Title

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Journal

Visual Neuroscience, 20(1)

ISSN

0952-5238

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Publication Date

2003

Peer reviewed

Visual Neuroscience (2003), **20**, 11–17. Printed in the USA.

DOI: 10.1017/S0952523803201024

Spectral properties and retinal distribution of ferret cones

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(RECEIVED August 16, 2002; ACCEPTED November 19, 2002)

Abstract

The spectral mechanisms of the ferret (*Mustela putorious furo*) were studied with electroretinogram (ERG) flicker photometry. Variations in adaptation state and flicker rate were used to define corneally based spectral sensitivities for the three classes of receptor present in the retina of this mustelid—rods (λ_{max} = 505 nm), S cones (430 nm), and L cones (558 nm). The retinal distributions of the two classes of cone were determined using opsin antibody labeling. Ferret retinas contain a total of about 1.3 million cones with L cones outnumbering S cones in a ratio of approximately 14:1. ERGs were also recorded using 18.75-Hz flickering stimuli that were designed to isolate signals from individual cone classes. The contrast/response functions for signals originating from both S and L cones were linear over low-to-moderate levels of contrast, but with greatly different slopes for the two cone types. The L:S contrast gain ratio derived from a comparison of these slopes, as well as inferences drawn from another experiment in which responses to various combinations of L- and S-cone activation were analyzed, suggest that contributions of these two cone types to the flicker ERG have a relative weighting of about 4:1 to 5:1 (L/S).

Keywords: Ferret retina, Cones, Visual pigments, Electroretinogram, Opsin antibody labeling

Introduction

The ferret (*Mustela putorious furo*) is a nocturnal carnivore. Studies of the visual system of the ferret were initiated more than 60 years ago, but it is only during the past two decades that these animals have been widely employed in vision research (Jackson & Hickey, 1985) Because time-bred animals are readily available, and because many developmental events in their visual system occur postnatally, the ferret has become a particularly attractive subject for studies of the development of the visual system largely replacing the cat for such purposes. Like that of the other carnivores, the retina of the ferret is heavily rod dominated (Braekevelt, 1983). Even so, however, ferret retinas do contain many cones that vary regionally across the retina increasing centrally from the peripheral margins so as to achieve a peak density in the region of the area centralis, a location where the rod/cone ratio drops to around 14:1 (Jeffery et al., 1994). Opsin antibody labeling reveals two populations of cone in the ferret retina, one reacting to an antibody that indiscriminately labels all middle- to long-wavelength (M/L) sensitive cones in the retinas of many mammals and the other sensitive to an antibody that labels ultraviolet (UV) and short-wavelength sensitive (S) cones in these same animals (Peichl et al., 2001). The purpose of our investigation was to characterize the spectral properties of ferret photoreceptors using an electrophysiological technique, electroretinogram (ERG) flicker photometry, and to map the distribution of cone types using antibody labeling. We were motivated to pursue these goals both because of a general utility of adding basic information about the visual system and vision in an important animal model and because there is apparently little or no information about such features for any of the approximately 65 species that constitute the family Mustelidae (Jacobs, 1993). An abstract reporting a preliminary version of this research has appeared (Calderone & Jacobs, 1997).

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Methods

Subjects

Adult sable ferrets (*Mustela putorious furo*) were obtained from Marshall Research Animals (North Rose, NY). Adults of both sexes were studied. All animal care and experimental procedures were in accordance with institutional animal care and use guidelines and with the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Recording experiments

In preparation for recording, ferrets were anesthetized with an IM injection of a mixture of xylazine hydrochloride (3 mg/kg) and ketamine hydrochloride (30 mg/kg). The pupil of the test eye was dilated with a topical application of atropine sulfate (0.04%). The animal was placed in a head holder that allowed for alignment of the eye with an optical system. ERGs were differentially recorded

using Burian-Allen style contact-lens electrodes. Except as noted, all the recording was done in a room with overhead fluorescent lighting that provided an ambient illumination of about 100 lux at the position of the test eye.

Full descriptions of the use of ERG flicker photometry to characterize the spectral properties of the eye have been published previously so only a brief summary is provided here (Jacobs & Neitz, 1987; Jacobs et al., 1996*a*). In this procedure, temporally alternating stimuli derived from two sources are presented as an interleaved train of pulses to the eye with each pulse having a 25% duty cycle. As in classical flicker photometry, one of these serves as reference light while the other is a test light. ERGs elicited by these two sources are electronically compared and over successive presentations the test light is adjusted in intensity until the ERG it produces is the same as that produced by the reference source. Repeat equations made with this technique typically vary by an amount less than 0.04 log units (Jacobs et al., 1996*a*) Repetition of this procedure for a range of test wavelengths permits a characterization of the spectral sensitivity of the mechanism(s) that are responsive to the lights. Variations in the details of the viewing situation, for instance in the adaptation state of the eye or the rate of temporal stimulation, are used to permit selective characterization of different spectral mechanisms. Analog hardware was used to window the amplified ERG signal with a sinusoid set to the frequency of the stimulus train. For any stimulus sequence, the position of the window was shifted to maximize its correlation with the ERG signal. Window positions could then be used to retrieve information about the relative timing of ERG responses. The comparison of responses to test and reference stimuli was based on averages of 50 responses elicited from each source.

