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Spectral mechanisms in the tree squirrel retina

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Summary. The retina of the gray squirrel (*Sciurus carolinensis*) contains rods and cones in a ratio of about 2:3. The spectral mechanisms in this retina were examined in behavioral and electrophysiological experiments. Tests of color vision revealed that this animal has a spectral neutral point at about 500 nm and, thus, dichromatic color vision. Recordings made from single optic nerve fibers and results obtained from an analysis of the flicker photometric electroretinogram (ERG) indicated that vision in the gray squirrel is based on three spectral mechanisms. One of these, presumably rod-based, has peak sensitivity at about 502 nm. The other two mechanisms reflect the presence of two classes of cone having average peak sensitivity of about 444 nm and 543 nm.

Introduction

An early examination of the tree squirrel retina yielded the hedged conclusion that it was 'probably pure-cone' (Walls 1942). Arden and Tansley (1955) were more certain; electrophysiological results suggested to them that these animals have 'a pure cone retina almost certainly containing only a single kind of cone.' This idea proved to be incorrect. Two types of photoreceptors arranged into tiers can be distinguished in the retina of the gray squirrel. About 40% of these receptors, those comprising the inner tier, have been described as being 'rodlike', the remainder have morphological features characteristic of other mammalian cones (Cohen 1964; West and Dowling 1975; Anderson and Fisher 1976). Both behavioral (Silver 1966; Dippner and Armington 1971; Jacobs 1974; Yolton 1975) and electrophysiological (Gouras 1964; Green and Dowling 1975; Yolton 1975) measurements have been employed to demonstrate duplex retinal function in several species of tree squirrel. A reasonable conclusion from these experiments is that visual duplicity is a functional reflection of the two receptor types.

Questions remain about the identity of the photopigments in the retina of the tree squirrel. One spectral mechanism has peak sensitivity close to 500 nm (Arden and Silver 1962; Jacobs 1974; West and Dowling 1975; Loew 1975; Yolton 1975). The spectral location of this mechanism and its relatively high sensitivity suggest this pigment is housed in the rods. That intuition is supported by direct microspectrophotometric (MSP) measurements of gray squirrel photoreceptors made by Loew (1975). A second spectral mechanism, presumably cone-based, is also evident, but its spectral peak has not been very accurately specified. The peak of this mechanism has been measured as lying somewhere in the range of 530 to 545 nm (Arden and Tansley 1955; Weale 1955; Dartnall 1960; Green and Dowling 1975; Loew 1975). Results from several behavioral studies indicate that tree squirrels have dichromatic color vision; the characteristics of the dichromacy imply that there must be a second cone type with peak sensitivity in the short wavelengths (Jacobs 1974, 1975; Yolton 1975). Two puzzles stand in the way of accepting the conclusion that these retinas contain a short wavelength cone. One is that in behavioral experiments it has not been possible to find convincing evidence for the presence of a neutral point at that spectral location which would be required for dichromatic color vision based on the operation of a middle and short wavelength cone pigment (Jacobs 1974; Yolton 1975). A second is that a direct search for

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a short wavelength cone pigment with MSP yielded negative results (Loew 1975). Partly as a result of these difficulties, Silver (1975) was led to suggest that the photopic vision of the gray squirrel might be accounted for solely by interactions of the 500 nm mechanism and the middle wavelength cone.

We have re-examined these questions about the spectral mechanisms in the retina of the tree squirrel by undertaking some new behavioral measurements and by measuring the spectral mechanisms in the retina through recordings of optic nerve fibers and the flicker photometric electroretinogram (ERG). The results verify that this animal has dichromatic color vision, establish that there are three separate spectral mechanisms in the retinas of tree squirrels, and provide estimates of their spectral sensitivities.

Methods

Subjects

Adult eastern gray squirrels (*Sciurus carolinensis leucotis*) of both sexes were examined in all of the experiments. In addition, ERG measurements were made on two red squirrels (*Tamiosciurus hudonicus*).

