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POLYMORPHISM OF THE MIDDLE WAVELENGTH CONE IN TWO SPECIES OF SOUTH AMERICAN MONKEY: *CEBUS APELLA* AND *CALLICEBUS MOLOCH*

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Abstract—The spectral sensitivity of the middle wavelength cone was measured in two species of South American monkey, *Cebus apella* and *Callicebus moloch*, using electroretinogram (ERG) flicker photometry. Both of these species were found to have a polymorphism of the middle wavelength cone. Eight male *Cebus* monkeys each had only a single type of middle wavelength cone having λ_{max} values of either 550 or 562 nm. Eight *Callicebus* monkeys (7 male, 1 female) showed a similar polymorphism of the middle wavelength cone (λ_{max} = 549 or 561 nm). A single female of this latter species had two types of middle wavelength cone. The cone polymorphisms of these species appear very similar to that previously described for the squirrel monkey (*Saimiri sciureus*).

Cone photopigments Polymorphism South American monkeys

INTRODUCTION

In the past several years it has been established that there is a pronounced polymorphism of color vision in the squirrel monkey (*Saimiri sciureus*). Behavioral tests indicate the presence of six distinct varieties of color vision in this species, three types of trichromacy and three types of dichromacy (Jacobs, 1984; Jacobs and Blakeslee, 1984). Microspectrophotometric (MSP) measurements of cone pigments in squirrel monkeys have further revealed that this variation in color vision is based on individual variations in the cone pigment complement (Mollon *et al.*, 1984; Bowmaker *et al.*, 1985; in preparation). Four types of cone pigment are found in this species. One of these, having peak absorbance (λ_{max}) at about 434 nm, is believed common to all animals. In addition, there are three pigments having λ_{max} values of about 536, 549, and 564 nm. Individual monkeys have either any one of these latter three and dichromatic color vision, or they have any pair of these three and are trichromats.

A unique feature of the color vision polymorphism of the squirrel monkey is that although all six of the phenotypes are represented among female monkeys, males express only the three dichromatic phenotypes. This difference led to the suggestion that the polymorphism and inheritance of color vision in the squirrel monkey could be explained if it were assumed that the three middle to long wavelength cone pigments arose from the activity of three alleles found at a single locus on the X-chromosome (Mollon *et al.*, 1984; Jacobs and Neitz, 1985). A recent examination of individual cone complements in a large sample of squirrel monkeys, including those for members of several families, has yielded evidence strongly supportive of this single locus model (Jacobs and Neitz, 1987).

The polymorphism of color vision in the squirrel monkey is strikingly different from the individual variations in color vision seen among Old World primate species, including man (Jacobs, 1983). In the species from that group that have been studied, individual variations in color vision are relatively rare or, for some species, perhaps absent entirely. That difference suggests the evolution of color vision may be at quite different stages in Old World and New World primates. In order to evaluate that proposition further it would be useful to know if the squirrel monkey represents an isolated case or whether a similar polymorphism of color vision might be found among other species of New World primates. We have begun an evaluation of this question through an examination of the middle-wavelength cone mechanisms in two other species from the family *Cebidae*, a group that includes the squirrel monkey. These species are *Cebus apella* (the Tufted Capuchin) and *Callicebus moloch* (the Dusky Titi). The spectral properties of the cone pigments in each of these

species were estimated through the use of a noninvasive electrophysiological technique involving the recording of a retinal gross potential, the electroretinogram (ERG). This response was examined using a flicker photometric procedure (Neitz and Jacobs, 1984; Jacobs, Neitz and Crognale, 1985).

METHOD

Subjects

Monkeys were tested at two locations. Eight adult *Cebus apella*, all male, were examined at the Animal Behavior Laboratory of the Department of Psychology, University of Arizona, Tucson. Nine adult *Callicebus moloch* (7 male, 2 female) were drawn from a larger colony of this species maintained at the California Primate Research Center, Davis.

Apparatus

The apparatus and procedures have been fully described in recent publications (Neitz and Jacobs, 1984; Jacobs and Neitz, 1987). Only brief descriptions of these features are thus included here.