Spectral sensitivity measurements were made for three different test conditions. In each case, the test and reference lights were presented in Maxwellian view (59-deg spot). The test light originated from a monochromator (15-nm band pass) equipped with either a 100-W tungsten-halide lamp or a 150-W xenon lamp. The reference light and a third beam used for accessory adaptation came from two other tungsten-halide lamps. All lamps were run from regulated power supplies. The three test conditions were as follows. (a) *Scotopic spectral sensitivity*. Animals were dark adapted for 20 min and the recording was then carried out in a darkened room. The pulse rate of the photometer was 4 Hz; the reference light was achromatic (2850 K; 14.7 td). Photometric equations were made for test lights taken at 10-nm steps from 440 nm to 610 nm. Two scans across this spectral range were completed and the results for the two were subsequently averaged. (b) *Photopic M/L spectral sensitivity*. In similar fashion, spectral sensitivity was measured with ERG flicker photometry under photopic test conditions that included a stimulus pulse rate of 25 Hz and an achromatic reference light (3702 td). Sensitivity was measured for wavelengths between 450 and 670 nm. (c) *Photopic short-wavelength spectral sensitivity*. To assess contributions from cones having maximum sensitivity in the short wavelengths, spectral sensitivity measurements were made using test conditions previously shown to enhance contributions from such receptors (Jacobs et al., 1996*b*). These included (1) use of a continuous long-wavelength adapting light to suppress long-wavelength sensitivity produced by using a long-pass filter having 50% transmission at 580 nm, (2) a short-wavelength reference light (450 nm; 1.12×10^{14} photons/s/sr), and (3) a 12.5-Hz stimulus pulse rate. Under these conditions spectral sensitivity was measured over the range from 400 nm to 500 nm using the procedures outlined above.

To further examine characteristics of cone-generated signals in ferrets, ERGs were also recorded using temporally modulated, square-wave stimuli generated on a computer-controlled color monitor (Radius Intellicolor, Model 0461) with the screen positioned perpendicular to the optic axis so as to provide a rectangular field of view 116 × 101 deg. Use of the monitor permitted the design and utilization of stimuli that isolated responses from single classes of cone. The setup used for such recording and the calibration procedures have been recently presented (Brainard et al., 1999; Jacobs et al., 2002). The stimulus was spatially uniform and modulated in time with a mean luminance of 50 cd/m^2. Cone isolation involves the substitution of a light with one spectral composition for a second with a different composition, the two being so designed that the quantal catch of only one cone type changes in the transition. To derive such cone-isolating stimuli, we used estimates of the spectral sensitivities of the ferret receptors that were obtained from the ERG experiments described above. In a first experiment, we recorded responses to S and L cone-isolating stimuli flickering at 18.75 Hz that were systematically varied in contrast. At each contrast level, the averaged amplitude of the stimulus locked component of the ERG was recorded for five samples of 50 responses each. This procedure was repeated for seven contrast levels of L-cone modulation (from 1.6% to 16%) and for six contrast levels of S-cone modulation (6.7% to 33.5%).

The goal of a second experiment done with cone-isolating stimuli was to draw inferences about the combination of cone signals in the ferret flicker ERG. This experiment follows closely the logic used in an earlier study of the human ERG (Brainard et al., 1999), and it involved the use of the flicker photometric procedure in which responses to test stimuli that modulated the two cone types to differing degrees were compared to responses given to a reference stimulus that modulated both together, that is, the L- and S-cone contrasts were identical in the reference stimulus (isochromatic). For this experiment the reference cone contrast levels were fixed at 5%. For any single measurement, the test stimulus was set to modulate the two cone types at some fixed ratio and the overall contrast of this stimulus was then repetitively adjusted until the responses to it and to the reference modulation were equivalent. This process was repeated a total of four times and the final result taken as the average of these four photometric balances. These equations were obtained for nine different proportional combinations of S- and L-cone modulation.

