond to large changes in absorbance, and (b) the short-wave region of a microspectrophotometrically measured absorbance spectrum is known to be the section most subject to

distortion, by wavelength-dependent scattering and by the presence of photoproducts (MacNichol *et al.* 1973).

Criteria for inclusion of cells

With the exception of those obtained from short-wave cones, absorbance spectra were excluded from further analysis if any of the following conditions held. (i) The peak value of absorbance was less than 0.01. (ii) The standard deviation of λ_{max} estimates obtained by fitting the template curve to the right-hand limb was greater than 8 nm. (iii) There was a difference of more than 5 nm between the estimate of λ_{max} from the right-hand limb of the absorbance spectrum and the estimate obtained by fitting the template to the top of the curve. (iv) The average short-wave absorbance between 420.5 and 440.5 nm fell outside the range 5–45 % (long- and middle-wave cones) or 10–50 % (rods).

A summary of the data for *Papio* has been previously published in the proceedings of a conference (Bowmaker *et al.* 1983); for the purpose of the present comparison, the individual absorbance spectra have all been re-analysed using the log-frequency method and have been subject to the criteria listed above.

Results

Catarrhine monkeys

Fig. 1 shows the distributions of λ_{max} values for the eight species of Old World monkey. Each cell of the histograms represents an individual outer segment. Mean values of λ_{max} are listed in Table 1. There is great similarity across species in the spectral positions of the photopigments: the λ_{max} values of the cones in each case lie close to 430, 535 and 565 nm. However, the Kruskall–Wallis test (a non-parametric analogue of analysis of variance) reveals small but significant variations between species, both for the middle-wave cones ($P<0.01$) and for the long-wave cones ($P<0.05$). It is possible that these small variations are experimental in origin, and they certainly should not be allowed to obscure the basic interspecific similarity of the mean values. We do note that the middle-wave pigment of the Diana monkey lies at a noticeably shorter wavelength than do those of macaques or of other guenons.

In three species there are single cells lying between the distributions for the rods and middle-wave cones (522 nm, *Macaca mulatta*; 518 nm, female *Papio papio*; 512 nm, *Cercopithecus petaurista*); these cells satisfy the criteria listed in Materials and methods and so we have no grounds for excluding them from the histograms.

Since Fig. 1 includes only those cells that satisfy the criteria, the histograms should not be taken as a direct guide to the relative numbers of particular cone types. More records from long-wave cones fail the criteria than do records from middle-wave cones. Two main factors may contribute to this bias: the tissue is dissected in dim red light, and the efficiency of the microspectrophotometer is optimized for the middle of the visual spectrum. However, in the case of catarrhine monkeys we are seldom in doubt as to whether a particular record is

Table 1. *Estimates of λ_{max} for each class of receptor in individual animals from the present study*

Species	Sex	Short-wave cones	Middle-wave cones	Long-wave cones	Rods
Cercopithecus aethiops	M	434.0 (1)	535.0±2.7 (13)	566.3±1.9 (11)	499.2±2.6 (4)
Cercopithecus diana	F	432.0±0.7 (2)	530.7±3.9 (11)	565.9±2.4 (18)	496.5±1.9 (4)
Cercopithecus petaurista	M	424.1 (1)	533.9±2.8 (11)	562.7±3.2 (8)	497.2±2.6 (11)
Cercopithecus cephus	F	431.6±2.1 (6)	533.0±1.7 (21)	565.3±2.9 (17)	497.8±1.5 (8)
Erythrocebus patas	M	431.6±3.5 (3)	533.0±2.3 (11)	566.3±1.9 (20)	499.2±1.5 (2)
Cercopithecus talapoin	M	429.2±3.4 (2)	533.3±2.8 (20)	564.0±3.9 (10)	494.9±3.3 (4)
Macaca mulatta	F	433.5 (1)	536.0±1.8 (12)	561.5±5.8 (6)	502.2±2.3 (16)
Papio papio (i)	M	430.5±3.5 (3)	536.4±4.3 (11)	564.3±3.7 (7)	500.5±3.5 (3)
Papio papio (ii)	M	–	535.2±3.3 (8)	565.7±1.6 (2)	–
Papio papio (iii)	M	–	538.8±4.3 (4)	564.4±7.0 (6)	502.3±2.7 (5)
Papio papio (iv)	F	426.0 (1)	535.6±3.5 (9)	567.8±2.8 (7)	500.9±1.5 (5)
Tupaia glis	M	445 (1)	554.5±3.5 (18)		498.1±0.3 (2)

Each λ_{max} is obtained by averaging the λ_{max} values of all individual cells that fall in a given class and pass the criteria.
The standard deviation is given after each λ_{max} value, and the number of cells contributing to the estimate is shown in brackets.

