Photopigment basis for dichromatic color vision in the horse

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Horses, like other ungulates, are active in the day, at dusk, dawn, and night, and they have eyes designed to have both high sensitivity for vision in dim light and good visual acuity under higher light levels (Walls, 1942). Typically, daytime activity is associated with the presence of multiple cone classes and color-vision capacity (Jacobs, 1993). Previous studies in other ungulates, such as pigs, goats, cows, sheep and deer, have shown that they have two spectrally different cone types, and hence, at least the photopigment basis for dichromatic color vision (Neitz & Jacobs, 1989; Jacobs, Deegan II, Neitz, Murphy, Miller, & Marchinton, 1994; Jacobs, Deegan II, & Neitz, 1998). Here, electroretinogram flicker photometry was used to measure the spectral sensitivities of the cones in the domestic horse (*Equus caballus*). Two distinct spectral mechanisms were identified and are consistent with the presence of a short-wavelength-sensitive (S) and a middle-to-long-wavelength-sensitive (M/L) cone. The spectral sensitivity of the S cone was estimated to have a peak of 428 nm, whereas the M/L cone had a peak of 539 nm. These two cone types would provide the basis for dichromatic color vision consistent with recent results from behavioral testing of horses (Macuda & Timney, 1999; Macuda & Timney, 2000; Timney & Macuda, 2001). The spectral peak of the M/L cone photopigment measured here, in vivo, is similar to that obtained when the gene was sequenced, cloned, and expressed in vitro (Yokoyama & Radlwimmer, 1999). Of the ungulates that have been studied to date, all have the photopigment basis for dichromatic color vision; however, they differ considerably from one another in the spectral tuning of their cone pigments. These differences may represent adaptations to the different visual requirements of different species.

Keywords: horse (*Equus caballus*), comparative color vision, dichromacy, cone photopigment, electroretinogram, ungulate

Introduction

In modern classification schemes, the ungulates are a diverse group of mammals that includes seven taxonomic orders (Nowak, 1999; Tudge, 2000). However, traditionally, only the hoofed mammals in Perissodactyla (odd-toed) and Artiodactyla (even-toed) were considered "true" ungulates, with a greater number of species belonging to Artiodactyla. Thus, in comparative studies of mechanisms underlying color vision, the majority of existing data for ungulates comes from the Artiodactylids (Neitz & Jacobs, 1989; Jacobs, 1993; Jacobs et al., 1994;

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Jacobs et al., 1998). We have examined a member of the order Perissodactyla, the domestic horse (*Equus caballus).*

Trichromatic color vision is found only in primates, and the most common form of color vision in nonprimate mammals is dichromacy (Jacobs, 1993). Dichromatic mammals have one cone photopigment maximally sensitive in the middle-to-long wavelength region of the spectrum and a second pigment with a spectral peak (λ_{max}) in the short wavelengths; either an ultraviolet (UV)-sensitive pigment, as found in many rodents (Jacobs, Neitz, & Deegan II, 1991; Jacobs, 1993), or a more traditional short-wavelength-sensitive (S)-cone pigment.

Information about the number of spectrally distinct photopigments an animal has can be obtained through an examination of their spectral sensitivity. An efficient and reliable method for obtaining spectral sensitivity functions in vivo is the flicker photometric electroretinogram (ERG) (Jacobs, Neitz, & Krogh, 1996). The ERG has been used previously to examine the cone pigments in a number of ungulates, cattle (*Bos taurus*), goats (*Capra hircus*), sheep (*Ovis aries*) (Jacobs et al., 1998), deer (*Odocoileus virginianus* and *Dama dama*) (Jacobs et al., 1994), and pigs (*Sus scrofa*) (Neitz & Jacobs, 1989). All have been found to have two different types of cone, an S cone (range in λ_{max}, 439–456 nm) and a single middle-to-long-wavelength-sensitive (M/L) cone (range in λ_{max}, 537–557 nm).

