COLOUR VISION VARIATIONS IN MONKEYS: BEHAVIOURAL AND MICROSPECTROPHOTOMETRIC MEASUREMENTS ON THE SAME INDIVIDUALS

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ABSTRACT

Behavioural and microspectrophotometric measurements made on individual squirrel monkeys show that the variations in colour vision seen in this species are due to variations in the cone pigments having maximal sensitivity in the middle to long wavelengths. Three classes of cones with peak sensitivity at 536, 550 and 564 nm are present singly or in pairs in correspondence with the presence of dichromatic or anomalous trichromatic colour vision.

INTRODUCTION

Definition of the spectra of the photopigments underlying normal and defective colour vision has been attempted in many different ways. Recent work shows that it is now possible to use microspectrophotometry (MSP) to obtain precise measurements of primate cone pigments (Bowmaker, Dartnall, Lythgoe and Mollon, 1978; Bowmaker, Dartnall and Mollon, 1980; Bowmaker and Dartnall, 1980; Bowmaker and Mollon, 1980). There is thus the inviting possibility of obtaining direct measurements of the cone pigments present in the retinas of known colour defectives. One difficulty in accomplishing this, however, is in obtaining suitable human retinas for such investigations. An alternative is to make use of a species of nonhuman primates whose colour vision is known to show significant within-species variations similar to those seen in man. Our work follows this latter approach – it involves joint behavioural and MSP measurements on squirrel monkeys (Jacobs, Bowmaker and Mollon, 1981).

BEHAVIOURAL MEASUREMENTS

The subjects were from the platyrrhine species *Saimiri sciureus*. Those examined were all of the subtype typically exported through Iquitos, Peru. The behavioural measurements were made in forced-choice discrimination tests in which the animal was trained to select one from among three small

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stimulus panels, the one (positive) being differently illuminated from the other two panels. The animal indicated its selection by touching one of the panels and, if correct, was rewarded with the delivery of a small food pellet. Over trials and test problems the nature of the difference between the positive and two negative panels was systematically changed so as to permit measurements of the subject's sensitivity to luminance or colour differences. In this context monkeys were tested on several different discrimination problems, each usually being run over a duration of many months.

One of the tests run on squirrel monkeys involved determining detection thresholds for monochromatic lights (540 and 640 nm) superimposed on achromatic backgrounds. Several years ago it was discovered that individual squirrel monkeys vary significantly in their sensitivity to middle and long wavelengths (Jacobs, 1972; 1977). Whereas there is no significant variation in sensitivity to a 540 nm light, there are large individual variations (more than 1 log unit) in sensitivity to 640 nm light. That these variations reflect real sensitivity differences, and nor merely some inadequacies in the measurement, is shown by the fact that thresholds measured on two occasions on individual animals show an average difference of less than 0.2 log units. In reporting this sensitivity variation it was noted that it is sex-linked in the sense that females tend to have higher sensitivity to 640 nm light than do males (Jacobs, 1977). Additional animals tested since the initial report verify the presence of large within-species variations among squirrel monkeys. However, it is now also clear that, although on average females are more sensitive to 640 nm than males, the sex difference in sensitivity is not an absolute one: individual female squirrel monkeys may be as insensitive to the long wavelength light as male monkeys.

These variations in visual sensitivity strongly suggested the presence of corresponding within-species variations in colour vision among squirrel monkeys. This expectation has been fulfilled. This report focuses on results obtained from six monkeys (five females, one male) who were tested for colour vision and then examined with the microspectrophotometer.

We consider here results from two tests of colour vision. One was an anomaloscope test in which the relative proportions of mixed red and green light that the animal was unable to discriminate from a yellow light was determined. In this case the mixed light was the positive stimulus while the other two were a fixed yellow (luminance = $6.2\,\mathrm{cd.m^{-2}}$). The animal was initially trained to discriminate alternately a pure red and a pure green light from the yellows. Once these discriminations were acquired, the animal was further tested for its ability to discriminate various red/green mixtures from the yellow. Potential luminance cues were controlled by (a) equating the mixed light and the yellows on the basis of previously determined spectral sensitivity values, and (b) then varying luminance randomly around this value (usually over a range of 0.6 log units in steps of 0.1 log units). The Rayleigh match was defined as encompassing those red/green mixtures the animal was unable to discriminate successfully at a level of greater than 60% correct.