Immunocytochemistry

Ferrets were given a lethal dose of sodium pentobarbital and perfused intracardially with 0.9% saline followed by 500 ml of 4% paraformaldehyde in 0.1 M sodium phosphate buffer. The retinas were dissected from the eyecups, postfixed for 2–5 h in 4% paraformaldehyde, rinsed in phosphate buffered saline (PBS), and placed in a 20% sucrose solution. A day later the tissue was shock frozen in liquid nitrogen, rinsed in PBS, bathed in 0.3% hydrogen peroxide, rinsed in PBS, and placed in 2% bovine serum albumin (BSA), 10% normal goat serum (NGS) solution for 3 h. Retinas were then reacted with the polyclonal antibodies JH492 (dilution 1:30,000) or JH455 (dilution 1:100,000), both kindly provided by J. Nathans (Wang et al., 1992). The tissue was exposed to the primary antibodies for 72 h, incubated for 24 h in a goat anti-rabbit biotinylated secondary antibody, and then for an additional 24 h in an avidin-biotin-peroxidase complex (Biomedia, Foster City, CA). Immunoreactive cones were visualized by reaction with diaminobenzidine and hydrogen peroxide.

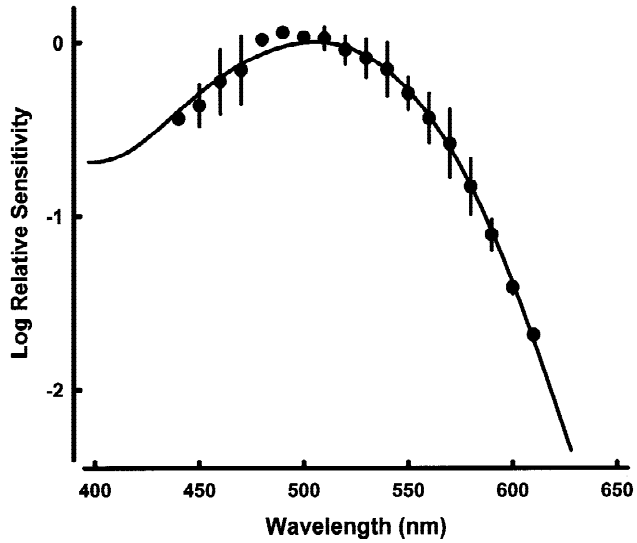

Fig. 1. Spectral sensitivity of the rod-based flicker (4 Hz) ERG. The data points, mean values obtained for two animals (± 1 SD), represent corneally based spectral sensitivity. The continuous line is the best-fitting photopigment absorption template (peak value of 505.6 nm). Note that here, as in Figs. 2 and 3, the error bars are frequently smaller than the width of the data points.

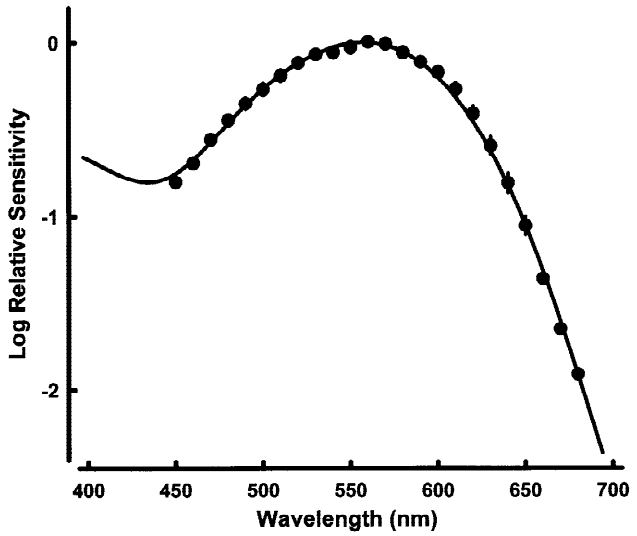

Fig. 2. Spectral sensitivity function for the ferret L cone obtained from ERG flicker (25 Hz) photometric measurements. The data points are mean values for six animals and the fitted pigment absorption function has a peak value of 557.6 nm.

Results

Spectral sensitivity

Spectral sensitivity functions obtained from two ferrets under conditions of dark adaptation were virtually identical. In Fig. 1, these averaged sensitivity values (solid circles) have been best fit to a photopigment template curve (Govardovskii et al., 2000). To accomplish that fit, the template was stepped along the wavelength scale in successive steps of 0.1 nm to determine the spectral position of the curve that yielded the best least-squares fit to the array of sensitivity values. The location of peak sensitivity (λ_{max}) providing the best fit under these test conditions was 505.6 nm.

