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SHORT COMMUNICATION

Photopigment basis for dichromatic color vision in cows, goats, and sheep

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Abstract

Electroretinogram (ERG) flicker photometry was used to measure the spectral properties of cones in three common ungulates—cattle (*Bos taurus*), goats (*Capra hircus*), and sheep (*Ovis aries*). Two cone mechanisms were identified in each species. The location of peak sensitivity of an S-cone mechanism varied from about 444 to 455 nm for the three species; analogous values for an M/L-cone were tightly clumped at about 552–555 nm. Each of these three species has the requisite photopigment basis for dichromatic color vision and they are, thus, similar to other ungulates examined earlier.

Keywords: Electroretinogram, Cone pigments, Cattle, Goats, Sheep, Color vision

Introduction

Ungulates are often described as having good vision, but that conclusion is based more on inference from casual observation than on direct measurements. In an early review, Gordon Walls suggested that the large eyes of such animals could have a high rod/cone ratio to insure sensitivity to low light levels while at the same time accommodating a large retinal image to allow reasonable acuity even with a relatively sparse cone mosaic (Walls, 1942). Behavioral studies that could establish visual capacities are particularly challenging to pursue with large animal subjects. An attractive alternative is to examine those features of retinas that can be most directly related to visual capacity. With that possibility in mind, we earlier used an electroretinographic (ERG) technique to study the spectral properties of cones in representatives from two families of ungulate: from the family Suidae, the domestic pig (*Sus scrofa*) (Neitz & Jacobs, 1989) and from the family Cervidae, white-tailed (*Odocoileus virginiana*) and fallow deer (*Dama dama*) (Jacobs et al., 1994). In each case, we found evidence for two classes of cone whose spectral properties can be used to predict various visual capacities, including the presence of dichromatic color vision. We now report results from a similar examination carried out on three common species from Bovidae, the most populous family of the artiodactyls. These were domestic cattle (*Bos taurus*), sheep (*Ovis aries*), and goats (*Capra hircus*).

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Methods

Subjects

Recordings were obtained from five cattle (all females younger than 1 year), five adult sheep (three female, two male), and six adult goats (three female, three male). The animals were drawn from herds at the University of Georgia, Athens, where the experiments were conducted at the D.B. Warnell School of Forest Resources. All procedures were in accord with the National Institutes of Health guidelines on the care and use of animals and were approved by the Institutional Animal Care and Use Committee.

Apparatus

The apparatus and general procedures used to obtain ERG-based estimates of cone spectra have been fully described in previous publications (Jacobs & Neitz, 1987; Jacobs et al., 1996). Briefly, ERGs are differentially recorded using bipolar contact-lens electrodes of the Burian-Allen design. The stimuli are delivered as square-wave modulated pulse trains derived from a three-beam optical system that yields a circular retinal field subtending 57 deg presented in Maxwellian view. In the train are interleaved pulses from two sources: from a high-intensity grating monochromator (half-band = 10 nm) that serves as the test light and from a tungsten-halide lamp (the reference light). A third beam is used to provide adaptation lights. The spectral properties and radiance values of the two latter sources are controlled through the use of interference and neutral-density filters. The lamps for all three beams were

underrun at 11 V from regulated DC power supplies. The duty cycles of both the test and reference light train was 25% so that when they are interleaved successive stimuli from test and reference lights are separated by intervals that contain neither. The ERGs elicited by the test and reference lights are frequency filtered and electronically compared. The details of signal processing are given elsewhere (Jacobs et al., 1996). Sensitivities to the test wavelengths were determined relative to the fixed reference light. The intensity of each test wavelength is iteratively adjusted until the response it produces is equivalent to the response produced by the reference light. Test light intensity was varied by adjusting the position of a 3.0 log unit neutral-density wedge.

Procedure

The sedation procedure was as described elsewhere (Riebold et al., 1982). The drug regime for each species was (1) Cattle: i.m. injection of xylazine hydrochloride (0.22 mg/kg) followed by i.m. injection of atropine sulfate (0.13 mg/kg); (2) Goats: i.m. injection of xylazine hydrochloride (0.22 mg/kg) and atropine sulfate (0.4 mg/kg) followed 10 min later by ketamine hydrochloride (11.0 mg/kg, i.m.); (3) Sheep: i.m. injection of xylazine hydrochloride (0.22 mg/kg) and atropine sulfate (0.13 mg/kg) followed after 10 min by 22.0 mg/kg of ketamine hydrochloride (i.v.). The pupil of one eye was dilated by topical application of atropine sulfate (0.04%) and phenylephredine HCl and the cornea was anesthetized by topical application of proparacaine hydrochloride. The animal was positioned in ventral recumbency for recording with the head firmly supported in an upright position. The contact lens electrode was placed on the eye and a ground electrode was positioned against the inside of the cheek. All measurements were made in a room brightly illuminated by overhead fluorescent lights.