The results of the anomaloscope test are shown in Figure 1. The top panel of that figure shows the range of the match for each subject as defined above; the □s represent the match midpoints. The two bottom panels of Fig. 1

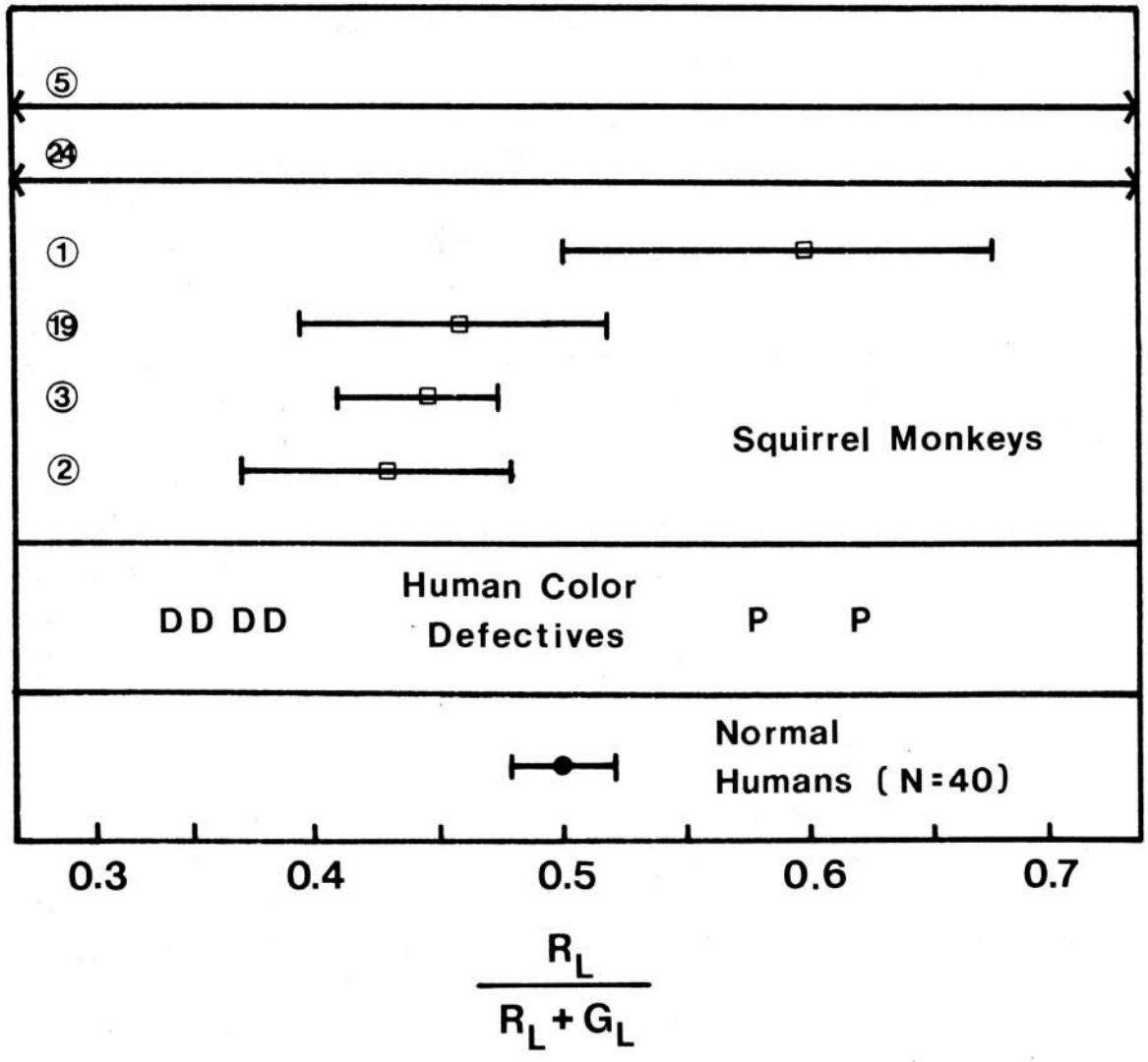


Fig. 1. Rayleigh matches for squirrel monkeys, human colour defectives, and normal human trichromats tested in the same apparatus. The techniques used for measurement are described in the text. For the squirrel monkeys the horizontal lines mark the range of the acceptable Rayleigh matches while the □s are the match midpoints. Subjects 5 and 24 were unable to discriminate any mixture combinations from yellow. Rayleigh matches are included for six humans having colour defective vision (D: deuteranomalous; P: protanomalous). For the normal trichromats the solid circle shows the mean match for 40 subjects while the horizontal line indicates two standard deviations.

provide a comparative perspective giving the Rayleigh matches for human subjects tested in the same apparatus. The middle panel shows match locations for six anomalous trichromats while the bottom panel shows the average match for 40 normal trichromats.

The three animals (Nos. 2, 3, 19) whose results are shown at the bottom of the upper panel all behaved very similarly. All succeeded at the discrimination over a wide range of different red/green mixtures. Their match ranges are relatively restricted with the mid-points being located slightly, but consistently, to the green side of that for the normal humans. The other three animals gave significantly different results. One of these (No. 1) had great difficulty in acquiring the discrimination, but was eventually able to discriminate some red/green mixtures from yellow. Her match range was large and the midpoint

is located well over on the red side relative to the normal humans. Even after extended training the final two animals failed to learn to discriminate yellow from either red or green.