The spectral sensitivity function obtained from ferrets under photopic test conditions using 25-Hz flicker is in Fig. 2 where the solid circles are means obtained from six animals. There is only relatively small variation among these subjects (error bars = ± 1 SD). As before, these values were best fit to a photopigment curve and that result appears as the continuous curve in Fig. 2. The curve best accounting for this data set has a λ_{max} of 557.6 nm. In most mammals there are many more M/L cones than S/UV cones and that fact seems correlated with a considerably smaller contribution of the latter to the flicker ERG. In such recordings, the spectral signature of S/UV cones usually only emerges when the sensitivity of the other cone class has been significantly depressed. To accomplish that goal, we recorded spectral sensitivity in the short wavelengths in the presence of an intense long-wavelength adaptation. In a preliminary experiment, the effectiveness of this adaptation was assessed by measuring the threshold for a 550-nm test light flickering at 12.5 Hz using an amplitude criterion of 6 μV. Threshold was measured in the absence of any accessory adaptation and then again in the presence of long-wavelength adaptation. For two animals so tested, this long-wavelength adaptation elevated the threshold to 550-nm test light (12.5 Hz) by 2.8–3.0 log units. With this adaptation in force, spectral sensitivity was then measured for three ferrets and the resulting function appears as Fig. 3. Long-wavelength adaptation elevated the threshold for test wavelengths longer than about 500 nm by an amount that made it impossible to assess sensitivity beyond that point. What remains (Fig. 3) is a spectral sensitivity function having a single peak in short wavelengths. The visual pigment template that best accounted for this spectral sensitivity function has λ_{max} at 429.7 nm.

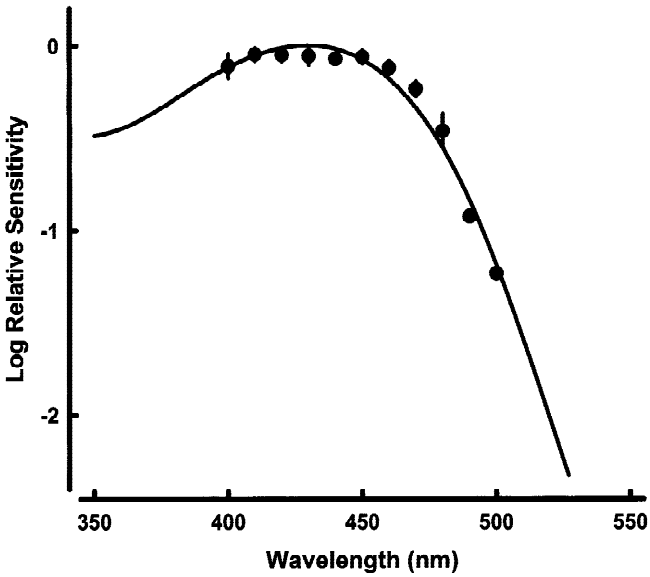

Fig. 3. Spectral sensitivity function for the ferret S cone obtained from ERG flicker photometry. The data points are mean values for three animals and the best-fit pigment absorption function has a peak value of 429.7 nm. Details of the measurement conditions are given in the text.

Ferret cone-isolating ERGs

As described in the Methods section above, we used the estimated spectra of the ferret receptors as defined in Figs. 1–3 to design stimuli that modulated the contrast seen by any one of the three receptor types (rods, S and L cones). In preliminary experiments, we assessed the effect of stimulus rate on the amplitude of the response by recording ERG responses at the maximum contrasts available for each of the three conditions of isolation. Both S and L cone-isolating stimuli gave small but reliable responses to 37.5-Hz stimulation (0.5 to 2 μV) and then significantly larger responses to stimuli presented at four slower rates (18.75, 12.5, 9.4, and 7.5 Hz). Over these four slower rates there was relatively small change in maximum amplitude for either test condition. No consistently reliable responses could be recorded for rod-isolating flicker at the highest rate and only small responses were detected for 18.75-Hz flicker (average of 2 μV for five animals). This latter rate was then selected for a further examination of cone contrast/response relationships.

For maximum contrast stimuli, the S- and L-cone responses obtained from six animals to 18.75-Hz stimulation were substantially antiphasic with S-cone responses lagging those recorded for L-cone modulation (mean difference of 197.5 ± 28.9 deg). The averaged contrast/response function obtained for five animals is shown in Fig. 4. The responses elicited by M-cone flicker were significantly larger than those obtained for S-cone flicker. The regression lines shown in Fig. 4 indicate that over the contrast ranges examined the contrast/amplitude relationship is linear for both ferret L and for S cone-driven responses (respective r^2 values of 0.95 and 0.99). The slopes of these functions define cone contrast gain and these differ significantly for L- and S-cone stimulation. So specified, the ferret L-cone contrast gain was 0.68 while the equivalent figure for the S cones was 0.13. The L/S cone contrast gain ratio for the ferret is thus 5.23.

Fig. 5 summarizes the photometric balances obtained between nine sets of stimuli designed to modulate the two cone types to differing degrees and a fixed contrast reference stimulus. Each data point in Fig. 5 represents the balance obtained for one of these conditions. Results are shown for two ferrets (squares and circles) from which it proved possible to obtain complete data sets. The responses from one subject were consistently less sensitive than that from a second animal. Nevertheless, the data from each subject are well described (r^2 values of 0.78 and 0.96) by a line, indicating that signals derived from the S- and L-cone activation contribute additively to the overall flicker ERG signal. The slope of the best-fitting line indexes the relative contribution of the S and L cones to the overall response. The slope of these lines are very similar for the two animals (−3.5, −3.8) implying that, on average, the relative contribution of the two cone types to the ERG flicker response is about 4 L to 1 S.