Three different types of experiments were conducted: behavioral tests, single unit electrophysiology, and ERG flicker photometry. The apparatus and procedures for each have been described in detail elsewhere so only brief summaries are provided here.

Tests of color vision

Apparatus. The apparatus was a general purpose device that permits the measurement of a variety of features of color vision and visual sensitivity in nonhuman subjects (Jacobs 1983). A three-alternative, forced-choice discrimination was employed. The apparatus contained three stimulus panels arranged along one wall of a small test chamber each of which was transilluminated by an optical system located outside of a test chamber. The subject was trained to select (by touching) the uniquely illuminated stimulus panel from among the three. The two negative panels were illuminated identically to one another but different from the positive panel. A correct choice was reinforced by delivery of a 90 mg food pellet. Over successive trials the position of the uniquely illuminated panel was randomly alternated among the three panels. The characteristics of the light illuminating the positive and negative panels was changed over test sessions so as to permit the measurement of discrimination thresholds. All of the functions of this test apparatus were computer controlled.

Procedure. A true dichromat should have a spectral neutral point (Pokorny et al. 1979). We sought to determine if gray squirrels have dichromatic color vision by searching for the presence of spectral neutral points. For this test, the light illuminating the positive stimulus panel was drawn from a monochromator (Instruments SA, Model H-10; half-energy bandpass = 16 nm). The two negative panels were illuminated by achromatic (4800 K) sources; panel luminance was held constant at 3.5 $cd \cdot m^{-2}$. The interior of the test chamber was diffusely lit (mean illuminance = 20 lux). Over test trials the animals were required to discriminate the chromatically illuminated panel from the two achromatic panels as the wavelength of the positive panel was varied from 470 to 515 nm in steps of 5 nm. The intensity of the monochromatic light was randomly varied in steps of 0.1 log unit over a range of ± 0.5 log unit around the value required for a human subject to set the monochromatic and achromatic lights to equal brightness. The assumption was made that this intensity range should include a setting of equal brightness for the subject. That this assumption was correct is indicated by the results presented below (see Fig. 1 and the accompanying discussion). Intensity of the test light was changed every 5 trials. The trials were 2 s in duration with 4 s intertrial intervals.

Two gray squirrels (one male, one female) were tested daily following 22 h of food deprivation. Each was first trained to discriminate 470 and 515 nm lights from achromatic ones over the full span of test light intensity. After sufficient experience that these discriminations were consistently correct, the intermediate wavelengths were tested. This process continued until no further changes in performance were noted. At that point a final 25 trials were run at each wavelength/intensity combination. These latter data are reported here.

Optic nerve recording

Apparatus. Details of the optical stimulator used and the recording preparation have been presented (Jacobs and Tootell 1981; Blakeslee 1983). A dual-beam optical system was used. One beam came from a monochromator (Bausch and Lomb, high intensity) set to yield a half-energy passband of 10 nm. The other beam, used to provide adaptation lights, came from a tungsten-filament lamp. These two lights were optically combined to illuminate one end of a fiber optic bundle. The intensities of these lights were varied by using neutral density step filters; stimulus presentation was controlled with electromagnetic shutters run from a digital timer. The output end of the fiber optic bundle was individually positioned for each unit recorded so that it was centered on the receptive field and subtended 6°. This stimulus is larger than the receptive fields of almost all optic nerve fibers in this species.

Procedure. Two different animal preparations were used (Blakeslee 1983). In one, the occurrence of eye movements was limited by a surgical procedure (Tootell and Jacobs 1979). In this case recording was conducted under urethane (2.1 g/kg) anesthesia. In the other preparation eye movements were limited through the use of a paralytic agent (pancuronium bromide, Pavulon). Animals were artifically respirated and end tidal CO_2 was held at 5–5.5%. In this case squirrels were initially anesthetized with a combination of pentobarbital sodium and chloral hydrate; anesthesia was maintained throughout the recording session by continuous infusion of urethane. For both preparations body temperature was kept at a normal level through the use of a circulating hot water heating pad. The pupil was dilated and accommodation paralyzed by topical application of atropine sulfate. No differences in results were found in the two preparations.