Wavelength discrimination was measured in a second colour vision test. The same test apparatus and general methods were used: in this case the positive light came from a monochromator while the two negative lights (the standard) were obtained by passing beams from tungsten-filament sources through Wratten filters. For any standard value, the animal was trained to discriminate the monochromator light set initially at values alternately shorter and longer than that of the standard. Once these discriminations were acquired, the wavelength of the monochromatic light was systematically varied in steps of 5 and 10 nm around that of the standard light. Wavelength discrimination values were defined as the difference between the wavelengths of the positive and negative lights required to support a discrimination at a criterial value of 70% correct. Eleven different spectral locations were tested. Potential luminance cues were eliminated through the use of the procedures described above for the anomaloscope experiment.

The wavelength discrimination results for the six subjects are summarized in Figure 2. The wavelength discrimination capacities of these animals fall into two homogeneous groups. Subjects 2, 3, and 19 (indicated by the open circles in Fig. 2) showed relatively good wavelength discrimination over the entire range tested. Their functions have twin minima at about 500 and 580 nm, and they are relatively poorer at discriminating through the greens and at the spectral extremes. Subjects 1, 5, and 24 also produced very similar wavelength discrimination functions. These animals also had good sensitivity at about 500 nm. However, at longer wavelengths than this their ability to discriminate declined rapidly, and they were unable to show any significant wavelength discrimination beyond about 550 nm.

If one wishes to describe the colour vision of these six monkeys in terms of human colour vision categories, the results from these two tests suggest the following conclusion. Monkeys 2, 3, and 19 performed nearly identically in both tests. As judged by the shapes of their wavelength discrimination functions, and the precision of their Rayleigh matches, they are trichromats. However, all required significantly more green in the Rayleigh matches than did the normal trichromats so, even though they are not as aberrant in this direction as some human deuteranomalous observers (see Fig. 1), they can be categorised as having deuteranomalous colour vision. The wavelength discrimination functions of subjects 1, 5, and 24 are similar to those obtained from human dichromats or extreme anomalous trichromats (Wright, 1947). Monkeys 5 and 24 were additionally unable to discriminate any anomaloscope settings so they were judged to be dichromatic. The remaining animal (No. 1) was able to make some discriminations in the anomaloscope task. Her performance thus suggests a severe protanomalous trichromacy, although results from similar tests on human colour defectives indicate that a diagnosis of protanopia might also be supported (Smith and Pokorny, 1977; Nagy, 1980).