Flicker photometric equations were determined by averaging the responses to the last 50 of 70 stimulus cycles. As noted, these equations were made by adjusting the position of a neutral-density wedge. The wedge values required to equate the responses to the reference and test lights were recorded to the nearest 0.01 log unit. For each stimulus condition, the equations were made twice during the experiment and these values were subsequently averaged. For each species, we attempted to measure the spectral properties of short-wavelength sensitive (S) and middle-to-long-wavelength sensitive (M/L) cones. In preliminary measurements, the following stimulus conditions were determined to be favorable for isolating signals from these two cone classes: (1) For M/L-cone recording a pulse rate of 25 Hz was used. A long-wavelength reference light was used. This was produced by placing a high-pass filter (50% transmission at 580 nm) in the reference beam of the optical system (corneal radiance = 226 μW/cm^2). Accessory short-wavelength adaptation was used to attempt to suppress signals from S-cones and rods. It was provided by placing an interference filter (peak 460 nm; half-band = 10 nm) in the third channel of the optical system (radiance = 181.2 μW/cm^2). This light was on steadily throughout the recording. The test light was varied from 510 nm to 650 nm in steps of 10 nm. (2) For S-cone recording, the pulse rate was 12.5 Hz. A short-wavelength reference light was used (460 nm; corneal radiance of 7.9 μW/cm^2). This was used in conjunction with a steady, intense (7400 μW/cm^2) long-wavelength adaptation light that was intended to suppress rod and M/L-cone responses. The latter was produced by placing a high-pass filter (50% transmission at 580 nm) in the adaptation channel of the optical system. The test light was varied in steps of 10 nm from 410 nm to 500 or 510 nm.

Results and discussion

Large ERG signals could be recorded for 25-Hz stimuli from each of the three species. Under the conditions favorable for eliciting responses from M/L-cones, spectral sensitivity functions were obtained from three cows, five sheep, and six goats. The solid circles in Fig. 1 give the averaged spectral sensitivity values obtained for each of the three species. The variability of the data across animals was quite small as indicated by the error bars of Fig. 1. Photopigment absorption curves were fit to each data set (continuous lines). To generate these fits, a standard photopigment absorption curve (Ebrey & Honig, 1977) was shifted in steps of 1 nm along a log wavenumber axis (Baylor et al., 1987) until the best least-squares fit between the data array and the pigment absorption curve was obtained. These fits are quite good and the spectral peaks (λ_{max}) so determined were 552 nm for both the sheep and goats and 554 nm for the cattle.

Signals from S-cones could be readily recorded using long-wavelength adaptation and a short-wavelength reference light. The long-wavelength adaptation was sufficiently effective that no responses could be recorded for any test lights longer than about 510 nm. The average spectral sensitivity functions obtained with these conditions for three cows, four sheep, and four goats are given in Fig. 2. The magnitude of the variability between animals was again small (note error bars.) The best-fitting photopigment absorption curves were determined as described above. The derived λ_{max} values varied somewhat among the species (goat = 444 nm; sheep = 446 nm; cows = 455 nm).

The presence of cones in the retinas of these common ungulates has been noted from light (Rochon-Duvigneaud, 1943) and from electron-microscopic (Nilsson et al., 1973) observations. Although there appear to be no detailed receptor distribution maps for any of

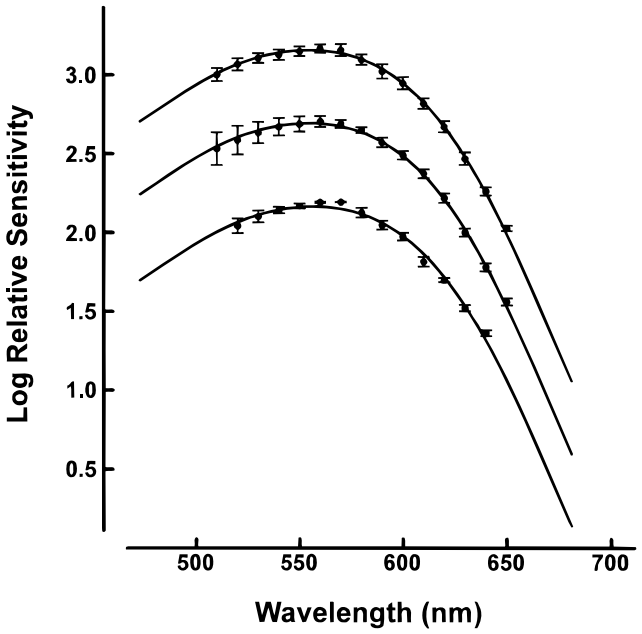

Fig. 1. Spectral sensitivity functions for M/L-cones of cows (top), sheep (middle), and goats (bottom). The data were obtained using ERG flicker photometry. Each solid circle is a mean sensitivity value (±1 S.D.). The data for the different species are arbitrarily positioned on the sensitivity axis. The curves are the best-fitting photopigment absorption functions determined as described in the text.