Single optic nerve fibers were recorded with glass-insulated tungsten microelectrodes. The electrodes were lowered through the intact brain into the optic nerve at a point just anterior to the optic chiasm. Amplified and electrically-shaped action potentials were fed to an electronic frequency counter.

The receptive field location was determined for each unit

on a tangent screen and the fiber optic bundle was then positioned as indicated above. The spectral response properties of the units were determined by presenting one sec flashes of monochromatic light and recording the impulse activitiy over three successive one second periods: prior to stimulation, during stimulation, and following stimulation. Test wavelengths were presented in random order. Stimuli were presented both with and without additional adaptation and multiple intensities were run at each wavelength to permit the determination of spectral sensitivity.

ERG flicker photometry

Apparatus. The apparatus and procedure used for ERG flicker photometry have been described (Neitz and Jacobs 1984). Stimuli were produced by a three-beam optical system. The test light, originating from a monochromator (half-energy = 10 nm), comprised one beam. A second beam was used as a reference light. It originated from a tungsten-halide lamp as did a third beam used as an adaptation light. The three beams were combined to form a 53° Maxwellian view.

ERGs were differentially recorded from a contact lens electrode with a ground electrode placed against the inside of the cheek of the animal. The stimulus was an interleaved pulse train from the test and reference lights. The pulses from the two sources were equal in duration and each pair was separated by an equal-duration dark interval. The ERGs elicited by the test and reference light were fed to active, narrow bandpass (0.2 × the center frequency) filters. The filtered outputs were subtracted from one another. The resultant combination was averaged and viewed on an oscilloscope.

Procedure. Squirrels were anesthetized with ketamine hydrochloride (15 mg/kg) and 0.15 mg/kg of acepromazine maleate and by subsequent injection of 10 mg/kg of sodium pentobarbital. The pupil of the test eye was dilated with atropine sulfate and Phenylephredine HCl. The animals were placed in a stereotaxic instrument and positioned in the test beam.

Spectral sensitivity functions were determined by establishing photometric equations between a monochromatic test light and an achromatic reference (0.05 mW). Such equations were made for test lights taken at 10 nm steps from 460 to 650 nm. At each wavelength a density wedge in the test light beam was adjusted until the response to the test light best nulled the response to the reference light. The reading of the density wedge at this point was taken as establishing a photometric equation. The pulse rate of the test and reference lights was 50 Hz. An accessory adaptation light of 440 nm (0.15 mW) was used. The purpose of this light was to reduce any possible contribution from short wavelength sensitive receptors.

Results

Neutral point

In the neutral point test there are two possible cues the animal subject might use to successfully discriminate between the achromatic light and the monochromatic lights: brightness differences and color differences. Variation in test light intensity at any given wavelength would be expected to have relatively little effect on either the presence or the magnitude of the potential color cue, but such variations should greatly alter the size of the potential brightness cue and, at some intensity setting, the brightness difference should be eliminated entirely. Figure 1 summarizes the results from the neutral point test. Plotted for each squirrel is the asymptotic performance in the discrimination of test lights between 470 and 515 nm. At each test wavelength the poorest performance recorded at any of the test light intensities is given. In every case, the test light intensity yielding poorest performance was located midway in the total range of test light intensities. These intensity settings are presumed to define the locations where brightness differences are eliminated for each subject and, thus, these discriminations must result solely from the use of color cues. Both subjects were able to accurately discriminate test wavelengths shorter than 490 nm and longer than 510 nm at all of their intensity values. Thus, in support of the earlier experiments, it proved to be relatively easy to demonstrate that gray squirrels have color vision.