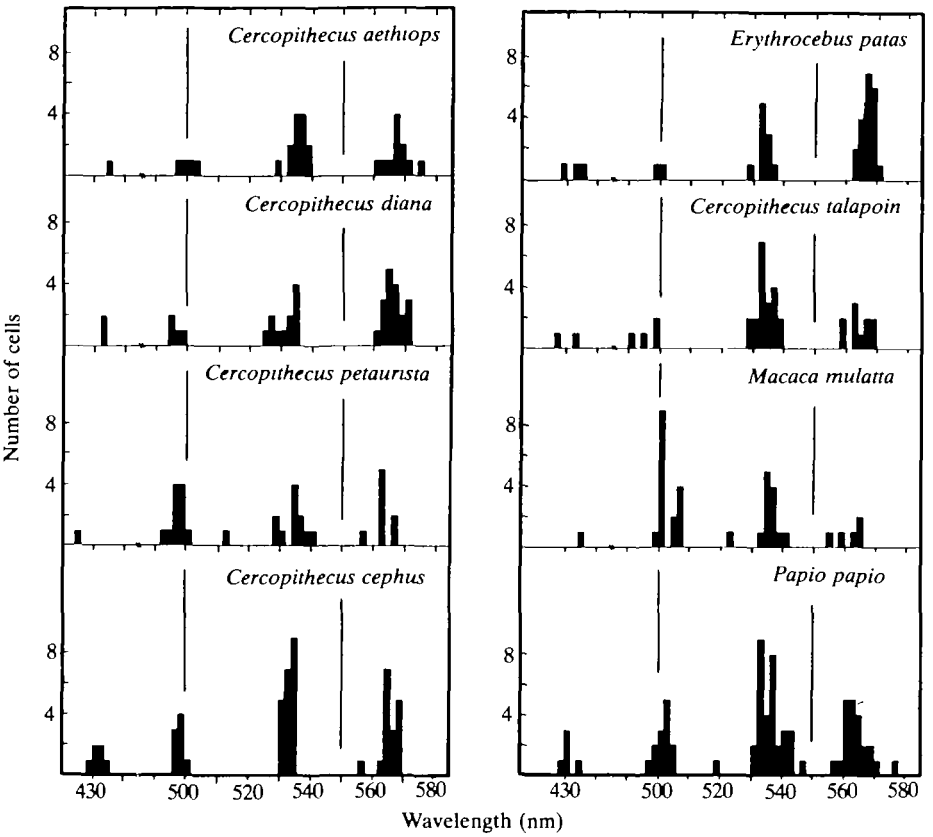

Fig. 1. Distribution of λ_{max} values of individual outer segments from eight species of Old World monkey. The bin size is 2 nm.

'middle-wave' or 'long-wave', and therefore we give in Table 2 the numbers of all middle- and long-wave records obtained from the monkeys of the present study. Although the samples obtained from individual animals are relatively small (typically 40–50 cones) and the individual ratios vary considerably, the overall ratio that we observe for 442 cells is 1.05.

Short-wave cones were rare in all species examined, although it should be remarked that the six short-wave cones recorded in the blue-faced *Cercopithecus cephus* were more than we have found in any other catarrhine or platyrrhine specimen.