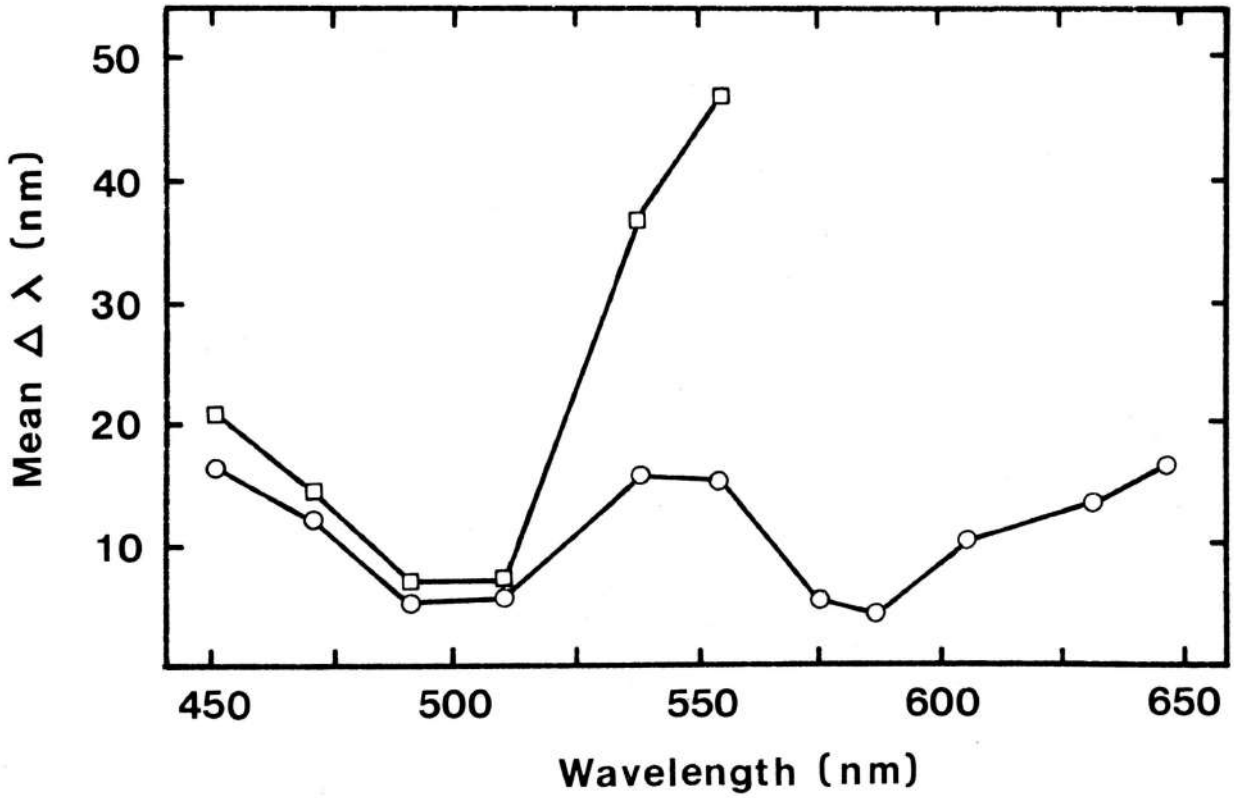


Fig. 2. Squirrel monkey wavelength discrimination functions. Shown separately are the mean wavelength discrimination values for monkeys 2, 3, and 19 (○——○) and monkeys, 1, 5, and 24 (□——□).

MICROSPECTROPHOTOMETRY

The MSP and behavioural measurements were conducted in a double-blind fashion so that the behavioural results were not known to the microspectrophotometrists and vice versa until both sets of data had been deposited with an independent third party. The behavioural experiments were conducted in Santa Barbara and the animals were then flown to London.

MSP measurements were made in London using a modified Liebman microspectrophotometer (Liebman and Entine, 1964; Knowles and Dartnall, 1977) run under computer control. The monkeys were narcotised with ketamine and then sacrificed with an overdose of pentobarbitone sodium. Measurements began within 1–2 hours after death. The preparation of tissue had been described previously (Bowmaker et al., 1980). Several samples of tissue were taken from each retina. The microspectrophotometer was programmed to step from 700 to 390 nm in 2 nm steps and then to return making measurements at the interleaved wavelengths. The measuring beam passed transversely through the outer segments of individual receptors and a reference beam passed through adjacent clear space in the preparation.

Extensive numbers of records were obtained from all six monkeys. Measurements were obtained from numerous rods (λ_{max} ca 500 nm). Several short wavelength cones (λ_{max} ca 430 nm) were also detected. These two classes of receptors will not be further discussed here. In addition, a total of 250 cones absorbing maximally in the middle and long wavelengths were