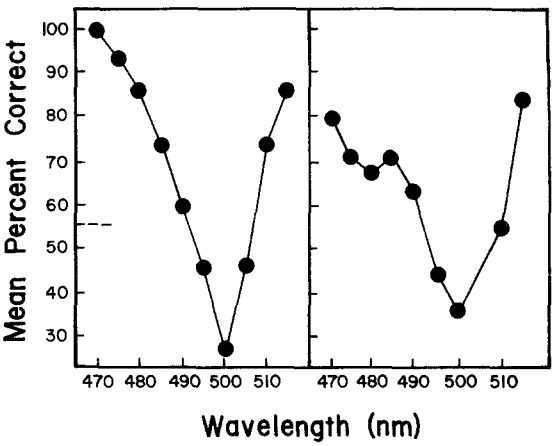

Fig. 1. Neutral point test results for two gray squirrels. The plotted points show asymptotic performance levels achieved in discriminations of monochromatic (470 nm to 510 nm) and achromatic lights. The horizontal dashed line in the left panel indicates the performance required to reach the 95% level of confidence

At wavelengths in the range between 490 and 510 nm both subjects experienced some difficulty in making the discriminations, and both failed entirely for some test wavelengths. The second of the two possible cues for successful discrimination, color differences, must therefore also have been eliminated within this wavelength band. Failure to make such discriminations indicate the presence of a spectral neutral point. The color vision of these squirrels is, thus, dichromatic rather than trichromatic. Neutral point locations were virtually identical for these two subjects. The midpoint of the spectral range over which performance

dropped to chance ($P<0.05$) was 499.5 nm for one subject and 500.5 nm for the other subject.

Spectral response properties of optic nerve fibers

Recordings were obtained from a total of 326 units in twenty gray squirrels. The types of units encountered in this sample appear qualitatively similar to those earlier found in recordings from the ground squirrel optic nerve (Jacobs and Tootell 1981). The patterns of spectral responses to large field stimuli allow a twofold division: spectrally opponent if the response of the unit showed a sign reversal as a function of variation in wavelength, and spectrally non-opponent if no sign reversal was seen. The non-opponent units fell into three major response classes: directionally selective units, contrast sensitive units (sustained and transient varieties), and BG/G units. Some observations on the directionally selective units have been reported (Blakeslee et al. 1985); this group of units is not further considered here. The spectrally opponent units could be subdivided into two classes: B/G and G/B, the former exciting to short wavelength stimuli (<ca. 500 nm) and inhibiting to longer wavelength lights with the latter behaving in the reverse fashion.

Figure 2 shows spectral response functions obtained from representatives of each of the several types of optic nerve fiber in the gray squirrel. Each panel plots the responses to monochromatic lights of equal energy. On-responses (filled circles) and off-responses (open circles) are given separately and each is expressed as the change in firing rate from the spontaneous level. Contrast sensitive units (Fig. 2, top) were broadly responsive to all spectral lights. The one illustrated in Fig. 2 increased its firing rate to stimulation; others showed a decrease in response to light onset. The spectral response pattern of the other type of non-opponent unit, the BG/G cell, is illustrated at the bottom of Fig. 2. These units show a change in the pattern of their response as a function of wavelength. For short wavelength stimuli these units respond in a sustained fashion; for longer wavelength lights (>ca. 500 nm) these units fire transiently at both light onset and offset. In the ground squirrel this response pattern is attributed to the fact that the center of the receptive field receives the same sign input from both short and middle-wavelength receptors while the antagonistic surround is driven by the middle wavelength receptor alone (Gur and Purple 1978). Spectral response functions for the two types of spectrally opponent units of the gray squirrel are shown in the middle two panels of