As an illustration of the absorbance spectra of catarrhine visual pigments, Fig. 2 shows the mean sensitivities of the rods and cones of the moustached guenon (*Cercopithecus cephus*). Each set of data points represents the average absorbance spectrum for all cells that fall within a given class and satisfy the criteria; the individual records were not normalized before averaging. The solid lines fitted to the absorbance data correspond to the rhodopsin template, translated on a log-frequency abscissa and fitted to the right-hand limb of the absorbance spectrum

Table 2. *Numbers of middle-wave and long-wave cones obtained from individual catarrhine monkeys*

Species	Sex	Middle-wave cones	Long-wave cones	Ratio
Cercopithecus aethiops	M	22	19	1.16
Cercopithecus diana	F	12	30	0.40
Cercopithecus petaurista	M	19	21	0.90
Cercopithecus cephus	F	26	22	1.18
Erythrocebus patas	M	25	35	0.71
Cercopithecus talapoin	M	27	28	0.96
Macaca mulatta	F	23	13	1.77
Papio papio (i)	M	21	14	1.50
Papio papio (ii)	M	20	9	2.22
Papio papio (iii)	M	14	15	0.93
Papio papio (iv)	F	17	10	1.70
Total		226	216	1.05

We have included all records in this table, whereas the values given for N in Table 1 refer only to those cells yielding absorbance spectra for which a peak wavelength could be precisely given.

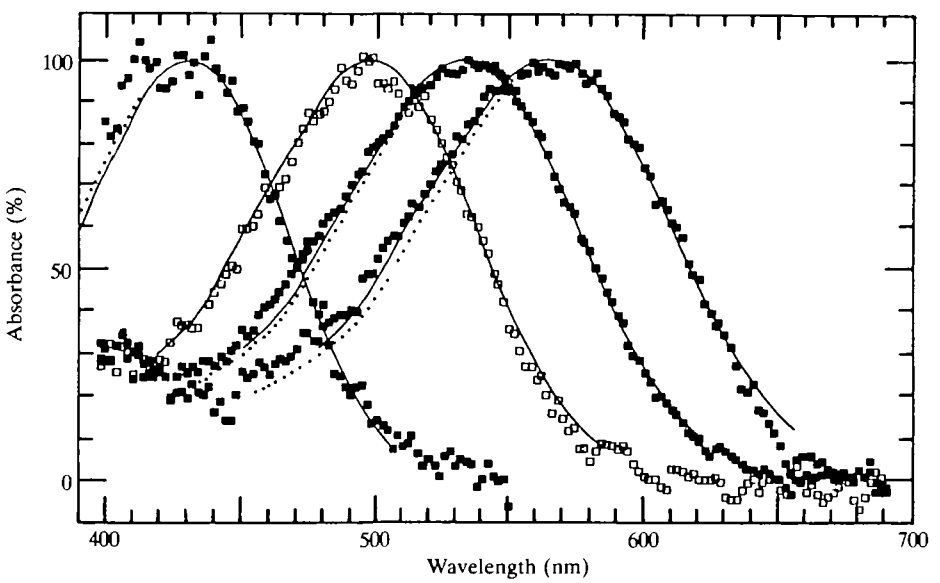

Fig. 2. The absorbance spectra of rods (□) and three classes of cone (■) in the moustached guenon *Cercopithecus cephus*. The solid lines represent the best-fitting position of the Dartnall template for frog rhodopsin when expressed on an abscissa of log frequency; the dotted lines represent the same template when expressed in terms of $\lambda^{1/4}$.

