Microspectrophotometric Results for Old and New World Primates

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Over the past few years we have been measuring, by microspectrophotometry, the absorbance spectra of the visual pigments of primates, from both the Old and the New World. The aims of these investigations were to establish with some precision the shape and spectral locations of the rod and cone visual pigments and to determine whether variations occur in the location of the peak sensitivities (λ_{max}) of these pigments either between species or within species. In the case of one species, *Saimiri sciureus*, we have been able to secure both behavioural and microspectrophotometric measurements from the same ten individual animals.

The microspectrophotometer is a modification of the instrument designed by P. A. Liebman for the former MRC Vision Unit at the University of Sussex (Liebman and Entine, 1964; Knowles and Dartnall, 1977). One beam is passed transversely through a receptor outer segment while a second beam, the reference beam, is passed through an adjacent area containing no cells. The dimensions of the beams could be adjusted to accommodate the dimensions of a particular cell, but were typically about 2 μm $\times$ 3 μm. To increase the proportion of light absorbed by the outer segments, the measurements were made with the *e*-vector of the beams perpendicular to the long axis of the outer segments. In the earlier measurements absorbance spectra were obtained by scanning from 700 to 400 nm and back to 700 nm, the double scan taking about 20 sec, and the output was displayed on an X$-$Y recorder. In the more recent measurements, after the addition of a DEC-MNC-11 laboratory computer, the monochromator was programmed to step from 700 to 390 nm in 2-nm steps and then to return making measurements at the interleaved wavelengths.

The animals were obtained from a number of sources. Before enucleation they were either sedated with phencyclidine hydrochloride or narcotized with ketamine and then sacrificed with an overdose of pentabarbitone sodium. Measurements normally began within 1−2 hours after death, but in some cases not until 4−5 hours after death, depending on the source of the animal; measurements continued for up to twelve hours after death. The preparation of tissue has been described previously (Bowmaker, Dartnall and Mollon, 1980).

In the present paper we summarize data from three species of Old World monkey, two types of macaque, *Macaca mulatta* and *M. fascicularis* (Bowmaker, Dartnall, Lythgoe and Mollon, 1978; Bowmaker *et al.*, 1980), and the baboon, *Papio papio*; and from two species of New World monkey, the capuchin monkey, *Cebus apella* and the squirrel monkey, *Saimiri sciureus*.

In the case of the macaque species the absorbance spectra were analysed by first drawing by eye a smooth curve through the trace obtained from the cell and another through the baseline trace obtained by passing both beams through an adjacent, tissue-free area of the preparation. The difference between the two curves, sampled at 10 nm-intervals, was used to calculate an absorbance spectrum and from the latter the λ_{max} was estimated as described previously (Bowmaker *et al.*, 1980). The data from *P. papio* and the *S. sciureus* were obtained after the addition of the computer and were analysed slightly differently. From the stored data points at each nm, pairs of absorbances for adjacent wavelengths were first averaged. Each of a set of twenty such values on the long-wave limb (corresponding to 40 nm and to per cent absorbances in the range approximately 45−90%) was then referred to an appropriate nomogram to establish the λ_{max}; this operation amounts to finding where the nomogram must be placed on a wavelength abscissa to yield the absorbance value under consideration. The twenty individual estimates of the λ_{max} were then averaged to give the values entered in the histograms.