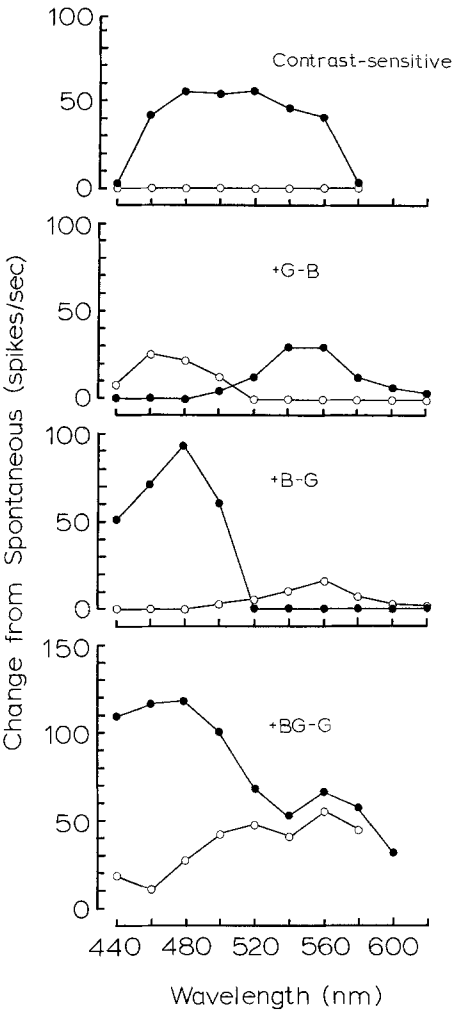

Fig. 2. Representative spectral response functions obtained from four different types of units in the optic nerve of the gray squirrel. Each function shows the average response obtained to monochromatic lights of various wavelengths all of which were equated in energy. On-responses are indicated by filled circles, off-responses are given by open circles

Fig. 2. Among the 326 units examined, the vast majority (274=84%) were contrast sensitive. The remainder of the units were classified as follows: directionally selective (32=10%), BG/G (12=3.6%), spectrally opponent (9=2.3%).

When examined under conditions of dark adaptation or under dim illumination the spectral response functions of most contrast sensitive units peaked near 500 nm suggesting that they receive principal input from rods. To evaluate this quantitatively, spectral sensitivity functions were determined for six such units recorded under these conditions. In each case the quantal intensity of the test light required to produce a change in response rate of 10 spikes/s was determined. This was done for each of ten test lights taken at 20 nm steps

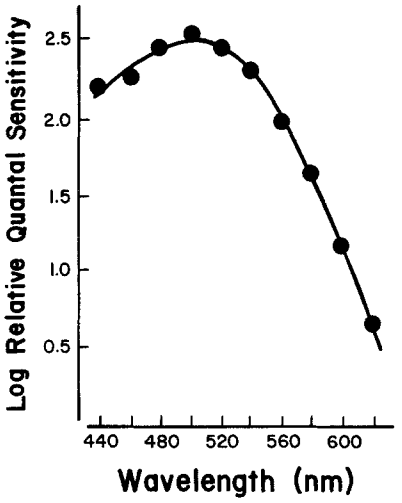

Fig. 3. The average spectral sensitivity function obtained from contrast sensitive units in the gray squirrel optic nerve. The recording was done under conditions of dark adaptation. The solid circles are mean values for six units. These have been corrected for preretinal absorbance in the eye of this species. The curve is that for the best fitting visual pigment nomogram (see text). The λ_{max} value for this curve is 502 nm

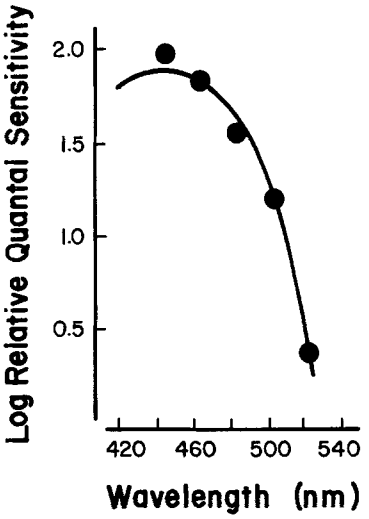

Fig. 4. Spectral sensitivity function for five units (3 B/G, 2 BG/B) in the gray squirrel optic nerve which had inputs from a short wavelength sensitive cone. These data were obtained while the eye was concurrently exposed to intense long wavelength light. The sensitivity values have been corrected for preretinal absorbance; the curve is that for the best fitting visual pigment nomogram ($\lambda_{max} = 444$ nm)

from 440 to 620 nm. To compare these functions to photopigment absorption curves, the spectral sensitivity values were corrected for absorption by the ocular lens of the tree squirrel (Yolton et al. 1974). These corrected values were fit to wavelength dependent visual pigment nomograms using a procedure previously described (Neitz and Jacobs 1984). In this procedure a computer is used to determine the spectral positioning (to the nearest nm) of the visual pigment nomogram which best fits the data.