Cone opsin labeling

As previously reported (Peichl et al., 2001), two different classes of cone can be labeled in the ferret retina with opsin antibodies. The spectral measurements reported above now identify these types as L and S cones. The antibody JH492 labeled a substantial population of L cones, while a smaller population of S cones was stained with the JH455. Labeled cones were counted at approximately 50 sites taken at 1-mm intervals across two ferret retinas for both sets of antibodies. The density of L cones (Fig. 6) varies as function of retinal eccentricity with peak densities in a region slightly temporal and superior to the optic nerve head. For the two retinas the densities of the L cones ranged from 10,250/mm^2 to 28,750/mm^2 and from 5500/mm^2 to 30,700/mm^2. Based on these

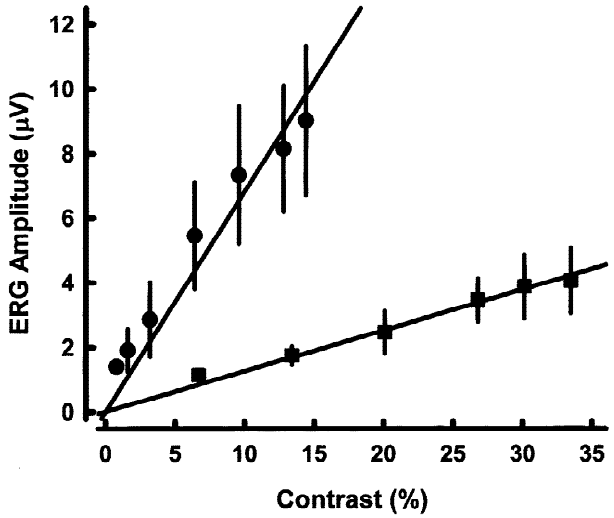

Fig. 4. Contrast/response functions obtained for isolated L- and S-cone stimuli (circles and squares, respectively). The test lights were flickered at 18.75 Hz. The data points are mean values obtained from six ferrets (±1 SD). The slopes of the best-fit lines define contrast gain (in μV/% contrast) for each of the cone types. For L-cone responses the contrast gain was 0.68; the corresponding value for responses to S-cone isolating stimuli was 0.13.

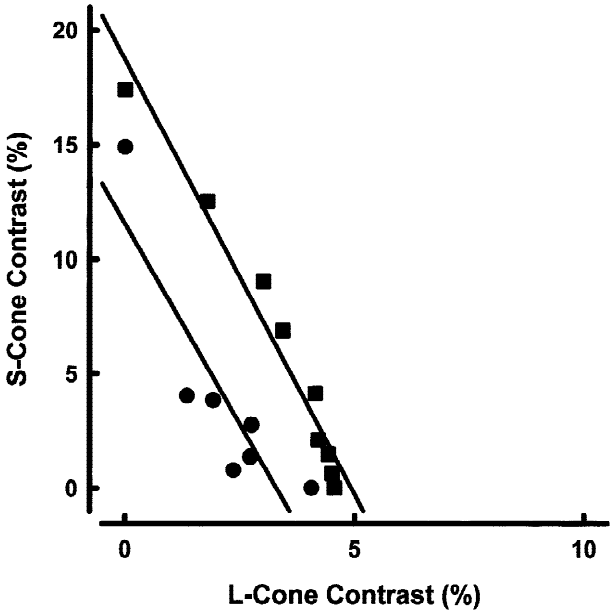

Fig. 5. Balance data obtained from two ferrets (circles and squares, respectively). Each data point represents the contrast required to obtain equivalent ERG signals for a specified test light that modulated the S and L cones in various proportions and an isochromatic reference light (contrast = 5%). The stimuli were full-field modulations generated on a computer-controlled monitor that was flickered at 18.75 Hz. The slopes of the best-fit lines for the two data sets are −3.5 and −3.8.