Stimuli were produced with a three-beam optical system. The output from this system was a 53° circular spot presented in Maxwellian view. One beam (the test light) came from a grating monochromator. A second beam used as the reference light originated from a tungsten–halide lamp as did a third beam that was used for accessory adaptation. Each beam contained a high-speed electromagnetic shutter controlled by a digital timer.

ERGs were recorded using a bipolar contact lens electrode. In the flicker photometric procedure, ERGs elicited by a train of pulses from the monochromator are compared to those elicited from an interspersed train of light pulses from a fixed reference light (Neitz and Jacobs, 1984). Over test presentations the radiance of the test light is varied by adjusting the setting of a neutral density wedge until the response elicited by the test light produces an ERG that is equal to the ERG elicited by pulses from the reference light. The dependent measure is the radiance required to produce this effect for each of a series of test wavelengths.

Procedure

Monkeys were anesthetized with initial intramuscular injections of ketamine hydrochloride ($15\,mg \cdot kg^{-1}$) plus $0.15\,mg \cdot kg^{-1}$ of acepromazine maleate and, subsequently, by $10\,mg \cdot kg^{-1}$ of sodium pentobarbital. For *Cebus* monkeys the latter was given as an intraperitoneal injection; for *Callicebus* this was delivered through a catheter inserted into the saphenous vein. Atropine sulfate was used to inhibit salivation and respiratory tract secretions. The pupil of the eye to be tested was dilated by topical application of atropine sulfate (0.04%) and Neosynephrine (phenylephrine HCl). The animals were positioned for recording using a specially designed head holder consisting of several large neoprene pads designed to conform to the contours of the skull of each species.

Photometric equations were made in the manner noted above; that is, by adjusting the radiance of a monochromatic test light until the ERG it produced best matched that elicited by a reference light. The pulse rate used throughout these experiments was 62 Hz. The reference light was achromatic (color temperature = 2850 K; corneal radiance = 0.05 mW). Sensitivity measurements were made at 10 nm intervals over the spectral range from 450 to 650 nm. Recordings were carried out in brightly illuminated experimental rooms.

The wedge settings obtained at each test wavelength were corrected for spectral variations in the output from the monochromator and in wedge transmittance. These corrected values were compared to wavelength-dependent visual pigment nomograms using polynomial expressions (Dawis, 1981). A computer was used to determine the spectral positioning of the nomogram that gave the best fit to the array of sensitivity values. This was accomplished by computing an index of goodness of fit between the data array and every possible nomogram (λ_{max} steps = 1 nm).

To determine if more than one middle-wavelength cone mechanism contributed to the response, each subject was also tested for the presence of a chromatic adaptation effect (Jacobs and Neitz, 1987). To accomplish this a flicker photometric equation was made between a 540 nm test light and a 630 nm reference light. These equations were alternately made in the presence of 540 and 630 nm accessory adaptation. The radiances of these adaptation lights were initially adjusted so that each elevated the threshold for obtaining a criterion response to the 540 nm test light by an additional 0.5 log unit.

RESULTS

Middle wavelength cones in Cebus apella

On the basis of earlier work (Neitz and Jacobs, 1984; Jacobs and Neitz, 1987) it seemed likely that with the stimulus conditions employed neither rods nor short-wavelength-sensitive cones would provide any contribution to the ERG responses. The chromatic adaptation test was consequently assumed to provide an explicit test of whether there was one or more than one spectral mechanism having maximal sensitivity in the middle to long wavelengths in the retinas of the *Cebus* monkeys tested. The results of this test were clear and consistent. In none of the eight animals was there any significant shift in the 540/630 nm photometric equation between the two conditions of adaptation. For five of the animals, in fact, the thresholds were identical under the two conditions while for the other three the threshold difference did not exceed 0.01 log unit. Squirrel monkeys known on independent grounds to have more than one type of middle to long wavelength cone pigment show threshold shifts in excess of 0.06 log unit under test conditions identical to those employed in this experiment (Jacobs and Neitz, 1987). This result thus provides evidence that the *Cebus* monkeys tested in this experiment each have only a single cone class in the middle to long wavelengths.