Old World Monkeys

The microspectrophotometric measurements of the Old World monkeys have established the presence of three cone types with maximum absorbance at about 565 nm, 535 nm, and 420−430 nm. In *M. mulatta* we have analysed records from 219 cones and 80 rods obtained from 17 animals. (Results from the first five animals have been published previously, Bowmaker *et al.*, 1978.) The cones fell into two groups, 92 long-wave cones with a mean λ_{max} at 566.2 ± 4.8 nm and 127 middle-wave cones with a mean λ_{max} at 534.8 ± 4.8 nm. No clear evidence was found for short-wave receptors. The distribution of the λ_{max} values of individual receptors is shown in Fig. 1. Some 58% of the long-wave receptors and 61% of the middle-wave receptors lie within 3 nm of the mean values, but the outliers of the distributions cover a
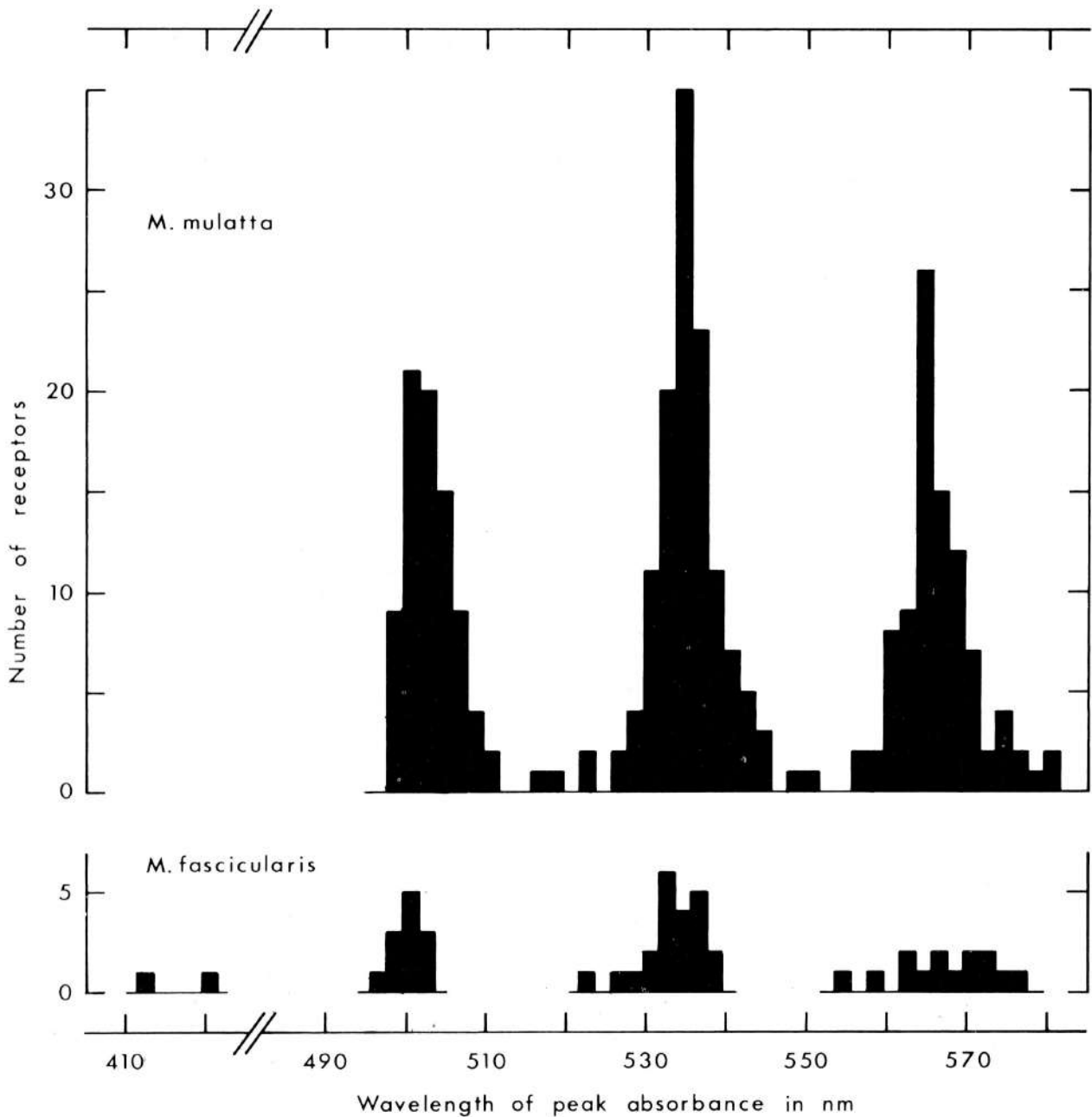
FIG. 1 Distribution of values of peak sensitivity of all individual records from 17 rhesus monkeys (upper panel) and from six eyes from cynomolgus monkeys (lower panel). The bin size is 2 nm. The λ_{max} of an individual record was obtained by fitting an appropriate nomogram to the absorbance spectrum as described in the text.

total of 25 nm for the long-wave and 35 nm for the middle-wave cones with no complete break between the distributions of the two classes. Most of the outliers are drawn from one animal and we have no grounds for discarding these records.

Similar data have been obtained from the closely related cynomolgus macaque, *M. fascicularis* (Bowmaker *et al.*, 1980) from which records for 12 rods and 38 cones from six different eyes were analysed. The rods had a λ_{max} of 500.1 ± 1.6 nm with a transverse absorbance ranging from 0.016 in cells of