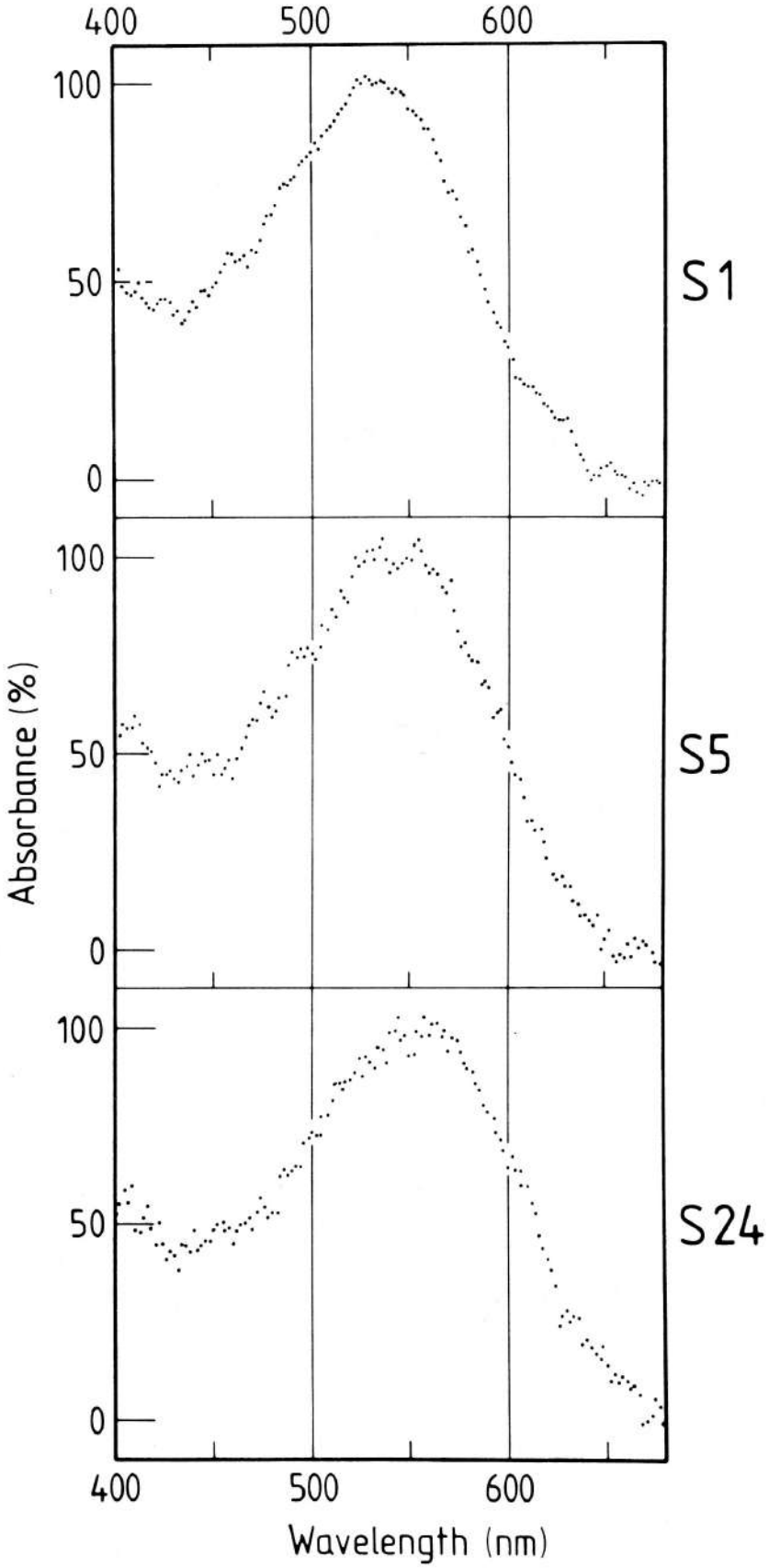


Fig. 3. Mean absorbance spectra for the long-wavelength receptors from squirrel monkeys S1, S5, S24. Each datum point corresponds to the average of values obtained at two adjacent wavelengths, one recorded in the descending scan of the microspectrophotometer, one recorded in the ascending scan. Absorbance values for different receptors were averaged before being normalised.