The spectral sensitivity functions determined with ERG flicker photometry fell into two separate classes. In three animals peak sensitivity was very close to 550 nm. The functions obtained from these animals are given in Fig. 1. It will be seen that over the spectral range from 460 to 650 nm these sensitivity measurements are extremely well accounted for by the functions generated from wavelength dependent visual pigment nomograms. For this group the mean spectral absorbance peak was 550.3 nm.

The other five *Cebus* monkeys formed a second, similarly homogeneous, group. The spectral sensitivity functions for these animals are given in Fig. 2. The data points are again very well fit by the nomograms. The mean λ_{max} value for this group was 562.2 nm.

Middle wavelength cones in Callicebus moloch

Chromatic adaptation produced no differential effect in the ERGs of eight out of nine of these monkeys. In each case the photometric equation established under one chromatic adaptation condition held equally well

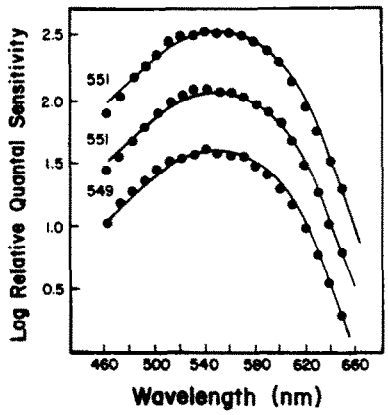

Fig. 1. Flicker photometric spectral sensitivity functions obtained from three *Cebus* monkeys. These animals have one of the two types of dichromacy found in this species. The solid circles are sensitivity values obtained by equating the effectiveness of monochromatic test lights and an achromatic reference light. The solid lines are the best fitting visual pigment nomograms, the spectral peak of which is given on each function. The curves are arbitrarily positioned on the sensitivity axis.

when the chromatic adaptation condition was changed. Thus each of these animals also appears to have only a single spectral mechanism in the middle to long wavelengths. And as for the *Cebus* monkeys, the spectral sensitivity functions from these eight *Callicebus* monkeys divide into two clearly differentiated groups. Figure 3 shows spectral sensitivity functions for four of these animals. These are all close to one another in spectral peak (mean λ_{max} = 548.8 nm). The fitted curves for the other four animals are given in Fig. 4. The peaks of these latter functions cover a short spectral range

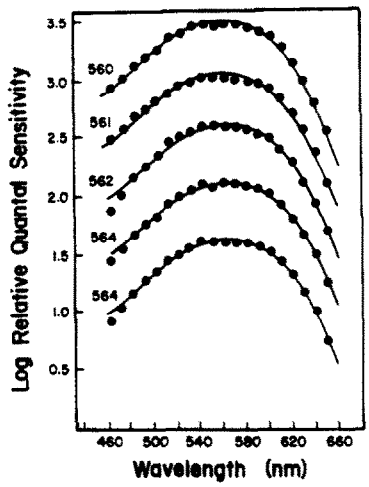

Fig. 2. Flicker photometric spectral sensitivity functions obtained from five *Cebus* monkeys. These animals have the second of the two types of dichromacy found in this species. Details are the same as those given for Fig. 1.

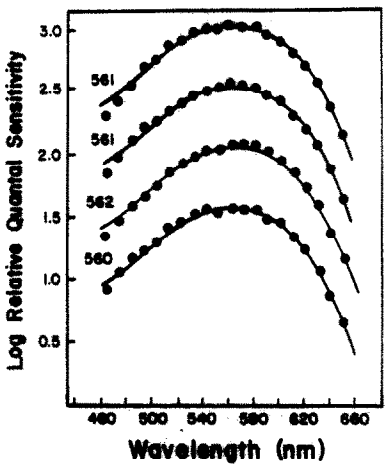

Fig. 3. Flicker photometric spectral sensitivity functions obtained from four *Callicebus* monkeys. These animals have the first of the two types of dichromacy found in this species. Details are the same as those given for Fig. 1.

from 560 to 562 nm (mean $\lambda_{max} = 561.0$ nm). The data for each of these eight animals are well accounted for by curves derived from visual pigment nomograms. There were no systematic deviations between the data points and the fitted nomograms for the four groups of subjects whose results are shown in Figs 1–4.