small diameter to 0.06 in large cells. The 38 cones fell into three distinct groups: 14 of the outer segments had values of λ_{max} ranging from 554 to 575 nm (Fig. 1) with a mean of 567.0 ± 6.1 nm whereas 22 cells yielded λ_{max} values from 521 to 538 nm with a mean of 533.3 ± 3.9 nm. When cells with high short-wavelength absorbance were excluded from the sample, the mean λ_{max} for the middle-wavelength cones shifted slightly to 535 nm (cf. MacNichol *et al.*, this volume). The remaining two outer segments, morphologically indistinguishable from the others, gave λ_{max} values between 410 and 420 nm with a mean transverse absorbance of 0.015. The mean absorbance spectra for the four classes of receptor from *M. fascicularis* are shown in Fig. 2. The λ_{max} values that we obtain for the two macaque species are in good agreement with the recent independent measurements of MacNichol *et al.* (see this volume); the only instance of a large discrepancy occurs in the case of the short-wave cones, for which data are limited.
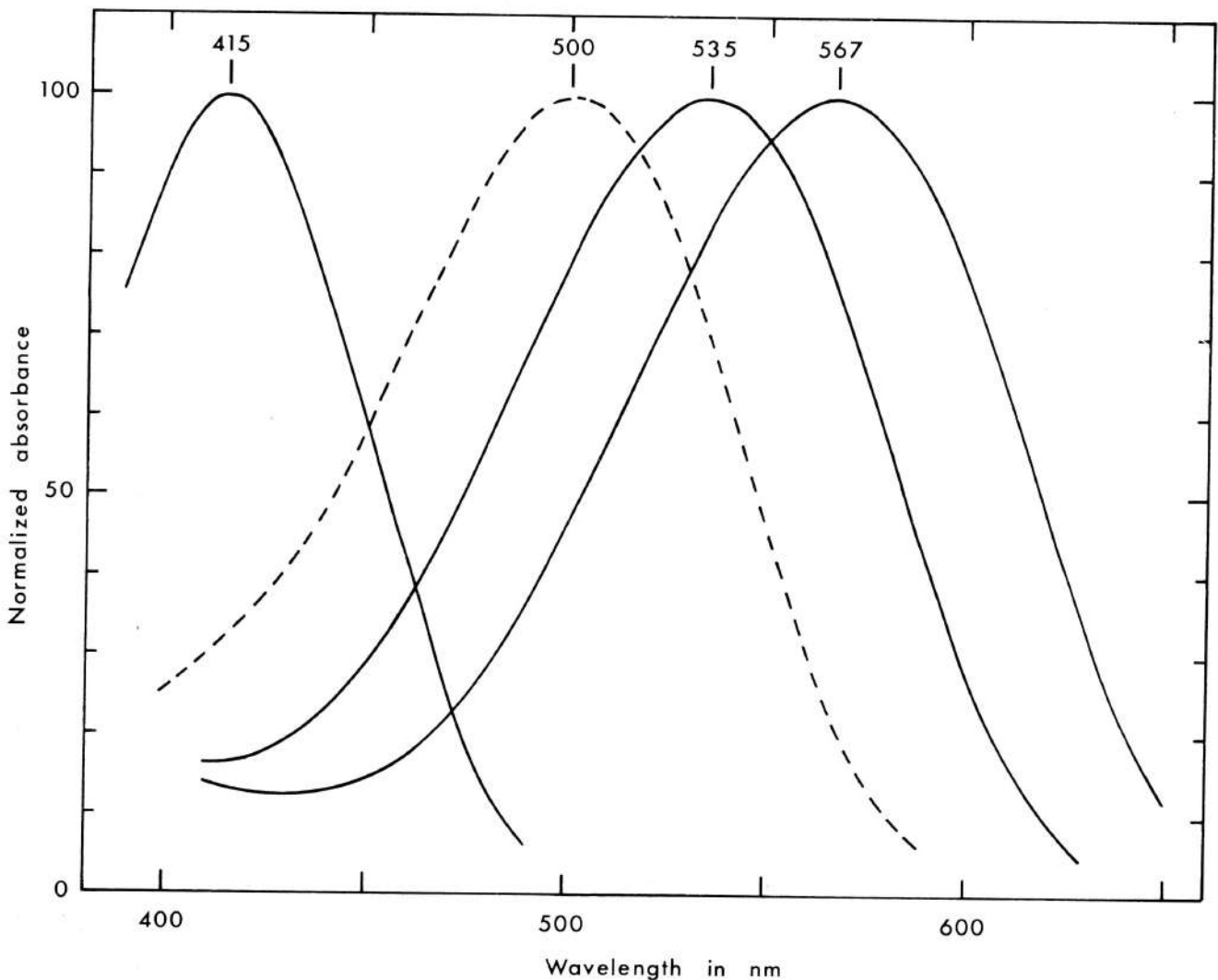

FIG. 2   Mean absorbance spectra for the four classes of receptor cell in *Macaca fascicularis*. The solid curves are for three classes of cone, the dashed line for the rods. The curve for the middle-wavelength cones is based on a selected subset of 16 cells (see text).