A recent behavioral study on the chromatic discrimination of the horse suggests that horses are at least dichromats (Macuda & Timney, 1999). In addition, the presence of two distinct cone types (an S type and an M/L type) has been demonstrated using immunohistochemical methods (Sandmann, Boycott, & Peichl, 1996). An M/L opsin gene from the horse has been cloned and sequenced, and when expressed in cultured cells, the pigment has a λ_{max} of 545 nm (Yokoyama & Radlwimmer, 1999). We used the flicker-photometric ERG to examine the spectral properties of the cone pigments in the horse in vivo. We found evidence for two cone types in the horse that provide the basis for dichromatic color vision.

Methods

Subjects

Recordings were obtained from six ponies. All animals were free of ocular disease as assessed by biomicroscopic and indirect ophthalmic examination. 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 of the University of Wisconsin-Madison. The experiments reported here adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Visual Research.

Apparatus

The apparatus used to obtain ERG-based estimates of cone spectra has been previously described (Jacobs et al., 1996; Jacobs et al., 1998; Carroll, McMahon, Neitz, & Neitz, 2000). Briefly, stimuli were presented using a three-channel Maxwellian view optical system. The light sources for the three beams were two Osram Xenophot HLX bulbs (50 W, 12 V underrun at 11 V). One beam was used for chromatic adaptation. The other two beams were used to produce a monochromatic test light and an achromatic reference light, respectively. A Varispec liquid-crystal electronically tunable filter (Cambridge Research & Instrumentation, Boston, MA) controlled the wavelength of the test light. The lights were superimposed to illuminate a circular portion of the retina subtending approximately 70 degrees. High-speed electromagnetic shutters (Uniblitz; Vincent Associates, Rochester, NY) allowed alternate presentation of the reference and test lights at varying frequencies (25 and 12.5 Hz). An off period (in which neither test or reference light was presented) was interposed between presentation of the test and reference lights. Thus, for example, for the 25-Hz flicker condition, the stimulus sequence was test light (10 ms), off (10 ms), achromatic reference light (10 ms), and off (10 ms). An active electrode, made with fiber from the DTL Plus™ electrode, was placed on the cornea. A reference electrode was secured subdermally above the eye, and a ground was positioned subdermally adjacent to the ear. Details of the signal-processing system are described elsewhere (Neitz & Jacobs, 1984; Jacobs et al., 1996). Spectral sensitivities were measured by comparing the response from a test wavelength to that from the fixed reference light. A circular neutral density wedge (3 log unit) was used to adjust the intensity of the test light while the intensity of the reference light remained constant. The intensity of each test wavelength was adjusted until a null of minimum ERG signal amplitude and intermediate phase was produced. This null was taken as the point when the effectiveness of the test light equaled that of the fixed reference light.

Recording Procedure

The horses were anesthetized with an intravenous injection of ketamine HCl (2 mg/kg) plus xylazine HCl (0.1 mg/kg). The horses were then intubated and maintained on halothane. The pupil of the eye to be tested was dilated by topical application of 2% atropine

sulfate and phenylphredine HCl. The eyelid was held open with a speculum and artificial tears were applied frequently to the open eye to prevent the cornea from drying out. The animal was positioned on its side with the head slightly propped up, though supported firmly. The ERG apparatus was maneuvered to present the visual stimuli parallel to the pupillary axis. Measurements were made in an open room under fluorescent lighting.