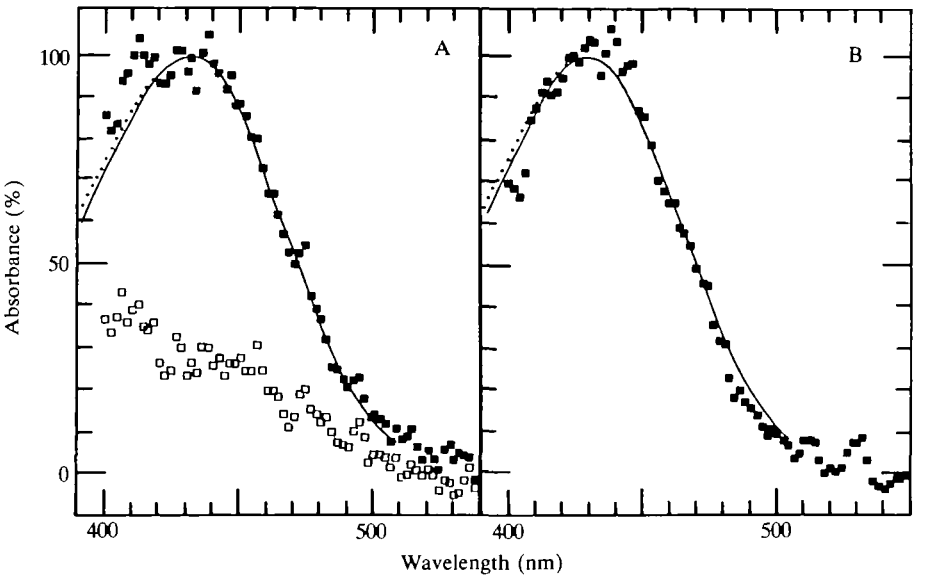

Fig. 3. (A) Mean absorbance spectra of six short-wave cones from *Cercopithecus cephus* before (■) and after (□) exposure to a 5-min bleach with white light. (B) Difference spectrum obtained by subtracting the post-bleach absorbance spectrum from the pre-bleach spectrum. In each case the solid line represents the best-fitting position of the rhodopsin template when expressed in terms of log frequency, whereas the dotted line represents the same template expressed in terms of $\lambda^{1/4}$.

(see Materials and methods); the dotted lines correspond to the same template translated on an abscissa of $\lambda^{1/4}$. The present example illustrates how small is the effect of choosing to translate the template in terms of log frequency rather than $\lambda^{1/4}$: the solid curves (for which log frequency was used) give peak sensitivities at 431, 498, 533 and 565 nm, whereas the dotted curves (for which $\lambda^{1/4}$ was used) give peak sensitivities at 430, 498, 534 and 567 nm.

Fig. 3A shows the mean absorbance spectrum of the six short-wave cones from *Cercopithecus cephus* before (filled symbols) and after (open symbols) exposure of the cell to a 5-min bleach with white light. The resulting difference spectrum is plotted in Fig. 3B. It is possible that the raw absorbance spectrum is contaminated by photostable components and by scattering, which do not affect the difference spectrum and which would be expected to shift the estimated λ_{max} to shorter wavelengths; conversely, short-wave photoproducts may be present after bleaching, and their effect would be to shift the peak of the difference spectrum to longer wavelengths (Mansfield, 1985). In fact, when the λ_{max} is estimated from the right-hand limb, our own data show only small differences between absorbance and difference spectra for short-wave cones: the difference between the two values of λ_{max} ranges from +1.5 nm in the case of *Cercopithecus talapoin* to −3.4 nm in the case (shown in Fig. 3) of *Cercopithecus cephus*.