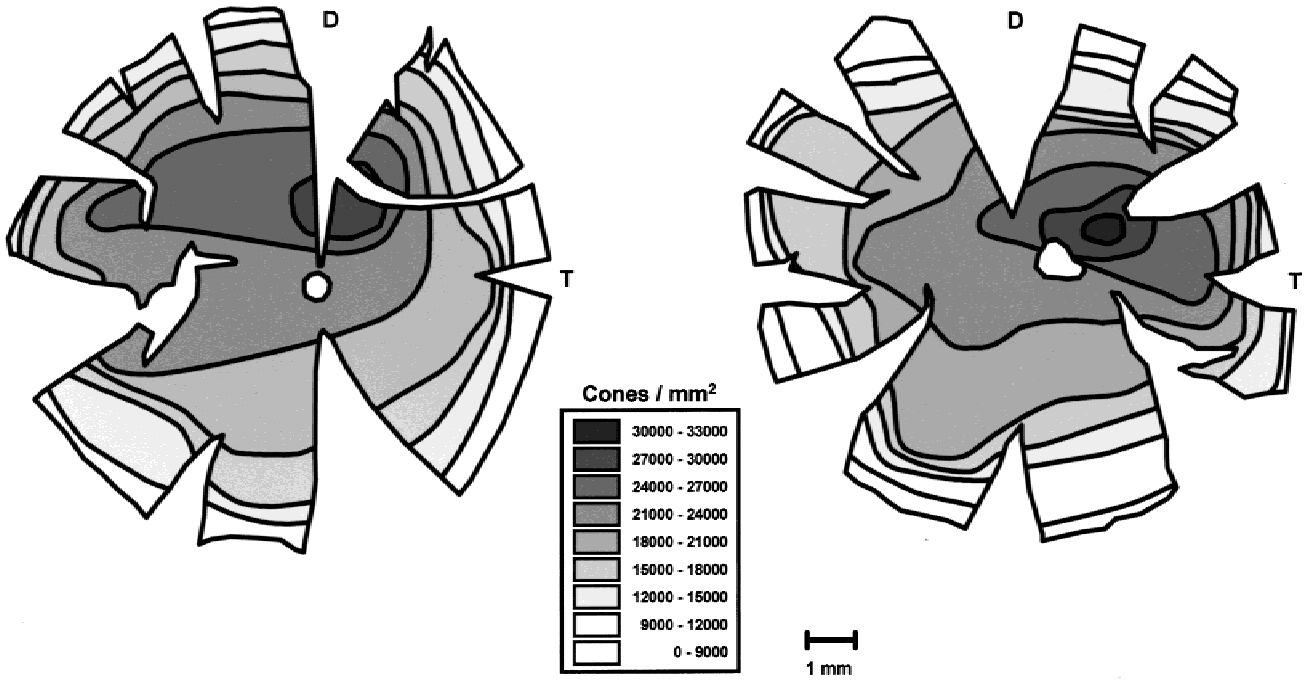

Fig. 6. Spatial distribution of L cones in the ferret retina. L cones were identified by their labeling to opsin antibody JH492. The isodensity contours were derived from counts made at approximately 50 sites taken at 1-mm intervals. The plots represent results obtained for two animals.

counts the total number of L cones in the ferret retina is estimated to be about 1.27 million. As for the L cones, ferret S cones (Fig. 7) have highest density in an area that is slightly temporal and superior to the optic nerve head. For the two ferrets the density ranges of S cones were 100/mm^2 to 3300/mm^2 and 200/mm^2 to 3700/mm^2. These counts imply a total S-cone population of about 98,000 and suggest that in the ferret retina L cones outnumber S cones by a ratio of about 13 or 14 to 1.