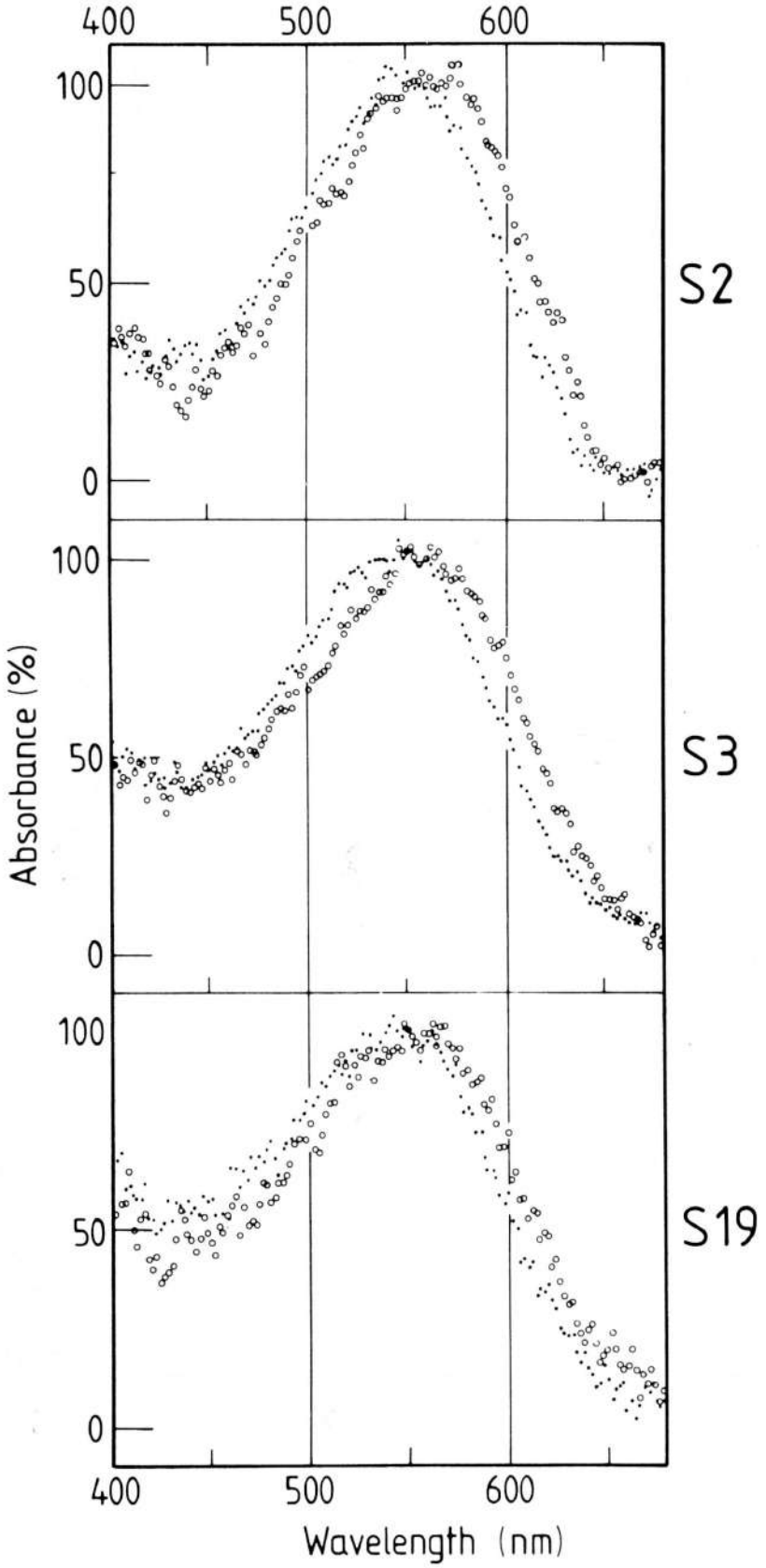
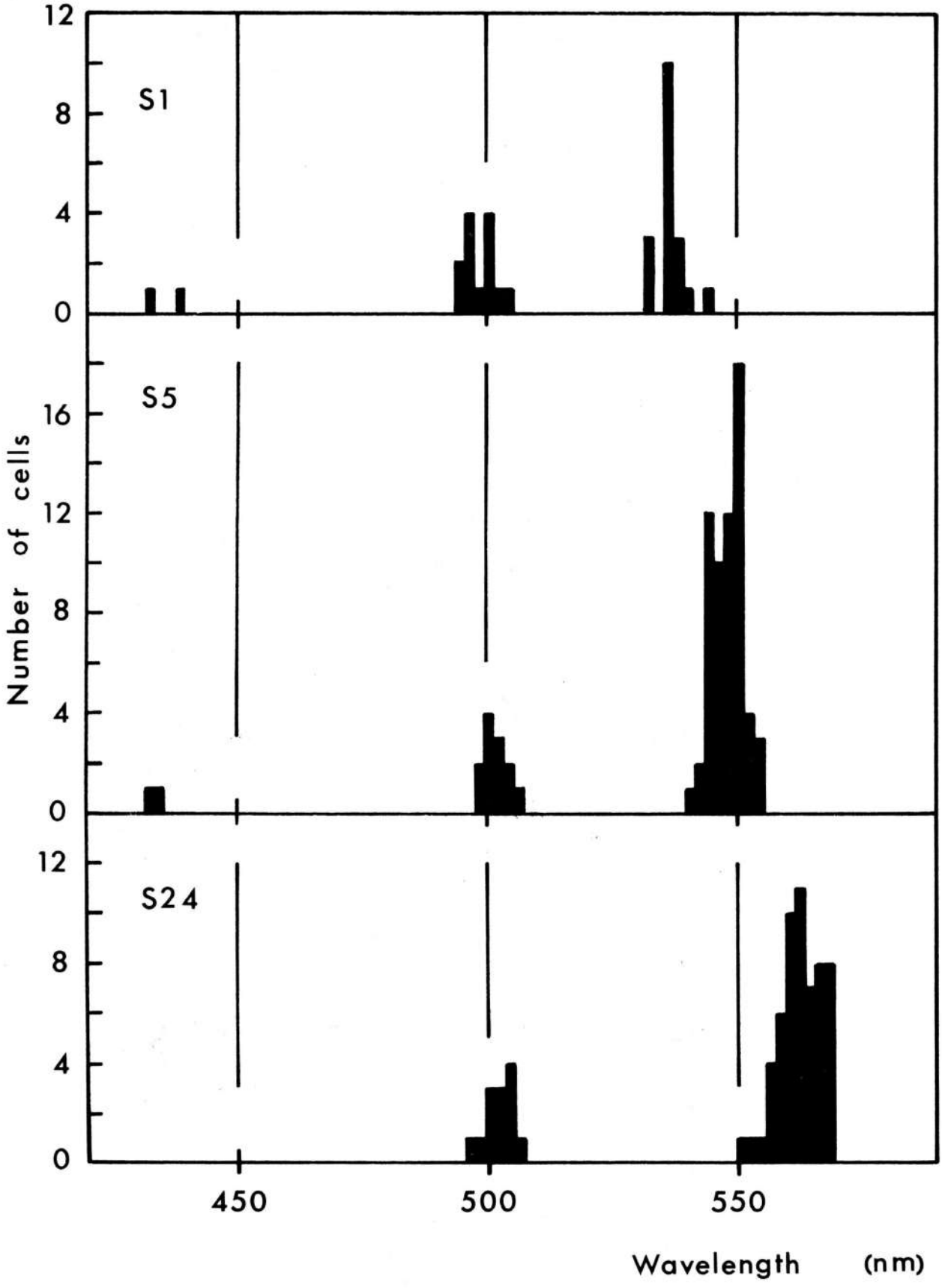


Fig. 4. Mean absorbance spectra for the two putative types of long-wavelength receptor from monkeys S2, S3, S19. On the basis of the distributions of λ_{max} values for individual records for S3 and S19 (see Figure 5), individual records were assigned to one of two classes according to whether the λ_{max} lay above or below 558 nm; the individual absorbance spectra were then averaged and normalised. In the case of each animal the two absorbance spectra show similar values at short-wavelengths; this would be unlikely to be the case if the apparent presence of two groups arose from photoproducts or from optical scattering.