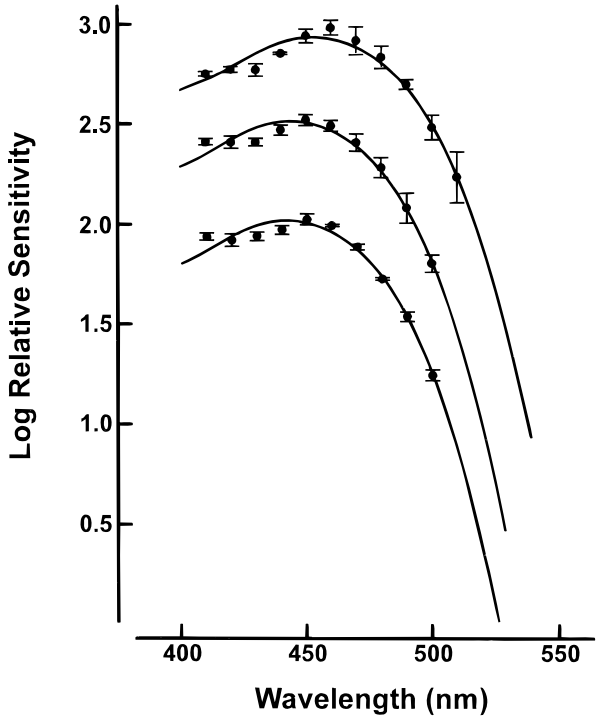

Fig. 2. Average ERG spectral sensitivity functions for S-cones of cows (top), sheep (middle), and goats (bottom). All other details are as described for Fig. 1.

these species, cones would appear to be reasonably abundant. In sheep retina, for instance, the rod/cone ratio is said to be 30–40:1 (Braekevelt, 1983). Earlier reports suggest that the bovine retina may contain a higher proportion of cones than that (a rod/cone ratio as low as 3:1 in some retinal locations—Rochon-Duvigneaud, 1943) and that there are a "substantial number of cones" in the goat retina (Prince et al., 1960). More recent evidence from immunocytochemical labeling and from the study of various molecular components of photoreceptor operation suggests the presence of both S- (Szel et al., 1988; Hamilton & Hurley, 1990) and M/L- (Ferreira et al., 1994) cone types in bovine retina. In addition, a bovine S-cone opsin gene has been isolated and sequenced (Chu et al., 1994), as has an M/L-cone opsin gene for the goat (Nei et al., 1997). Thus, the demonstration of the presence of two active classes of cone in these retinas is hardly unexpected.

The use of cone spectra recorded from intact eye to estimate the spectral absorption properties of the photopigments present is potentially subject to error if there are sizable contributions from spectrally selective inert ocular filters. The two most prominent possibilities in the present cases are absorption of light by the lens and reflection from the tapetum. We are not aware of any measurements of the spectral absorption properties of the lenses for any of these species. There is unlikely to be any differential absorption by the lens over the wavelengths used to estimate the M/L-cone spectra (510 nm and beyond), but there could well be some necessary correction for the short wavelengths tested in the experiments on S-cone spectra. The effect of any substantial short-wavelength attenuation by the lens would be to shift the estimated peak of the S-cones to longer wavelengths. Estimates of S-cone peaks obtained from fitting only the long-wavelength limb of the S-cone functions differ only slightly from those derived from the fitting the full functions (see below). This suggests that differential spectral absorption probably has only a minor effect on the spectral estimates reported here.

Like many other ungulates, all three of the species studied also have prominent tapeta. The spectral reflection from these structures has been qualitatively described as varying from "a brilliant blue" in cattle eyes (Rodieck, 1973) to a "greenish blue" or even "yellow" in sheep (Bellairs et al., 1975), to "bright yellow" or "yellowish green" in the goat (Prince et al., 1960). There are no measurements of the spectral reflectivity of these structures, and in any case it would be difficult to know how to correct for the presence of a structure that covers only a restricted portion of the retina. The use of reference and test lights from the same parts of the spectrum in each of the two experiments was designed in part to minimize any differential effects of tapetal reflection. The generally excellent fits of photopigment absorption curves to the M/L-cone spectra suggest that there was little tapetal contribution to these spectral sensitivity functions. However, the standard pigment absorption curves fit the derived S-cone functions less well and, in particular, the similar pattern of mismatch between data and fitted curves for all three species at wavelengths 460 nm and shorter suggests that there may have been some distortion introduced by an ocular absorption or reflection that is common to the eyes of all three species.