The results from the ninth *Callicebus* monkey, a female, were quite different. This animal showed a consistent change in the photometric equation obtained under the two conditions of chromatic adaptation: over three independent determinations this animal required on average 0.16 log unit more of the 630 nm mixture component in the presence of long wavelength adaptation then it did when the adaptation was shifted to 540 nm. This indicates the presence of more than one spectral mechanism in the middle to long wavelengths in this animal. Given that, it is not surprising that the full spectral sensitivity function derived from this animal likewise differed from that of the other eight monkeys in that it was relatively poorly fit by any single pigment nomogram. Indeed, the single nomogram that gave the best fit to the results from this subject had a fitting error more than four times greater than that of the nomogram used for the data of the animal giving the next poorest fit.

To attempt to account for the spectral sensitivity function obtained from this animal, we followed a strategy previously used with success on squirrel monkeys. This involved employing a computer to attempt to best fit the array of sensitivity data using all summative pairwise combinations of the curve for three cone mechanisms having max values at 538, 551, and 561 nm respectively. These three are the middle wavelength cone mechanisms found in the squirrel monkey. In this approach the fit provided by each pair in all possible summative combinations of that pair (step size = 1%) is computed and the pair selected by the computer as best fitting is taken as indicating which two pigment classes are present in the retina (Jacobs and Neitz, 1987). The results of the procedure are illustrated in Fig. 5 where it can be seen that the summative combination of components having max values of 538 nm (57%) and 561 nm (43%) provides a reasonable account of the measured spectral sensitivity in this animal. Further discussion of this result is given below.

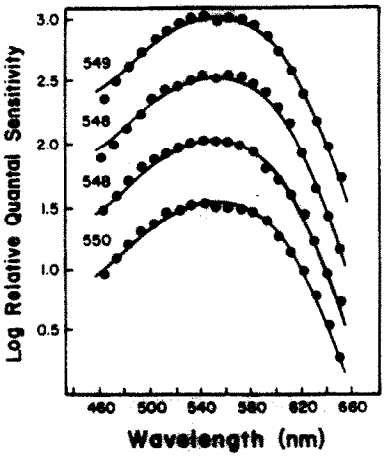

Fig. 4. Flicker photometric spectral sensitivity functions obtained from four *Callicebus* monkeys. These animals have the second of the two types of dichromacy found in this species. Details are the same as those given for Fig. 1.

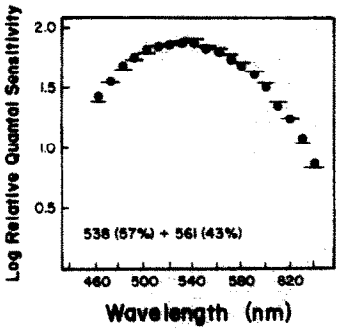

Fig. 5. Flicker photometric spectral sensitivity function obtained from a female *Callicebus* monkey. The solid circles are the sensitivity values obtained from equating the effectiveness of monochromatic test lights and an achromatic reference light. The horizontal dashed lines are the best fit values obtained from a linear summation of two cone photopigment curves. The λ_{max} values for these two and the proportion required to complete the fit are indicated at the bottom.

DISCUSSION

We have now found individual monkeys possessing only a single middle wavelength cone mechanism in three genera of South American monkey from the family *Cebidae*. In the squirrel monkey such individuals are known to have dichromatic color vision (Neitz and Jacobs, 1984) and it is hard to believe that this is not also true for the *Cebus* and *Callicebus* monkeys whose retinas contain only a single middle wavelength cone. Such an association is assumed in the remainder of this discussion.

The mean λ_{max} values derived for dichromatic *Cebus*, *Callicebus*, and *Saimiri* using the flicker photometric ERG are shown in Table 1. There is very close agreement among the three species in the spectral positioning of two cone mechanisms with the average peak locations for these two cone classes covering a range of only about 1 nm. As assessed by ERG flicker photometry, therefore, the best estimate is that these Cebid species have two classes of middle wavelength cone having λ_{max} values of about 550.2 nm (N = 20 monkeys) and 561.3 nm (N = 25). A third middle wavelength cone having peak sensitivity at about 538 nm was found among squirrel monkeys (Jacobs and Neitz, 1987). Although no dichromatic *Cebus* or *Callicebus* monkeys having a cone class at this location were found in the present sample, there is strong reason to believe that some members of these species, like some individual squirrel monkeys, will be found to have a third type of middle wavelength cone. Different evidence supports this conclusion for the two species.