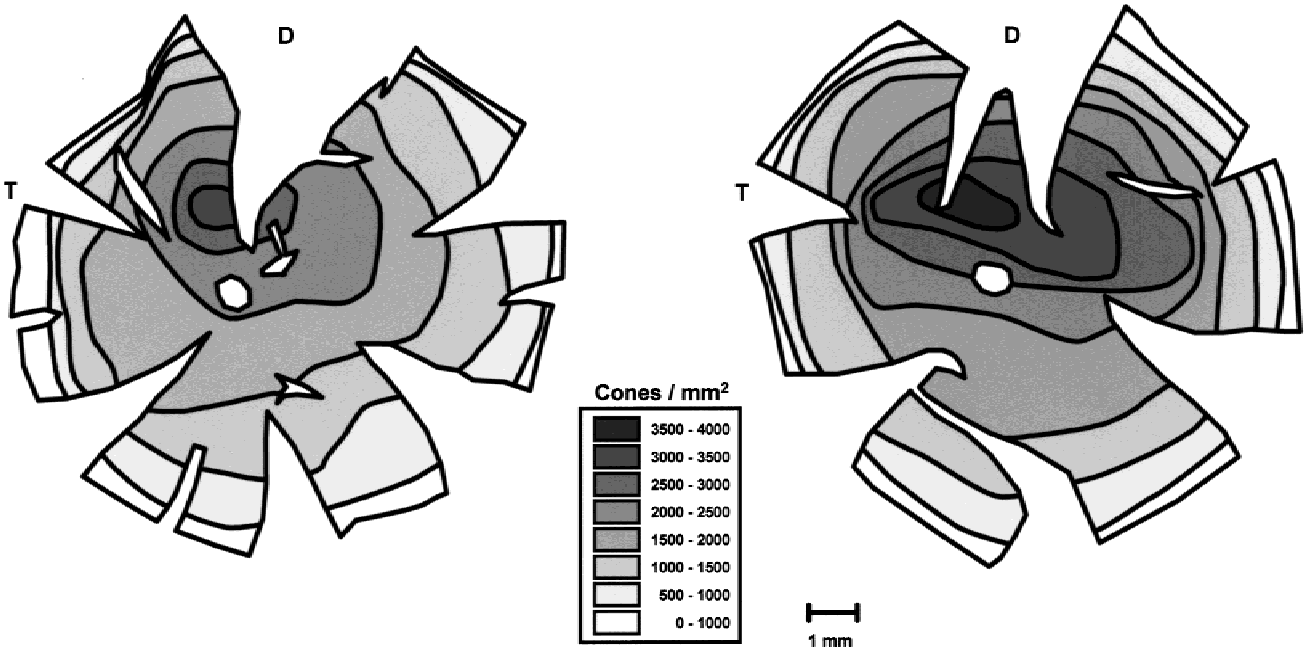

Fig. 7. Spatial distribution of S cones in the ferret retina. S cones were identified by their response to the opsin antibody JH455. Other details are as for Fig. 6.

Discussion

Recordings of spectral sensitivity identify three distinct mechanisms in the ferret retina and the conditions of measurement imply these principally represent contributions to the ERG from rods, L cones, and S cones. As in all such measurements made in an intact eye, the relationship between these spectra and the absorption properties of photopigments depends on assumptions about other properties of the eye that can condition spectral sensitivity. For the ferret there are three such factors not accounted for in the spectra of Figs. 1–3—lens absorption, tapetal reflectivity, and pigment self-screening. There are no direct measurements available to provide information as to the potential influence of any of these features on the measured spectral sensitivity, but several observations may be relevant. In an early study involving measurement of the absorption properties of squirrel lens (Cooper & Robson, 1969), a passing comment is made to the effect that no signs of specific absorption of light in the visible or near UV regions of the spectrum were detected in the lenses of a number of mammals, including that of the ferret. To the extent there is some small preferential absorption of short-wavelength lights by the ferret lens, it would tend to shift the estimated peaks of the three mechanisms toward shorter wavelengths, having its greatest influence on the S-cone mechanism and with proportionally smaller shifts for other two mechanisms. These effects are not apt to be very large. For example, correcting the ferret spectral sensitivity functions using lens absorption measurements that we have made (unpublished) for another nocturnal carnivore, the domestic cat, shifts the S-cone peak from 429.7 nm to 428.2 nm while a similar correction shifts the estimated rod peak shorter by 0.5 nm and the peak of the L-cone spectral sensitivity function by 0.4 nm.

As in most carnivores, the ferret eye also contains a tapetum comprising a region triangular in shape that is located above the optic disc (Tjalve & Frank, 1984; Wen et al., 1985). Its reflective properties have not been documented, but in any case its influence on spectral sensitivity would not be expected to be very substantial in measurements made with large test fields such as ours where signals generated by the tapetal and much more expansive nontapetal portions of the retina get averaged together. Note also that there appear to be no obvious deviations of spectral sensitivity from the pigment templates as might be expected if tapetal reflectivity had exerted a significant effect. Finally, self-screening broadens photopigment absorption spectra and can shift the location of spectral peaks estimated from template fits. Although there are no data-based estimates of pigment density in ferret receptors, the general effect of self-screening is to shift the peaks of the spectral sensitivity slightly toward longer wavelengths. The magnitude of the effect varies as a function of the location of the spectral mechanism such that, for example, increasing the assumed optical density (OD) of the L-cone mechanism from insignificant up to 0.3 shifts the peak 1.3 nm while the same operation applied to the rod spectral sensitivity curve yields a shift of 2.3 nm (from 505.6 nm to 503.3 nm). Considering that cones in general have relatively lower OD values, and that the measurements of cone spectra were made in highly light-adapted eyes, it seems unlikely that much correction for self-screening would be appropriate. How much of an effect self-screening might have on estimates of the rod peak remains an open question. In any case, the measurements of both L-cone and rod spectral sensitivity probably provide reasonably close estimates of the spectra of the photopigments themselves. Measurements of S cones were obtained more indirectly and that fact could make the estimate of the S-cone pigment peak somewhat less secure.

The spectral mechanisms identified in the ferret retina appear to be quite similar to those measured in other carnivores. For instance, using effectively the same techniques as those employed here, ERG measurements of the spectra of L cones were earlier obtained for cats and several species of canid (Jacobs & Neitz, 1986; Jacobs et al., 1993). With the same fitting assumptions as those employed for Fig. 2, we have recalculated the spectral positioning of the L cones of three species to be as follows: domestic dog (λ_{max} = 555.9 nm; SD = 1.74; n = 3), cat (λ_{max} = 556.9 nm; SD = 1.53, n = 7), ferret (λ_{max} = 557.6 nm, SD = 1.93; n = 6). No corresponding S-cone estimates are available for cat, but those obtained from ERG studies of the dog yielded a peak of about 430 nm, a value similar to that found here for ferret. There is similar agreement for comparisons of spectra based on rod signals for dog and ferret. Within measurement error it seems likely that these carnivores share in common their photopigments and, since both dog and cat have been shown to be capable of performing color discriminations (Neitz et al., 1989; Tritsch, 1993), this result also predicts that ferrets likewise have some capacity for color vision.