Each of the six contrast sensitive units examined under these conditions yielded spectral sensitivity data that could be well fit by single visual pigment nomograms. The spectral positioning of the best fitting nomograms for these six units fell over a relatively small spectral range with peak sensitivities (λ_{max}) from 500 to 506 nm. The averaged data from the six units are shown in Fig. 3. They are closely accounted for by a visual pigment nomogram having a λ_{max} of 502 nm. This value is close to previous estimates of the rod pigment for this species and defines one of the spectral mechanisms found in the retina of the tree squirrel.

Under most conditions of examination the spectral response functions for the contrast sensitive units were broader than those shown in Fig. 3 having peaks shifted toward the longer wavelengths. We attempted to fit the spectral sensitivity functions for such units to visual pigment nomograms in the same fashion as described above. This exercise made it clear that under conditions of light adaptation these contrast sensitive units typically receive inputs from more than one photopigment type. For instance, for seven such units the λ_{max} values for the best fitting pigment nomograms covered a large range, from 520 to 533 nm, and the individual fits were uniformly much poorer than those for the units of Fig. 3. The implication from this result is that the contrast sensitive units receive joint inputs from the 502 nm pigment and from a cone having peak sensitivity at some longer wavelength. Although the responses of these cells were examined under a variety of adaptation conditions, it proved impossible to obtain an accurate specification of the spectral location of the long wavelength cone from these single unit experiments. That goal was accomplished in the ERG experiments described below.

Both the BG/G units and the spectrally opponent units (Fig. 2) clearly receive an input from a pigment having maximum sensitivity in the short wavelengths. To characterize this mechanism, spectral sensitivity functions were obtained from five of these units. Each was recorded in the presence of intense (0.69 mW) long-wavelength adaptation. This adaptation was produced by placing a high pass filter (50% cutoff = 590 nm) in the second beam of the optical stimulator. With such adaptation the longest wavelength test light that still produced a response was 520 nm. Spectral sensitivity of these units was determined in the same man-

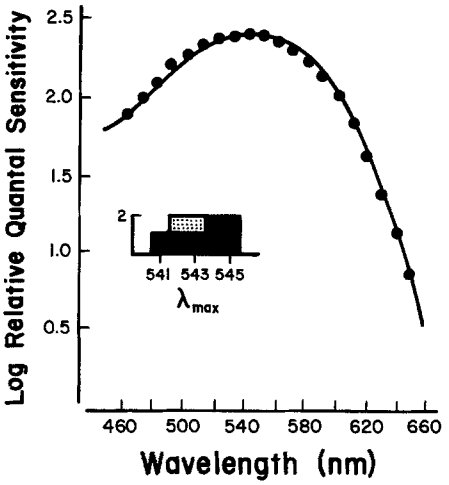

Fig. 5. Flicker photometric spectral sensitivity function obtained from gray squirrels under conditions favorable for isolation of the long cone (50 Hz flicker, additional short wavelength adaptation). The solid circles are average sensitivity values for seven animals obtained by equating the effectiveness of monochromatic test lights and an achromatic reference light. These values are corrected for preretinal absorption. The solid line is that for the best fitting visual pigment nomogram (λ_{max} = 543 nm). The inset shows the distribution of λ_{max} obtained from nine individual squirrels (solid-gray squirrels, stippled-red squirrels). Note the small range of variability in λ_{max}