Spectral sensitivity measurements were made for two test conditions. Both were designed to eliminate rod contributions. The first set of conditions was designed to maximize contributions from M/L cones by employing a high flicker rate (25 Hz). Spectral sensitivity measured under these conditions was obtained from four of the horses. The second set of measurements was made using conditions designed to maximize contributions from S cones by suppressing contributions from M/L cones, and these measurements were made on four horses, two of which also had M/L cone measurements. This was done using a slower flicker rate, 12.5 Hz, and an intense long-wavelength adaptation light that was produced using a high pass filter (50% transmission wavelength = 600 nm; corneal irradiance = 3027 μW). In the conditions designed to elicit responses from M/L cones, the test light was varied by 10-nm increments from 480 to 630 nm, and for recording responses from S cones, relative sensitivity was determined at 10-nm increments over a range of 440 to 600 nm. The final spectral sensitivity values are reported as quantal intensities. Intensities measured at the cornea were corrected for absorption by the lens using previously measured lens densities in the horse (C.J.M., unpublished data, 1999). However, the absorption by the lens is rather small; for example, the absorption is 0.20 at 460 nm, and diminishes to zero in the middle-to-long wavelengths (beyond 540 nm).

Results

Cone Spectral Sensitivities of the Horse

Figure 1 shows the average spectral sensitivity data from four horses obtained under the conditions designed to elicit signals from M/L cones. The spectral peak (λ_{max}) of the M/L photopigment in the horse was estimated by fitting the data to a template curve that was designed to accurately describe the spectral shape of any vitamin A_1-based photopigment (Carroll et al., 2000). When the optical density (OD) value was fixed at 0.35, the best-fitting template curve had a λ_{max} of 539 nm.

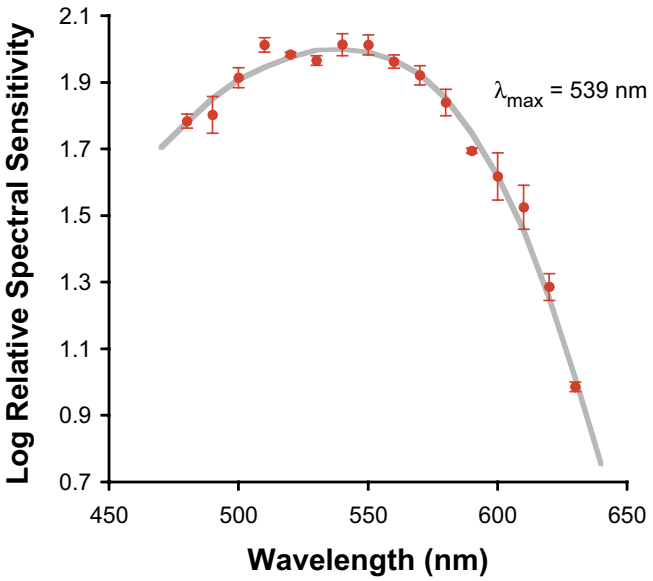

Figure 1. Average ERG flicker photometric spectral sensitivity function for four horses obtained under conditions designed to maximize contributions from the M/L cones. The solid circles are average sensitivity values obtained by equating the effectiveness of monochromatic test lights to that of the achromatic reference light. Error bars represent ± 1 SEM. The solid curve represents the best-fitting photopigment template; it has a λ_{max} of 539 nm.

The S cone of the horse was detected using an intense long wavelength adaptation light to suppress responses from the M/L cones. Under these conditions, even though the M/L cone responses are greatly suppressed, they do have a residual contribution to the ERG signal. It was assumed that the contributions of the two cone types under these conditions are linearly summed (Jacobs, Deegan II, Crognale, & Fenwick, 1993). Using an M/L cone with a spectral peak of 539 nm, the best-fitting combination of S-cone proportion and S-cone peak sensitivity was determined using a computer program. Figure 2 shows the results of this procedure. The λ_{max} of the best-fitting S-cone pigment curve was 428 nm with a relative weighting of 95% S, 5% M/L contribution.

Discussion

Classification of the Horse Pigments

The horse M/L pigment was recently measured in vitro as part of a larger study of spectral tuning of mammalian M/L photopigments (Yokoyama & Radlwimmer, 1998; Yokoyama & Radlwimmer, 1999).