The isodensity maps for S and L cones we obtained (Figs. 6–7) are quite similar to earlier results reported for overall cone density in the ferret retina and, as well, the range of cone density values are also in general agreement with earlier estimates offered for this species (Jeffery et al., 1994; Peichl et al., 2001). In comparison to other terrestrial carnivores, the peak densities of M and S cones for the ferret are like those reported for other species like the domestic cat (Linberg et al., 2001) and several species of canid (Peichl et al., 2001).

Our counts suggest that ferret S cones comprise about 7% of the total cone population and their relative representation is thus similar to that of a number of other mammals (Szel et al., 1996; Calkins, 2001). S cones make no obvious contribution to spectral sensitivity in most flicker-based measurements as evidenced by the fact that full spectral sensitivity functions, as derived with either ERG or psychophysical techniques, can be accounted for by the spectral absorption properties of the M/L cones alone (Lennie et al., 1993). Under such measurement conditions, the presence of signals from S cones can be made apparent when contributions from the much more abundant M/L cones are suppressed, as they were in the ferret ERG through the agency of intense chromatic adaptation (Fig. 3). Recently, an alternative strategy that employs cone-isolating stimuli has been introduced as a tool for dissecting contributions of different receptor types to the overall human ERG signal (Usui et al., 1998; Kremers et al., 1999; Brainard et al., 1999). These experiments were principally directed toward understanding M- and L-cone signals and their combination. These successes and the recent demonstration that reliable ERG signals can also be recorded in response to human S-cone modulation (Scholl & Kremers, 2000) prompted us to examine the ferret ERG using cone-isolating stimuli.

As for human M and L cones (Usui et al., 1998; Brainard et al., 1999), at low levels of contrast the amplitude of the 18.5-Hz ferret ERG is linearly related to cone contrast for both S- and L-cone stimulation (Fig. 4). And for these same stimulus conditions, signals from ferret S and L cones combine linearly (Fig. 5), just as they do for human M- and L-cone signals (Brainard et al., 1999; Kremers et al., 1999). Estimates obtained from comparison of the contrast gains for ferret S and L cones and from the balance experiment are in agreement in suggesting that inputs to the 18.5-Hz ferret flicker ERG have an L:S cone weighting of about 4 or 5 to 1. This weighting is clearly different from what would be

predicted based simply on the relative prevalence of L and S cones (a ratio of about 14:1). One explanation for the discrepancy may be that flicker ERG signals do not principally index cone signals directly but rather mostly reflect bipolar cell activity (Bush & Sieving, 1996; Kondo & Sieving, 2002). If so, the difference in cone weighting seen in the ferret ERG might reflect gain changes in the outer plexiform layer applied to signals originating from S and L cones. The L- and S-cone weightings derived from experiments using cone-isolating stimuli may also seem inconsistent with the interpretation of spectral sensitivity functions derived from ordinary flicker ERGs. These two procedures presumably tap signals from the same site, yet no hint of an S-cone contribution appears in the flicker spectral sensitivity functions (see Fig. 2), certainly nothing of the magnitude that would be predicted from an L- to S-cone weighting of 4 or 5 to 1. To our knowledge there are no analogous comparative examinations of S- versus L- and M-cone signals in the human ERG, although there is a hint from comparison of the contrast gains reported for L- and M-cone modulation (Kremers et al., 1999) and for isolated S-cone modulation (Scholl & Kremers, 2000) for the existence of a similar disparity. Reconciliation of that difference may require an examination of how the differences in phase between S- and L-cone signals documented under conditions of cone isolation may influence the ordinary flicker ERG. It seems also likely that the linear and additive properties of these cone-based signals measured at low contrast levels change dramatically at the high levels of contrast inherent in ordinary flicker ERGs and that fact could greatly influence the inferred ratios of cone representation. In any case, ERGs can be straightforwardly obtained under conditions of cone isolation both for humans and other animals and this makes it attractive to examine further the relationships between the characteristics of signals derived from the separate cone classes under conditions of isolation and their combined effects as recorded in traditional flicker ERGs.

Acknowledgments

This research was supported by a grant from the National Eye Institute (EY02052). We thank Jess Deegan and Carrie Basila for their participation in some of the experiments and Ben Reese and David Brainard for helpful advice and cooperation.

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