The results identify two cone types in each of the three species, the spectral peaks of which are fairly similar (S-cone: 444–455 nm; M/L-cone: 552–555 nm). To determine whether these species are likely to have identical pigment complements, we fit spectral absorption functions to the data obtained for each animal individually. To attempt to minimize any potential influence of intraocular filters, only the longer wavelength portions of each data set were used (for M/L-cone, 580 nm and longer; for S-cone, 450 nm and longer). This yields the summary data shown in Table 1, which contains for each species the mean λ_{max} values and the number of subjects that contributed to the estimate. A one-way analysis of variance was run on the spectral peak estimates. It indicates significant variation between species for the S- ($F = 19.51$, $P = 0.001$, $df = 2, 7$) and the M/L-cone estimates ($F = 8.20$, $P = 0.007$, $df = 2, 22$). *Post-hoc* tests (Scheffe) show that neither the S- or M/L-cone positions differ significantly between sheep and goat ($P > 0.05$), but that each species has S- and M/L-pigment positions that are significantly different from those of the bovine retina ($P < 0.01$). These apparent small differences between species probably have little practical implication and they should be interpreted very cautiously in light of the possible contributions from intraocular filters. Table 1 also includes a listing of the pigments found in earlier ERG measurements of other representative ungulates. Although all of these species have two classes

Table 1. *ERG estimates of the spectral peaks (in nm) of cones in ungulates*

Species	S-Cone	M/L-Cone
Cow	451.3 (3)	555.3 (4)
Sheep	445.3 (4)	552.2 (5)
Goat	443.3 (4)	552.5 (3)
Pig	440.7 (3)	556.7 (3)
Fallow deer	453.6 (4)	542.2 (6)
White-tailed deer	456.0 (4)	536.8 (6)

of cone pigment, there is some modest variation in the estimated spectral positioning of the pigments. These seem particularly clear in the case of the deer M/L-cones that are significantly displaced toward the shorter wavelengths relative to the M/L-cones in the other ungulates.

The deduced amino acid sequence has been recently reported for the goat M/L-opsin (Nei et al., 1997). For primate M/L-photopigments, a considerable amount has been learned about the relationship between the amino acid sequence of the opsin and the spectral absorption peak of the photopigment (Neitz et al., 1991; Williams et al., 1992; Merbs & Nathans, 1992; Asenjo et al., 1994). Changes at two closely spaced amino acid positions (positions 277 and 285) together produce the large spectral shift that separates these photopigments into M- and L-classes. However, there are five other amino acid locations at which substitutions have also been shown to influence the spectral peak of these M/L-pigments. For primates, it is possible to closely predict the spectral peak of the M/L-pigments from knowledge of the identities of these seven dimorphic amino acids (Asenjo et al., 1994). The goat opsin has the same amino acids as do primate L-pigments at positions 277 and 285, thus placing it in the L-class. For three other spectrally active positions (116, 180, and 233), the amino acids of the goat opsin are those that correspond to photopigments slightly shifted toward the shorter wavelengths, while the amino acids at positions 230 and 309 predict shifts to slightly longer wavelengths. The combination of the amino acids at all of these positions predicts the goat photopigment to a peak somewhere in the interval between 551 and 555 nm. This predicted location is close to that observed in our measurements. One implication of this correspondence is that the tuning sites in ungulate opsins are probably the same as those established for primate opsins.

The most immediate functional implication of the present results is that each of these three ungulates has the requisite photopigment basis to support dichromatic color vision. Over the years, there have been a number of studies that attempted to establish color vision in several species of the family *Artiodacytla* (reviewed in Jacobs, 1993). Generally, the claims for the presence of color vision have been positive. Although many of the studies can be criticized on various technical grounds, they seem likely to have reached the correct conclusion.

Note added in proof

Radlwimmer & Yokoyama (1997) recently reported measurements of the spectra of the photopigment artificially expressed from the goat M/L opsin gene [Radlwimmer, F.B. & Yokoyama, S. (1997). Cloning and expression of the red visual pigment gene of goat (*Capra hircus*). *Gene* **198**, 211–215.] The peak they report (553 nm ± 1) is the same as that reported here.

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