In the baboon, *Papio papio*, we have analysed spectra from a total of 144 receptors obtained from five animals. Again these fall into four classes, rods and 3 types of cones (Fig. 3). Analysis of 35 rods yielded a λ_{max} of
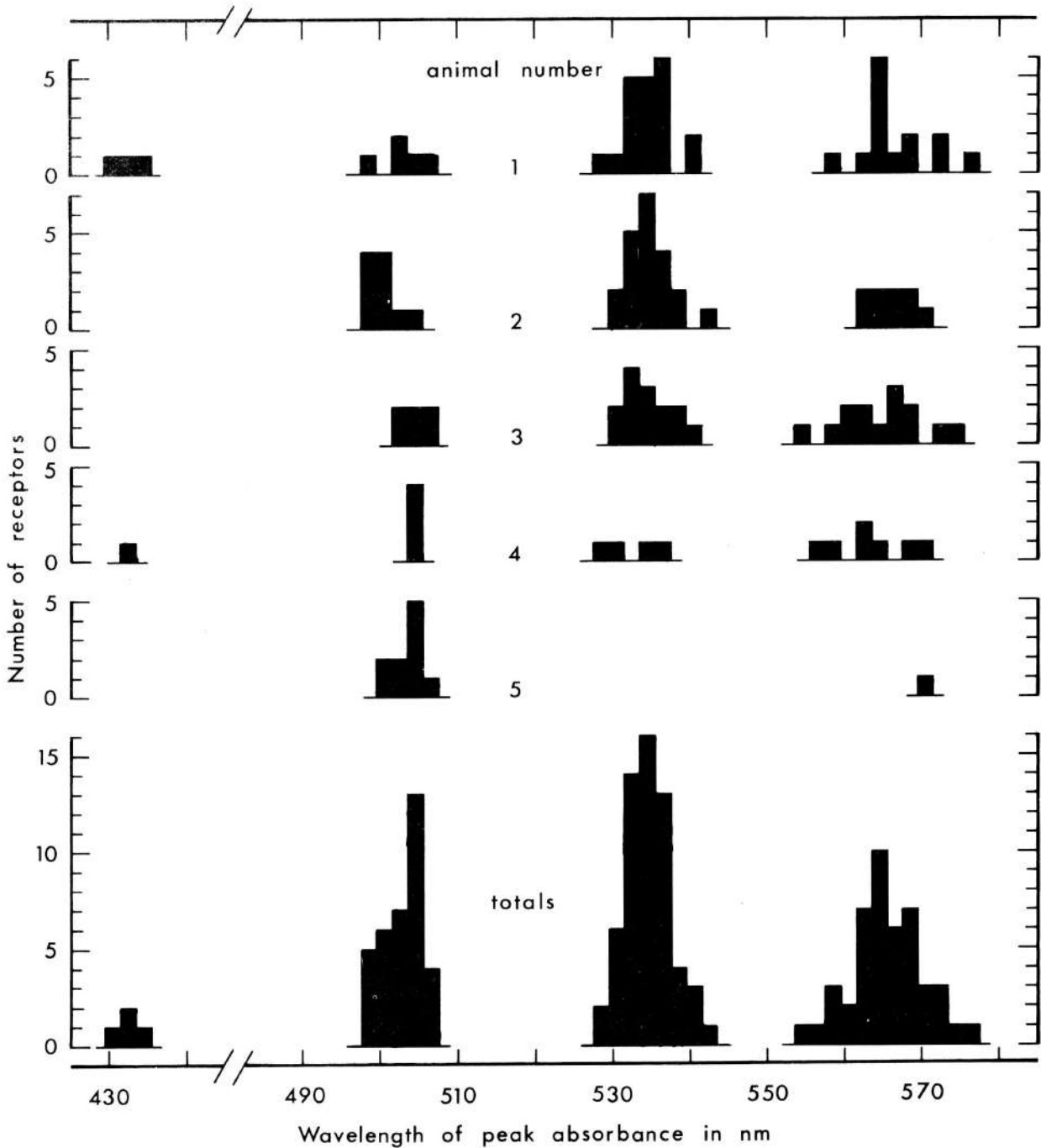

FIG. 3 Distribution of values of peak sensitivity of all individual records from five baboons. The bin size is 2 nm. The λ_{max} for an individual record was obtained using the method described in the text.

502.9 ± 2.4 nm. The long-wave cones had a mean λ_{max} of 565.5 ± 3.6 nm ($n = 45$) and the 60 middle-wave cones had a mean λ_{max} of 534.7 ± 3.2 nm. Four cones were found absorbing maximally at short wavelengths, with a mean λ_{max} of 432.5 ± 1.7 nm.

These results from Old World primates show a remarkable consistency. The mean λ_{max} values for the rods lie close to 500 nm within a 3 nm range, and similarly, the long-wave and middle-wave cones have maxima close to 565 nm and 535 nm falling within a range of only 2 nm in both classes. Less agreement is found between the short-wave cone data where the range is about 15 nm. However, these so-called "blue" cones are rare and only 6 out of a total of 493 receptors have been analysed. In addition, at short wavelengths, the signal to noise ratio of the microspectrophotometer is very low so that analysis becomes more difficult with a subsequent uncertainty as to the exact location of the λ_{max}.

It seems increasingly likely that the Old World monkeys, though very consistent among themselves, are not the exact model for human trichromats that they have sometimes been taken to be. Rather, their photopigments tend to have λ_{max} values several nm longer than those found for the majority of human observers (see Table 1, and Dartnall, Bowmaker and Mollon, this volume).