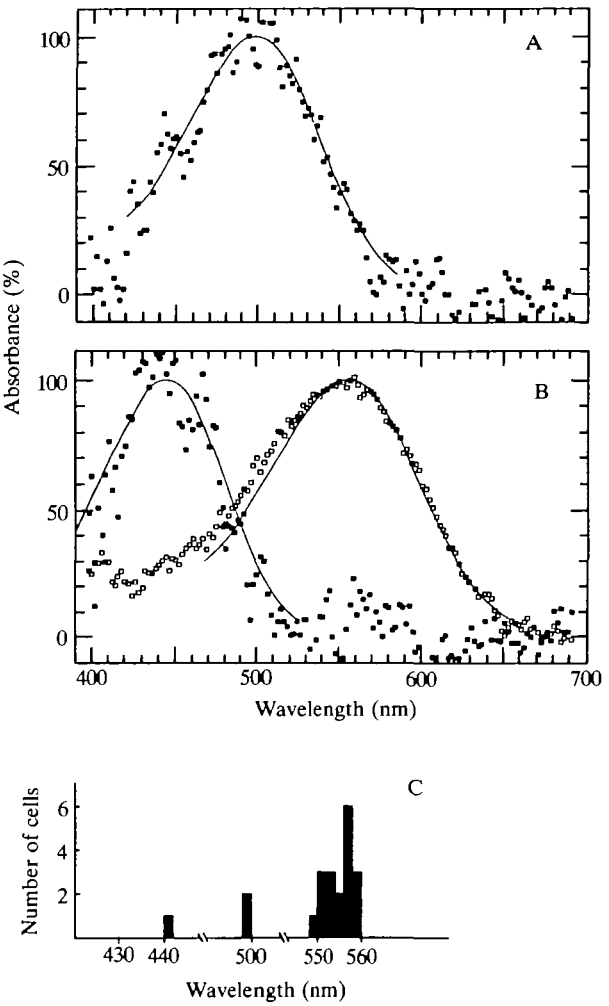

Fig. 4. Visual pigments of the tree shrew *Tupaia glis*. (A) Mean absorbance spectrum for two rods. In A and B, the solid lines represent the best-fitting position of the rhodopsin template when expressed in terms of log frequency. (B) Mean absorbance spectra for one short-wave cone (■) and 18 long-wave cones (□). (C) Distribution of λ_{max} values for individual outer segments. The bin size is 2 nm.

Tree shrew

Twenty-one analyzable records were obtained from the tree shrew. A single short-wave cone exhibited a λ_{max} between 440 and 445 nm. Eighteen further cones exhibited peak sensitivity in the long-wave spectral region (Fig. 4). The latter form a single distribution that does not differ from normality either by Geary's test (a=0.8761; see D'Agostino, 1970) or by Shapiro and Wilk's test (W=0.9466; see Shapiro and Wilk, 1965). The mean λ_{max} of these 18 cones was 555 nm. We also confirm the presence of rod-like structures: two such cells had a mean λ_{max} of

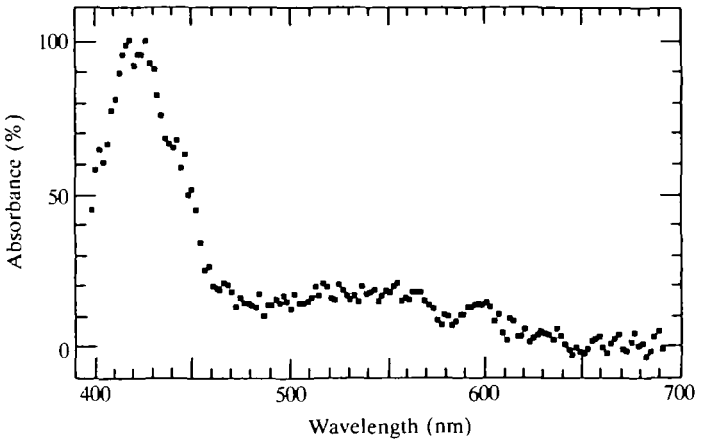

Fig. 5. Absorbance spectrum of a photostable pigment recorded in the inner segment of cones from *Tupaia glis*.

498 nm. Mean absorbance spectra for the three types of photoreceptor are shown in Fig. 4.

Inner segments

In Fig. 5 we illustrate a curious photostable pigment that we have recorded in cone inner segments from *Tupaia glis*. We have previously reported such a photostable pigment in inner segments from *Macaca mulatta* (Mollon and Bowmaker, 1979); and we have obtained similar records from all the species of the present study, as well as from man, from the cynomolgus monkey (*Macaca fascicularis*) and from platyrrhine species such as *Callithrix jacchus* and *Saimiri sciureus*. The absorbance spectrum of this photostable pigment is narrower than that of a photopigment; its peak sensitivity lies close to 420 nm; and in most of our records it has a characteristic shoulder at 440 nm. When the inner segment is measured transversely, the absolute value of the absorbance at 420 nm is 0.02.

In any given species we have obtained only a few records from inner segments; and only a minority of these inner segments were attached to an outer segment from which we could obtain a microspectrophotometric record. Across species, however, we have found the photostable pigment in inner segments attached to long-, middle- and short-wave cones and to rods. However, the pigment is not detected in every inner segment; and, when it is found, it is confined to the ellipsoid region, close to the base of the outer segment. The photostable pigment does not exhibit any marked dichroism: we have not detected any significant difference in absorbance between records made with the *e*-vector of the beam parallel to the axis of the inner segment and those made with the *e*-vector perpendicular.