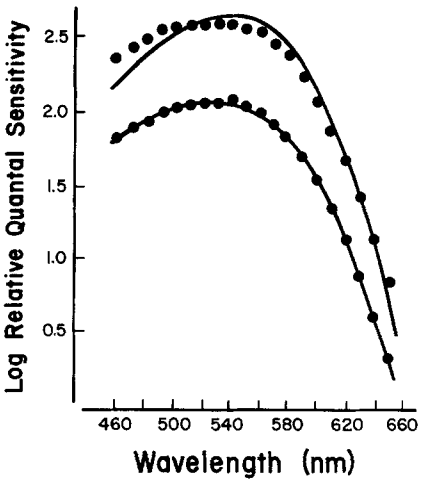

Fig. 6. Flicker photometric spectral sensitivity functions obtained from one gray squirrel. The conditions of the experiment were identifical to those used to obtain the results of Fig. 5 except that accessory short-wavelength adaptation was not used. At the top the curve is that for the best fitting visual pigment nomogram (λ_{max} = 536 nm). Note that, by comparison to Fig. 5, these data are poorly accounted for by the curve for a single visual pigment. The bottom curve shows the same data, but here they were best fit by linearly summing two pigment nomograms having λ_{max} values of 502 nm (45%) and 543 nm (55%). The closeness of this latter fit suggests that under these test conditions the recorded signal reflects contributions from both rods and long wavelength cones

ner as described above. The averaged data obtained from these units appears in Fig. 4. The continuous line is that for the best fitting visual pigment nomogram. That pigment had a λ_{max} value of 444 nm. As can be seen, that function provides a reasonable account of the data.

Flicker photometric ERG

We noted above that the long-wavelength cone mechanism could not be clearly defined from optic nerve recordings. The specification of that mechanism was the goal of this experiment. Two features of the ERG test situation were designed to minimize any possible contribution to the recorded signal from rods or short wavelength cones: the use of relatively rapidly flickering lights and additional short-wavelength adaptation. The combination of these measures proved to be effective. The spectral sensitivity functions obtained from the 50 Hz flicker photometric ERG were well fit by nomograms derived for single photopigments. Complete functions were obtained from seven gray squirrels. The λ_{max} values for the fitted pigments varied only modestly across this sample, from 541 nm to 545 nm. The mean values for these animals are given in Fig. 5. The nomogram that provides the best fit to these data has a λ_{max} value of 543 nm. Two red squirrels were also tested in the same manner. The curves obtained from these animals were indistinguishable from the data of the gray squirrels. The inset in Fig. 5 shows the distribution of λ_{max} for all nine tree squirrels. There is only small variability in the λ_{max} values (mean = 543.2 nm; SD = 1.39 nm). It appears that this procedure provides a good indication of the spectral properties of the long wavelength cone mechanism in the tree squirrel.

Although the test procedures outlined above produced a clear picture of the spectral properties of the long wavelength cone, we discovered that under less stringently adaptive conditions the 50 Hz flicker curve often showed indications of contribution from more than one spectral mechanism, behaving in fact much like the spectral sensitivity curves obtained from contrast sensitive units of the optic nerve. Figure 6 shows one such example. The data at the top (solid circles) represent flicker photometric equations obtained using 50 Hz flicker, but without additional short-wavelength adaptation. The solid line is the best fitting curve for any single pigment nomogram. As can be seen, that best fit curve provides a poor account of the data (compare to Fig. 5). The elevation of

the data points for wavelengths 500 nm and shorter suggest contribution from a second mechanism. That this is indeed the case can be seen at the bottom of Fig. 6. The same spectral sensitivity data have been refit to a combination of pigment nomograms. In doing so it was assumed that the spectral sensitivity function results from the summative contributions of two spectral mechanisms having λ_{max} values of 502 nm (Fig. 2) and 543 nm (Fig. 5), respectively. The combination of the two mechanisms provides an excellent account of these spectral sensitivity data.