New World Monkeys

The first New World monkey that we examined, a single male specimen of *Cebus apella*, confirmed the classical view of the Platyrrhini, that their colour vision corresponds to that of human protanopes. We were able to identify only a single class of cones in the red–green region of the spectrum with a mean λ_{max} of 534.3 ± 1.6 nm ($n = 19$), a value very similar to that obtained for the middle-wave receptors of the Old World monkeys. No short-wave receptors were found. The rods also gave a mean λ_{max} similar to other primates with a value of 499.3 ± 3.6 nm ($n = 18$).

However, as has become quite clear from behavioural results (see Jacobs, this volume), the classification of colour vision in New World monkeys is much more complicated and raises many interesting questions. In a collaborative study we have examined a group of ten squirrel monkeys, *Saimiri sciureus*, in which, from behavioural studies, there occur clear variations in colour vision similar to those found in the small percentage of the human population that is considered colour deficient. Correspondingly, we have found, by micro-spectrophotometric analysis, systematic variability in the spectral locations and number of the cone pigments between the individual monkeys.

The behavioural tests, carried out in GHJ's laboratory in Santa Barbara (see Jacobs, this volume) included wavelength discrimination, Rayleigh matches and measurements of sensitivity at 540 nm and 640 nm. The tests were all carried out in a forced-choice discrimination apparatus in which the monkeys viewed three circular, trans-illuminated panels. They were taught to touch one of the three panels which was illuminated differently from the other two, in order to receive a food pellet. Which of the three panels was positive

(i.e. differently illuminated) varied randomly from trial to trial. The behavioural results were not known to the microspectrophotometrists. The animals were flown to London where the microspectrophotometric measurements were made. It proved possible to obtain up to 75 records from a single animal. (Preliminary reports of the results from the first two and first six monkeys have already been published, Jacobs, Bowmaker and Mollon, 1981, 1982.)

From the microspectrophotometric data at least four cone visual pigments were identified. The short-wave cone type was rare, as it is in Old World primates, with only 14 being identified out of a total of about 475 cones, and none being found in two of the ten animals. The mean λ_{max} of these cells was 433.5 ± 2.5 nm, a value similar to that found for *P. papio*. In all animals we recorded rods with maximum absorbance at approximately 500 nm (Table 1).

TABLE 1 λ_{max} of primate visual pigments

	Short	Rod	Middle		Long
Old World					
Macaca mulatta	?	503	535		566
M. fascicularis	415	500	535		567
Papio papio	433	503	535		566
Homo sapiens[*]	419	496	531		558
New World					
Cebus apella	?	499	534		
Saimiri sciureus					
S2, 3, 19	432	501		550	564
S7	433	499	535	545	
S1, 21, 22, 25	435	499	535		
S5	433	503		549	
S24	?	501			562

[*] Data from Dartnall, Bowmaker and Mollon, this volume.