Discussion

Photopigments

It has been held that the opsins of visual pigments are capable of very rapid evolution (Knowles and Dartnall, 1977). For example, inter-specific variations occur in the photopigments of salmonid fish that have relatively recently been isolated in land-locked glacial lakes (Bridges and Yoshikami, 1970; Bridges and Delisle, 1974). In the case of man, Nathans *et al.* (1986*a*) have suggested that the homology of the juxtaposed middle- and long-wave genes allows frequent instances of unequal crossing-over and thus the ready formation of hybrid genes that code for pigments with intermediate spectral sensitivities.

Given the belief that visual pigments can evolve rapidly, the very consistency of the cone pigments in Old World monkeys becomes a matter for remark. We have sampled animals from different geographical regions of Asia and Africa, and from a wide range of ecological niches. The monkeys differed considerably in body weight and in colourings. Some were exclusively arboreal and others predominantly terrestrial. Yet all of them appear to be trichromats and always the long- and middle-wave cone pigments exhibit λ_{max} values close to 565 and 535 nm, respectively, the total range of inter-species variation being approximately 5 nm in both cases. This relative homogeneity of photopigments is in contrast to the variation seen between and within New World species (Mollon *et al.* 1984; Jacobs, 1986).

We do not say that the photopigments are the same in all the catarrhines sampled, only that their spectral positions are very similar. Small variations are present in the measurements; and even where the absorbance spectra are indistinguishable, it is quite possible that the opsins will prove to differ in their amino acid sequences. But we do conclude this: either there must be ecological or functional factors that constrain the peak sensitivities always to lie near 535 and 565 nm, or else it must be difficult (more difficult than has been thought) for nature to alter the absorbance spectra of photopigment molecules.

What the present species do have in common is that they are all frugivorous. In the case of *C. cephus*, for example, fruit constitutes 85 % of the diet (Sourd and Gautier-Hion, 1986). Fruit-eating may be the common factor that constrains the spectral position of the photopigments in all the monkeys that we have examined. The idea that coloured fruits and primate trichromacy co-evolved is not new (see, for example, Polyak, 1957; Cooper *et al.* 1986), but new evidence for the hypothesis can be seen in ecological results (Gautier-Hion *et al.* 1985), which demonstrate a class of fruit that is specialised for being dispersed by monkeys: such fruits are coloured yellow or orange, weigh between 5 and 50 g, and are either dehiscent with arillate seeds or are succulent and fleshy. Without trichromacy, the catarrhine monkey would share the difficulty of the human dichromat in detecting ripe fruit against the dappled background of forest foliage (Mollon, 1989; Steward and Cole, 1989). It is primarily at wavelengths above 500 nm that the spectral reflectance curves of yellow or orange fruit differ from those of background leaves (Cooper *et al.* 1986). Thus, the dichromatic consumer can seldom detect such fruit

by colour, since his colour vision (see below) depends on his short-wave cones, which absorb negligibly above 500 nm. And he cannot depend on lightness, because the leaves, lying at varying angles to the illuminant, provide a masking background that varies randomly in lightness.

The present study does not include any predominantly leaf-eating species, such as *Colobus*: if variant or polymorphic forms of catarrhine colour vision do exist, we suggest that they are most likely to be found among folivorous species.

Relative numbers of long- and middle-wave cones

Although our samples were limited to 40 or 50 cells per species, they were consistently drawn from near-foveal regions and it is interesting that the overall ratio of middle- to long-wave cones is very close to unity (Table 2). On the basis of psychophysical studies of human foveal vision (Vos and Walraven, 1971; Cicerone and Nerger, 1989), a value of 1:2 has been postulated for the corresponding ratio. Our monkey sample comprises a total of 442 cones and it is statistically very improbable that our empirically obtained ratio could be drawn from a parent population that exhibits a ratio of 1:2 ($\chi^2=55.8$; $P<0.001$). Nevertheless, there is a large variation in the ratios that we find for different animals and a second χ^2-test shows that our samples are unlikely all to have been drawn from one single parent population ($\chi^2=20.04$; d.f.$=10$; $P<0.05$). This latter result could merely indicate differences between individuals or species. On the other hand, the average ratio is suggestively close to unity, and our results would be compatible with a model in which (a) a random factor controls whether the long- or middle-wave opsin gene is expressed in a given cell (giving an average ratio of 1:1 for each species), but (b) this random decision is made in cells that are precursors of the measured cones (giving local regions in which there is a bias in favour of one or other type of cone).