S1
S5
S24
Number of cells
0
4
8
12
16
450
500
550
Wavelength (nm)

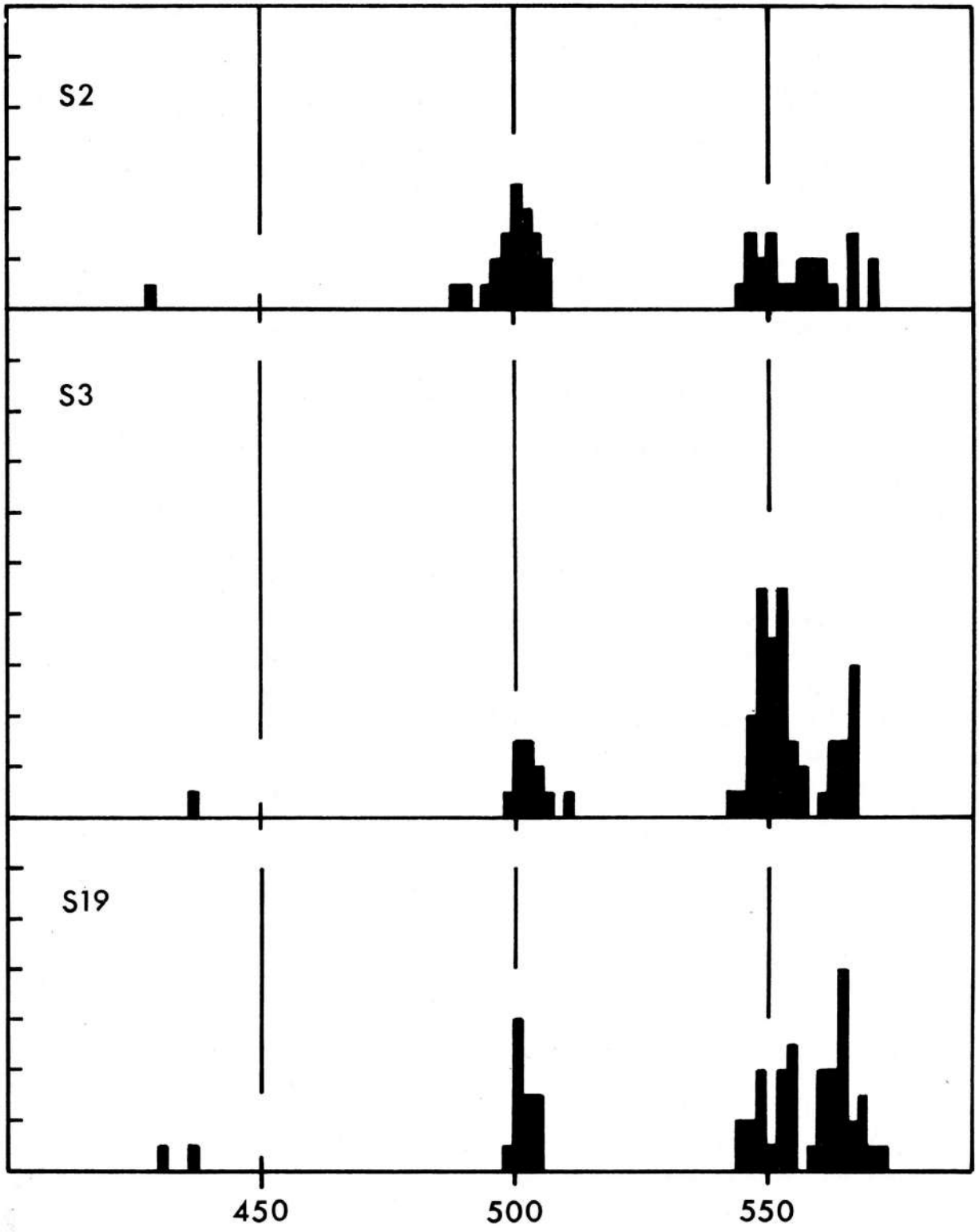


Fig. 5. Distribution of values of peak sensitivity of all individual records from six squirrel monkeys. The bin size is 2 nm. The λ_{max} of an individual record was obtained by fitting an appropriate nomogram to the absorbance spectrum using a modification of the procedures described earlier (Bowmaker et al., 1980; Jacobs et al., 1981). Absorbance values for pairs of adjacent wavelengths were first averaged. Each of a set of twenty such values on the long-wavelength limb (corresponding to 40 nm and to percent absorbance in the range approximately 45–90%) was then referred to an appropriate nomogram to estimate the λ_{max}; this operation amounts to finding where the nomogram must be placed on a wavenumber abscissa to yield the absorbance value under consideration. The twenty individual estimates of the λ_{max} were then averaged to give the value entered in the histogram.

measured. As defined by locations of peak absorbance, these cones appear to fall into three different classes. Figure 5 gives the distribution of peak sensitivities for all individual records recorded from all six animals while Figures 3 and 4 show for each of the monkeys the mean absorbance curve for each putative class of photoreceptor.