In six of the animals we found only one class of cone in the red–green region of the spectrum, rather than the two we have found in all of the individual Old World monkeys. However, the spectral location of the single pigment was not constant (Fig. 4). In four monkeys, S1, 21, 22 and 25, it appeared to be very similar, the mean λ_{max} values being 537, 534, 535 and 535 nm. This cone type corresponds with that found in the single *Cebus* monkey and also with the middle-wave cones of the Old World monkeys. On this basis these four dichromats can be classified as protanopes, if the analogy with human colour vision is valid. Such a diagnosis is basically in concordance with the behavioural evidence. Animals S21, 22 and 25 failed to make a Rayleigh match, being
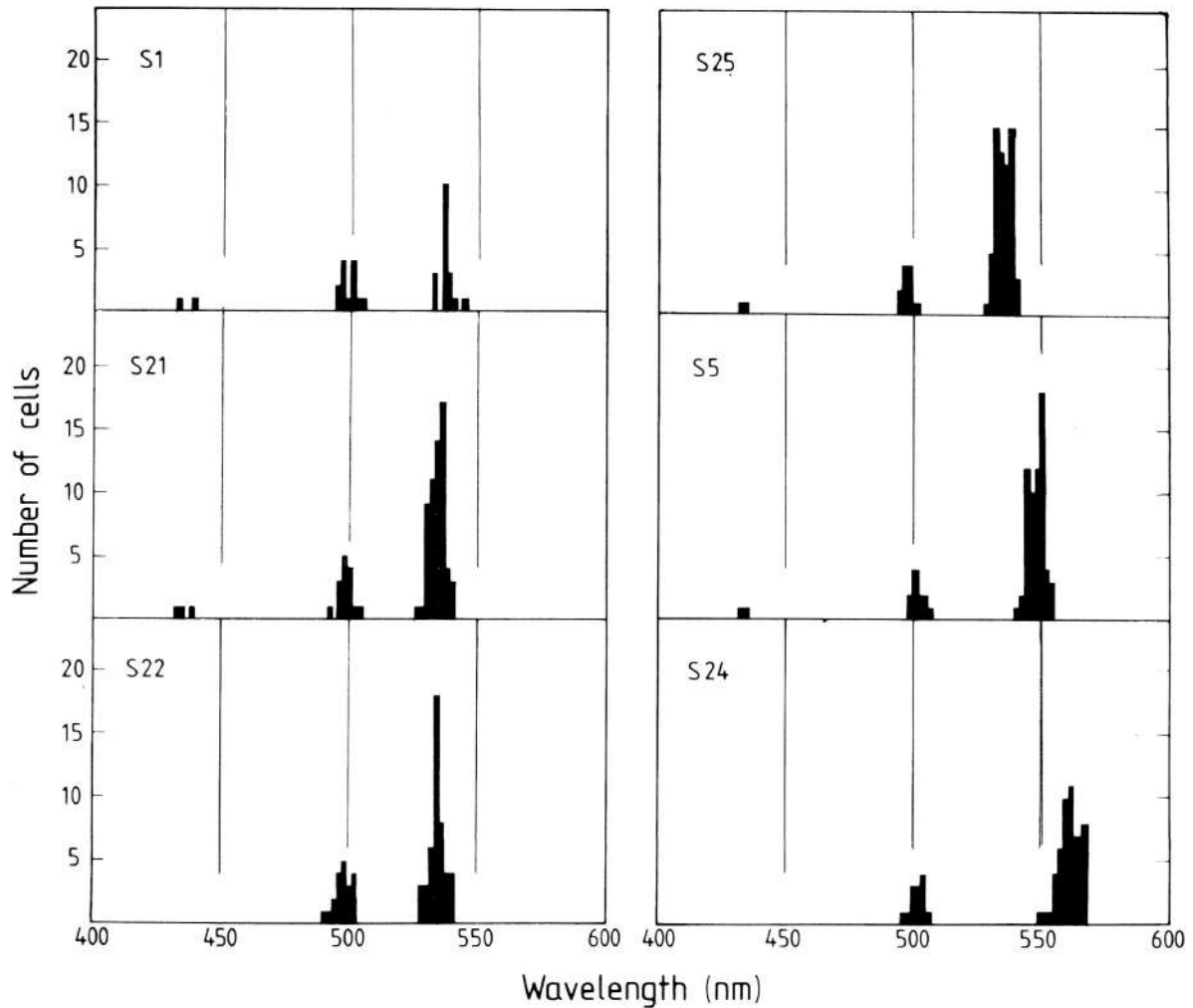
FIG. 4 Distribution of values of peak sensitivity of all individual records from 6 squirrel monkeys that had only a single pigment in the red−green region of the spectrum. S1, 21, 22 and 25 are assumed to contain the same pigment, λ_{max} 535 nm. The mean λ_{max} for S5 is 549 nm and for S24 is 562 nm.

unable to distinguish a pure red or green from yellow, a failure echoed in the wavelength discrimination functions for S21 and S22, which show good discrimination up to about 525 nm, with a minimum at about 490 nm (Fig. 5), but with increasingly poorer discrimination at longer wavelengths. The fourth of this group S1, proved slightly problematical in that after extensive trials it was able to make a Rayleigh match, accepting a very wide range of mixtures on the protan side of the normal human match. In addition, the wavelength discrimination function of S1, although showing only a single minimum at about 490 nm, showed better discrimination than S21, 22 and 25 at wavelengths above 525 nm (Fig. 5). We must leave open the possibility that S1 was an extreme protanomalous subject.