Aberrant cells

Very occasionally (see Results) we have recorded cells with peak sensitivities lying in the region 512–522 nm. These cells may represent some unrecognized experimental error or they may reflect the existence of an additional opsin gene with a low frequency of expression.

Tree shrew

Our results for *Tupaia glis* contrast with those so consistently found in catarrhines but they agree, in most respects, with the microspectrophotometric measurements already published by Petry and Hárosi (1987, 1990) and the electroretinographic measurements of Jacobs and Neitz (1986). The tree shrew retina is dominated by one class of cones with peak sensitivity at 555 nm, while rods and short-wave cones are sparsely present. In its dichromacy, the tree shrew is quite distinct from the catarrhine monkeys and more resembles non-primate mammals (Kraft, 1988; Neitz *et al.* 1989; Neitz and Jacobs, 1989) or male platyrrhine monkeys (Mollon *et al.* 1984; Travis *et al.* 1988).

Short-wavelength cones

Short-wavelength cones with λ_{max} values close to 430 nm were found in all the monkey species examined here, including *Macaca mulatta* – a species in which we had previously been unsuccessful in finding such cones (Bowmaker *et al.* 1978). The present estimates of the spectral position of catarrhine short-wave cones are consistent with those obtained microspectrophotometrically for macaques by Mansfield *et al.* (1984) and Hárosi (1987) and with the value obtained electrophysiologically by Baylor *et al.* (1987); but the short-wave receptors of the human retina exhibit a λ_{max} at a shorter wavelength (Dartnall *et al.* 1983). Our single short-wave cone from *Tupaia glis* exhibits a λ_{max} near 445 nm, a value greater than the values we measure in monkeys, and greater than the average value (428 nm) given by Petry and Hárosi (1990) for five short-wave cones from *Tupaia glis*.

Evolution of primate colour vision

Whereas the amino acid sequences of the human long- and middle-wave opsins exhibit 98% homology, the sequence of the short-wave cone pigment is as different from those of the long- and middle-wave pigments as it is from that of the rods (Nathans *et al.* 1986*b*). These molecular findings suggest that the short-wave cones diverged from other receptors a long time ago (Applebury, 1987) and support the long-held idea that the trichromacy of the Old World primates was preceded by a dichromatic form of colour vision in which the signal of the short-wave cones was compared with that of a single class of cone in the red–green spectral region (Ladd-Franklin, 1892; Jacobs, 1981; Gouras, 1984). The latter class of cone subserved the main business of vision – the detection of movement, flicker, and spatial detail – and short-wave cones were added sparingly to the receptor array to provide a primordial system of colour vision. In the present study, short-wave cones proved to be rare even in the retinae of cercopithecine monkeys, a group of species that employ blue colorations of face and genitals as social signals – although it is our impression that short-wave cones are nevertheless more readily found in cercopithecines than in macaques. To explain why the short-wave cones are rare in modern primates, we should probably look to the chromatic aberration of the eye and to the physical dilution of shadows by non-directional sky light, which together ensure that the short-wave component of the retinal image is almost always degraded. Given the optics of the primate eye, there would be little to be gained by sampling the short-wave image more finely.

This ancient subsystem of colour vision, which depends on a comparison of short- and long-wave signals, is probably shared by most diurnal mammals. In the primates, the chromatic information provided by the short-wave cones appears to be carried by morphologically distinct classes of bipolar and ganglion cells (Mariani, 1984; De Monasterio, 1979; Rodieck, 1988); and the subsystem may remain independent at least as far as Area 17 (T'so and Gilbert, 1988). Relatively recently, in phylogenetic terms, a second subsystem of colour vision was overlaid on the first. The second subsystem depends on a comparison of the rates at which

quanta are caught by the long- and middle-wave pigments. With the exception of female members of platyrrhine species, the second subsystem appears to be confined to the Old World monkeys, the apes and man. By contrast with the ancient subsystem, the retinal and geniculate neurones that carry the second dimension of colour vision are also sensitive to spatial contrast (Derrington *et al.* 1984). For detailed discussion, see Mollon and Jordan (1988/1989).