Discussion

Two previous behavioral studies of color vision in tree squirrels failed to provide compelling evidence that these animals have spectral neutral points, and thus dichromatic color vision (Jacobs 1974; Yolton 1975). Despite these earlier failures, it proved relatively easy to demonstrate color matches characteristic for dichromatic subjects in the present experiment (Fig. 1). Although there is no certain way to resolve this discrepancy, we believe it simply may result from better control of luminance-related cues in the current experiment. It was perhaps the use of such cues that allowed animals in the earlier experiments to make discriminations that a dichromatic subject ought not to be able to make. Whatever the explanation for the earlier results, there is now unequivocal evidence that this species has dichromatic color vision.

Dichromatic color vision requires the presence of two photopically active photopigments. Measurements from flicker photometry place the average λ_{max} value for the longer of these at 543 nm. This is a somewhat longer value than that indicated by most of the earlier gross potential studies (see above). However, this estimate agrees very well with the results of direct MSP measurements of gray squirrel cones (Loew 1975), and with spectral sensitivity data derived from intracellular recordings made on a single cone from a gray squirrel (Leeper and Charlton 1985). The long-wavelength pigment of gray squirrels and red squirrels appears identical.

There are no previous measurements of the second cone type in these retinas although, as noted, its presence can be deduced from a number of behavioral measurements. The spectral sensitivity functions derived for this mechanism from optic nerve recordings suggest that this cone has a spectral peak at about 444 nm. Although that value represents the best present estimate of the λ_{max} value for the short wavelength cone in the tree squirrel, we emphasize that the estimate depends on the adequacy of two assumptions: (a) that the chromatic adaptation conditions used to obtain those records were effective in minimizing influences from other receptor types, and (b) that the corrections used to account for spectral filtering by the lens were appropriate. These assumptions seem reasonable.

In his MSP survey of the gray squirrel retina Loew (1975) encountered no short wavelength cones which led him to suggest that if such existed they must constitute only a very small fraction of the total cones. Our results are consistent with his conclusion. Signals derived from the short wavelength cone were sparsely represented in the optic nerve of this animal; only about 6% of all the optic nerve fibers sampled showed any indication of input from the short wavelength cone. This can be compared to results obtained from similar experiments conducted on the ground squirrel, another dichromat. Fibers in the optic nerve of that species were categorized into the same response classes as found in the gray squirrel. Whereas only about 6% of all optic nerve fibers in the tree squirrel receive inputs from the short-wavelength cone, the comparable figure for the ground squirrel is approximately 30% (Jacobs and Tootell 1981). In the ground squirrel about 7% of the total cone complement is believed to consist of short wavelength cones (Ahnelt 1985). If tree squirrel and ground squirrel retinas have similar sorts of connections between short wavelength cones and ganglion cells, this result would imply that the proportion of short wavelength cones in the tree squirrel is probably lower than it is in the ground squirrel, i.e., less than 7%. Irrespective of the accuracy of these estimates, it is in any event interesting to note that the proportion of short wavelength cones appears to be roughly similar in the retinas of these dichromatic rodents and in the retinas of trichromatic primates (e.g., Mollon 1982).

Measurements of the third spectral mechanism ($\lambda_{max} = 502$ nm) in the tree squirrel retina are in agreement with earlier estimates for the rod pigment in the tree squirrel. There is some indication from both the ERG and single unit experiments that this spectral mechanism may operate under conditions of adaptation where typical rods do not. As we have noted, in the optic nerve recording experiments it was virtually impossible to find contrast sensitive units that did not receive joint inputs from the 502 nm and 543 nm mechanisms, even in the light-adapted eye. Even in the ERG experiments, where it was possible to employ much more stringent test conditions, the spectral sensitivity re-

sults frequently showed strong contribution from the 502 nm mechanism (e.g., Fig. 6). These observations suggest the possibility that although the inner tier layer of photoreceptors appear rodlike (West and Dowling 1975), and contain a pigment having a traditional rod spectrum, they may not have a typical rod physiology.

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