The remaining two dichromats, S5 and S24 were found to have their single pigment in the red−green range at longer wavelengths than the protans. S5 contained a pigment with λ_{max} 549 nm whereas S24 contained a pigment with λ_{max} 562 nm. The longer-wave pigment is similar to the long-wave pigment found
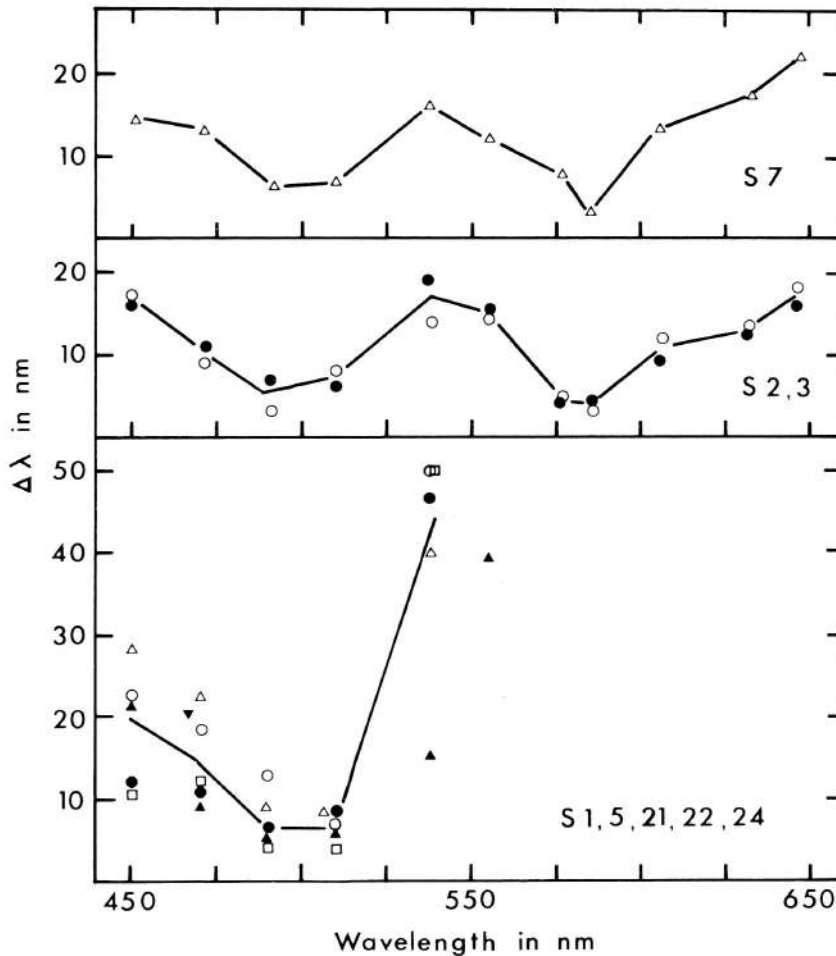
FIG. 5   Wavelength discrimination functions for eight individual squirrel monkeys. The values ($\Delta\lambda$) indicate the magnitude of the wavelength change required at each spectral location to support discrimination between two equiluminant spectral lights at a criterion level of 70% correct. The values are averages for differences in both spectral directions except at 452 nm.

S7, S2 and S3 are diagnosed as trichromats while S1, 5, 21, 22 and 24 are diagnosed as dichromats. S1, possibly severely protanomalous, is represented by the filled triangles in the bottom panel. S19 and S25 were not run on this test.

in Old World monkeys, but the class of cone with λ_{max} 549 nm has not been reported from other primate species. Behaviourally, S5 and S24 did not differ: they were unable to make a Rayleigh match and both showed only a single minimum at 490 nm in their wavelength discrimination function (Fig. 5). However, they differed from the protanopes in that they were more sensitive at 640 nm, as would be expected from the spectral location of their pigments. In terms of human colour vision, S24 would be classified as a deuteranope and S5 may correspond to the rare type of human dichromat said to accept matches of both protanomalous and deuteranomalous observers and thought to share the "anomalous" pigment with them both.