Cebus monkeys have been subjects in a number of behavioral experiments involving measurements of color vision (reviewed by Jacobs, 1981). Although there are some inconsistencies in the outcomes of these experiments, two general conclusions may be drawn: (a) not all *Cebus* monkeys have the same color vision capacities (Gunter *et al.*, 1965), and (b) at least some members of this species behave in a manner similar to human protanopic dichromats (Grether, 1939; Malmo and Grether, 1946). The former conclusion can now be understood to reflect the cone polymorphism in this species that is documented here. The latter conclusion would be consistent with the idea that some *Cebus* monkeys must have a cone peaking in the vicinity of 530–540 nm. More direct evidence that this is the case comes from microspectrophotometric measurements made on cones from a single male *Cebus apella* by Bowmaker and Mollon (1980). That animal was found to have a single class of middle wavelength cone having an average λ_{max} value of 534 nm. It seems likely that although no *Cebus* monkeys in the present sample had the 534 nm cone, some members of this species in fact have such a cone. If so, the middle wavelength cone types of *Cebus apella* are very similar to those of the squirrel monkey.

There are, apparently, no previous investigations of color vision or its mechanisms in *Callicebus*. Our confidence that this genus, like *Saimiri* and *Cebus*, has a third class of middle wavelength cone peaking in the 530–540 nm range comes from an analysis of the results from the only trichromatic animal encountered in this sample. To account for the spectral sensitivity results from that animal we attempted to fit the data using all three of the cone combinations present in the squirrel monkey (i.e. λ_{max} = 538, 551 and 561 nm). The 538/561 combination provided by far the best fit of the three combinations (Fig. 5). In addition, the size of the chromatic adaptation effect measured on this animal was in line with that found for squirrel monkeys having the 538/561 nm cone combination, but considerably larger than that characterizing squirrel monkeys having the other two cone combinations (Jacobs and Neitz, 1987). Although this evidence is more indirect than is

Table 1. Mean λ_{max} values for dichromatic animals from three Cebid species

Species	Mean λ_{max} (nm)		
Cebus apella	—	550.3 (1.15) (N = 3)	562.2 (1.78) (N = 5)
Callicebus moloch	—	548.8 (0.96) (N = 4)	561.0 (0.82) (N = 4)
Saimiri sciureus	537.8 (0.80) (N = 14)	550.6 (1.59) (N = 13)	561.1 (1.59) (N = 16)

The number of animals in each sample is indicated. Values given in parentheses are standard deviations. The results for *Saimiri* are from Jacobs and Neitz (1987)

desirable, the most reasonable hypothesis is that this animal has a cone class having λ_{max} at *ca* 538 nm. In sum, there is a near identity of two classes of middle wavelength cones in *Cebus*, *Callicebus* and *Saimiri*. The third cone class found in *Saimiri* was not directly observed in any of the *Cebus* and *Callicebus* monkeys tested, but indirect evidence strongly suggests that these genera also share this third cone type. Just why we did not find individual dichromatic *Cebus* or *Callicebus* with this cone class in the samples examined remains unknown. The limitations on the sample size could be the critical feature, although the possibility that the 530–540 nm cone class is present at significantly lower frequency in these two genera than in *Saimiri* cannot be discounted.

Only male *Cebus* monkeys were available for examination and thus we cannot be sure that the single locus model that appears to account for the inheritance of squirrel monkey color vision (above) is appropriate for *Cebus*. However, the fact that the males tested were polymorphic for the middle wavelength cone, and uniformly dichromatic, is in accord with that idea. All of the *Callicebus* males tested were likewise dichromatic, and they too showed a polymorphism in the middle wavelength cone. In addition, one of the two females tested had two classes of middle wavelength cone and would presumably be a trichromat. All of these facts lead us to conclude that in both specific cone mechanisms and in the mode of their inheritance these three Cebid species seem to be very similar, if not identical. Whether this is also true for other species from this same family remains an interesting and open question. In any case the present results make it even clearer that there are some significant differences between Old and New World primates in the mechanisms used for the genetic transmission of the pigments required for color vision.

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