The MSP measurements made on monkeys 1, 5 and 24 are summarised in Figs. 3 and 5. All of these animals were found to have only a single class of photopigment in the middle to long wavelength portion of the spectrum, but no class was common to two animals. The subject classified as a severe protan by behavioural tests (No. 1) had a single class of photopigment having a λ_{max} of 536 nm (n = 18); the standard deviation of the estimated values of λ_{max} for individual cells (Fig. 5) was 3.1 nm. The other two subjects classified as dichromatic also had only a single class of photopigment: for No. 5 the mean λ_{max} was 549 nm (n = 62; SD for individual estimates = 3.2 nm) and for No. 24 the mean λ_{max} was 562 nm (n = 56; SD = 4.3 nm).

Figures 4 and 5 provide a summary of the MSP measurements made on monkeys Nos. 2, 3 and 19. All of these animals had been previously characterized as deuteranomalous. For each of these subjects, photoreceptors having a broad range of peak absorbances were recorded, covering a total range from 542 to 572 nm. On the basis of data from one of these monkeys, we suggested previously that more than one class of photoreceptor was present (Jacobs et al., 1981). Figure 5 supports this suspicion and suggests a clear bimodality in the long-wave cones of animals 3 and 19. Summed across the three animals, one of these classes has a λ_{max} of 550.2 (n = 67), while the other has a mean λ_{max} of 564.1 nm (n = 47).

DISCUSSION

The MSP measurements thus far completed on squirrel monkey retinas indicate the presence of three different classes of photoreceptors absorbing maximally in the middle to long wavelengths. Two of these classes (λ_{max} = 536 and 564 nm) have absorbance peaks closely similar to the middle and long wavelength cones previously measured in the retinas of humans (λ_{max} = 534 and 563 nm), cynomologus monkeys (λ_{max} = 535 and 567 nm), and rhesus monkeys (λ_{max} = 536 and 565 nm) (Bowmaker and Dartnall, 1980; Bowmaker et al., 1980; Bowmaker et al., 1978). The third class (λ_{max} = 550 nm) has not been previously detected in primate retinas.

Although more detailed comparisons will be made in subsequent reports, it is obvious that to a first approximation the MSP and behavioural results show very good agreement. The three animals judged to be trichromats were found to have two classes of photopigments in the middle to long wavelengths while the two dichromats have only one pigment present in this spectral region. The animal (No. 1) for whom the behavioural measurements indicated either severely anomalous or dichromatic colour vision had only one class of photopigment, and as judged from the MSP perspective the second diagnosis might be argued to be correct. These results also conform to general expectations from colour vision theory. From that perspective one

dichromat (No. 1) is a protanope, one is a deuteranope (No. 24), and one has a dichromacy based on the sole presence of a 550 nm photopigment. There have been occasional reports of human dichromats who resemble monkey 5 and who, it has been suggested, have a single pigment common to both deuteranomalous and protanomalous observers (De Vries, 1948). Perhaps the most compelling case is that of the three deuteranomalous squirrel monkeys. The two pigment classes found in the retinas of these animals have spectral absorbance peaks close to those hypothesized to account for simple deuteranomalous colour vision among humans (cf. Pokorny, Smith, Verriest and Pinckers, 1979). We emphasize that the monkeys whose data are reported here may not be exhaustively representative of all colour vision variations in these species. Indeed, behavioural results already available strongly suggest that at least one other characteristic cone pigment pattern awaits detection by MSP.

There are large within-species variations in visual sensitivity and in colour vision among squirrel monkeys. The MSP measurements establish that these variations are due to variability among the cone photopigments. These results raise intriguing questions about the normal functional roles of such variation, the mechanisms that produce the variation, and the evolutionary backgrounds against which this variation has arisen. None of these issues can be understood at the present, but it is worth noting that within-species variations of this kind may also be characteristic of other New World monkeys. The classical view that all New World monkeys have protan defects is clearly wrong. Although the evidence is less extensive than for the squirrel monkey, it appears that there are within-species variations in colour vision among both Cebus monkeys (Gunter, Feigenson and Blakeslee, 1965; Lepore, Lassonde, Ptito and Cardu, 1975) and spider monkeys (Blakeslee and Jacobs, 1981).

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