If we consider all the known spectral locations occupied by cone pigments in catarrhine and platyrrhine species, it is interesting that the only location common to all simian species (565 nm) has not so far been found in any prosimian or other mammal. It is often asked whether it is the long-wave or the middle-wave catarrhine pigment that represents the ancestral pigment from which the other developed by gene duplication and mutation. But, in fact, the ancestral pigment may well have occupied an intermediate spectral position: once two distinct genes were present on the X-chromosome, selective pressures might lead to opposite displacements of λ_{max} values until the present, possibly optimal, spectral positions were occupied. By this account, man counts an anomalous trichromat among his ancestors.

The photostable pigment of the inner segments

Identity

In the ellipsoid region of inner segments we have repeatedly recorded a pigment that exhibits an absorbance curve which peaks near 420 nm and is narrower than that of a photopigment. In its location within the inner segment and in its absorbance spectrum, but not in its optical density, this photostable pigment recalls that of the ellipsosomes described in fish by MacNichol *et al.* (1978) and by Avery and Bowmaker (1982). The ellipsosomes of fish appear to consist of modified mitochondria, and in primates the ellipsoid region is a site of concentration of mitochondria. The pigment of ellipsosomes resembles reduced cytochrome *c* (MacNichol *et al.* 1978) and a plausible hypothesis would be that absorption in the primate inner segment is due to cytochrome *c* and other respiratory enzymes. Liebman (1969) has attributed to cytochromes a photostable pigment that he measured in the ellipsoid region of the inner segments of *Necturus* cones.

However, the site of our photostable pigment also corresponds to that occupied by carotenoid-containing oil droplets in avian and reptilian retinae and the absorbance spectrum could alternatively be that of a carotenoid. A carotenoid spectrum closely resembling the present one is that obtained for crocetin, when dissolved in hexane (Karrer and Jucker, 1950, p. 330).

Function

To reach the outer segments, light must pass through the pigment shown in Fig. 5 and therefore must be subject to selective absorbance by this photostable filter. We emphasize, however, that the optical density of the present pigment is

lower than that of the ellipsosomes by an order of magnitude. Micrographs of human inner segments from near-foveal regions (Boycott and Dowling, 1969; Hogan *et al.* 1971; Steinberg *et al.* 1977) suggest that the length-to-width ratio of the mitochondrial region is of the order of 2.5:3.0; our transverse measurements would thus imply a peak axial absorbance of approximately 0.05, a value that would be of only marginal significance for vision in a spectral region where the absorbance of the lens is of the order of 0.5 (Wyszecki and Stiles, 1982, p. 109). The fact that the photostable pigment can be associated with any of the four types of catarrhine receptor argues against the possibility that its *raison d'être* lies in selective spectral screening of the outer segment.

If the function of the photostable pigment is not optical, then we must assume that the absorbance is the accidental consequence of the presence of respiratory enzymes (such as cytochrome *c*) or of a carotenoid (such as crocetin). With respect to the latter possibility, we note that oxygen availability is likely to be at its lowest in the inner segments, which lie intermediate between choroidal and retinal blood supplies, and that crocetin has been reported to enhance oxygen diffusion in other physiological systems (Gainer and Chisholm, 1974; Holloway and Gainer, 1988).

Relationship to macular pigment

The photostable pigment of Fig. 5 is clearly distinct from the macular pigment, which exhibits peaks at much longer wavelengths, near 460 and 485 nm (Wyszecki and Stiles, 1982, p. 112). We have never recorded macular pigment in the body of an inner segment. This result is consistent with the belief that the macular pigment, in so far as it occurs in receptors, is confined to the fibres of Henle, the long, and very narrow, terminal processes of foveal cones (Segal, 1950; Snodderly *et al.* 1984).

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