The remaining four squirrel monkeys had distributions of cones in the red−green range that were clearly different from the previous six animals (Fig. 6). None of the distributions show the well separated long- and middle-wave
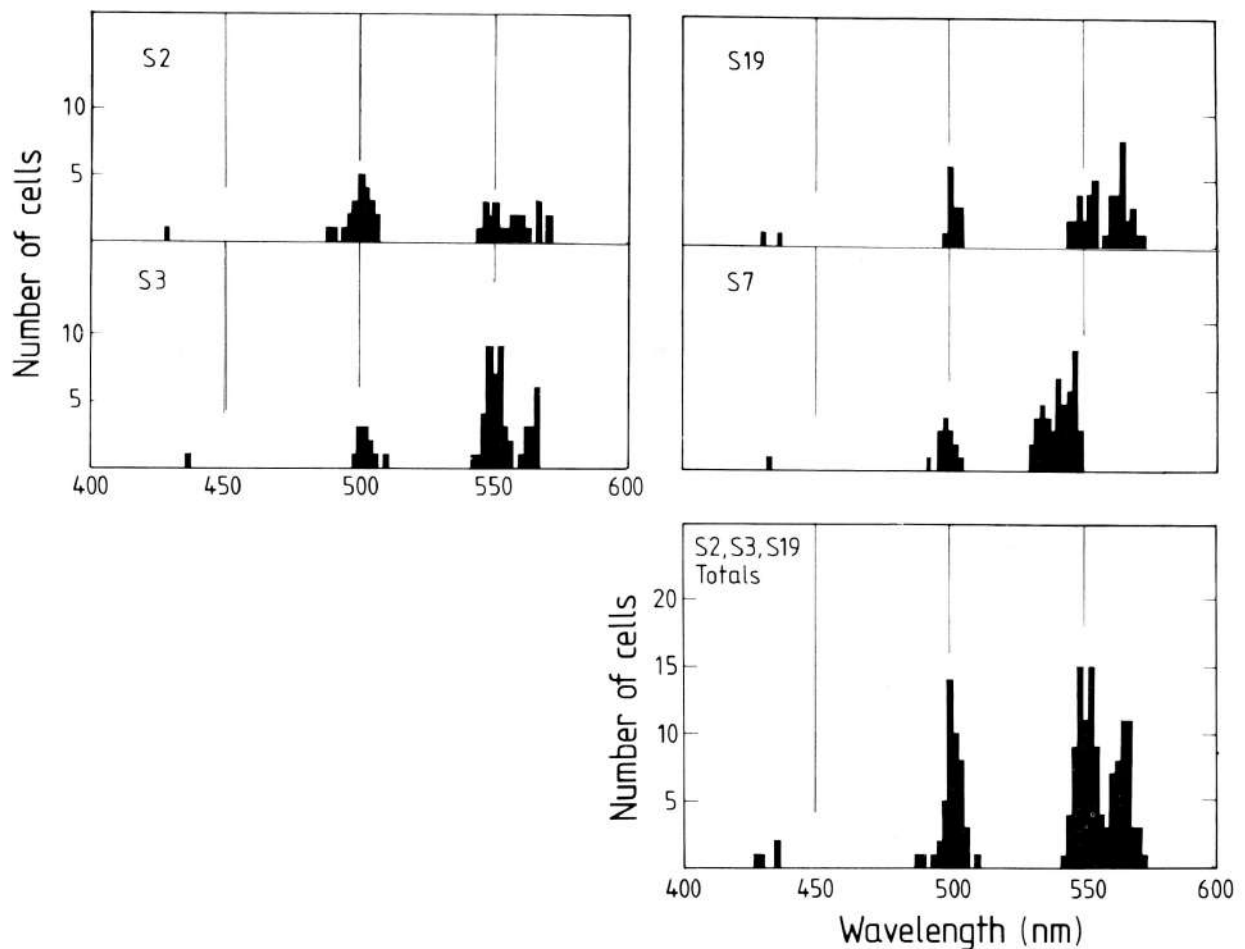
FIG. 6   Distributions of values of peak sensitivity of all individual records from four squirrel monkeys shown behaviourally to be trichromatic. S2, 3 and 19 are thought to be similar, having two presumptive pigments in the red−green range with λ_{max} at 550 and 564 nm. S7, a behaviourally protanomalous animal, has presumptive pigments with λ_{max} at 535 and 545 nm.

cones found in Old World primates, but they can be distinguished from the distributions of the six behavioural dichromats by their variance: the standard deviations of the dichromats are in the limited range of 2.4 to 4.2 nm, whereas the standard deviations of the other four monkeys are in the range 5.2 to 8.5 nm.

Three of the animals S2, 3 and 19 had long-wave receptors in a similar range with clear indications at least in S3 and S19 of a bimodal distribution, a suggestion supported by the total distribution obtained by summing the data from the three animals (Fig. 6). On the assumption that the distribution represents two separate classes of cone we took the mean of the individual λ_{max} values of the two hypothetical classes and obtained λ_{max} values of about 550 and 564 nm (Table 1). The longer of the two pigments corresponds with the long-wave pigment of the Old World primates and the pigment of the dichromat S24, whereas the shorter of the two corresponds with the pigment of the dichromat S5.

The suggestion that S2, 3 and 19 were trichromatic is in agreement with the behavioural results. Wavelength discrimination functions are available for S2 and 3, and show good discrimination over the entire range tested with two minima at about 490 and 580 nm (Fig. 5). All three animals accepted a limited range of Rayleigh matches, though lying to the deutan side of the normal match. The three monkeys can thus be classified, in human terms, as deuteranomalous.

The fourth animal of this group, S7, had receptors with a range of λ_{max} values from about 530 to 550 nm, clearly different from the three deuteranomalous monkeys, with an indication, though slight, of a bimodal distribution. On this slender evidence we divided the distribution into two hypothetical classes of cone with mean λ_{max} at 535 and 545 nm. The shorter of the two presumptive pigments corresponds to the middle-wave pigment of Old World primates and with the pigment of the four squirrel monkeys diagnosed as protanopes. The longer of the two pigments may correspond with the shorter of the two pigments found in the deuteranomalous animals (Table 1).

The tentative suggestion from the microspectrophotometric data that S7 is trichromatic is supported by the behavioural tests. The wavelength discrimination of S7 was good throughout the entire spectral range tested with two minima at about 500 and 580 nm (Fig. 5). The monkey could also make a Rayleigh match, accepting a limited range of mixtures on the protan side of the normal human match.

Thus in contrast to the Old World primates, squirrel monkeys show large variations in their colour vision that are due to variations in the cone pigments in the red–green range. At least three classes of cone, with peak sensitivities at about 535, 550 and 564 nm are present, singly or in pairs, in correspondence with the presence of dichromatic or anomalous trichromatic vision, giving at least five behavioural types. It is interesting that we have not found an animal that resembles a normal human observer or an Old World primate.

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