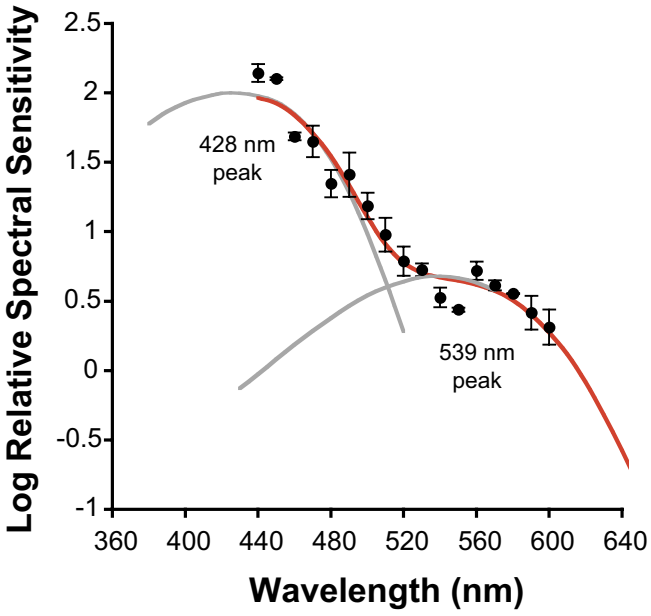

Figure 2. Average spectral sensitivity functions obtained from four horses using adapting conditions designed to isolate the S-cone class. The data were best fit by combining an M/L cone with a peak sensitivity of 539 nm (see Figure 1) and an S cone whose proportion and λ_{max} were allowed to vary. The λ_{max} of the S-cone pigment determined in this manner was 428 nm. Gray lines represent the M/L- and S-cone spectra, whereas the red line represents the best-fitting summed contribution from the two cone types (95% S, 5% M/L). Error bars represent ± 1 SEM.

The horse M/L opsin was expressed in cultured cells and reconstituted with 11-*cis*-retinal. A difference spectrum with a λ_{max} of 545 nm was obtained. In addition, from knowledge of the spectral tuning sites of other mammals (Neitz, Neitz, & Jacobs, 1991; Asenjo, Rim, & Oprian, 1994), a spectral peak near 546 nm was predicted for the horse. This supports the idea that the spectral tuning sites are highly conserved among the mammals. These values obtained from molecular methods are in reasonable agreement with the value obtained in vivo here (539 nm). The small discrepancy between the two measurements may be attributable in part to experimental error. In addition, other factors may contribute to the difference. For example, the in vitro measurements were done on pigments with negligible ODs; however, in the living eye, the photopigments have a significant OD. We used an OD value of 0.35 in estimating λ_{max} of the horse pigments, assuming different OD values for the horse pigments change the estimate of λ_{max}. For example, an OD of near zero yields a λ_{max} of 542 nm for the horse, whereas using an OD of 0.5 yields a corresponding λ_{max} of 537 nm. Also, the λ_{max} value obtained might be expected to be slightly shorter than the predicted values because of tapetal reflection in the horse. The bluish-green reflection (Martin, 1990) would augment the short wavelengths and thus might shift the estimated peak measured in vivo toward shorter wavelengths. In addition, it should be kept in mind that in these experiments λ_{max} is simply a parameter of fitting the data with a certain template, and thus the exact value depends on what template was used (Govardovskii, Fyhrquist, Reuter, Kuzmin, & Donner, 2000). Therefore, comparisons between methods may not be generally valid, and minor disagreements between different methods do not necessarily reflect real differences in the data.

In humans, dichromatic color vision is mediated by a single S-cone class, with a λ_{max} near 415 nm (Fasick, Lee, & Oprian, 1999), along with either an L- or M-cone class. The human L-cone pigments peak near 560 nm, whereas M-cone pigments peak near 530 nm (Neitz & Neitz, 1998). Most of the ungulates studied previously have an M/L pigment with a spectral peak close to the typical human L-cone pigment. An exception to this is the deer, which has an M/L-cone type more like that of a human M cone. In addition, all the ungulates studied to date have an S pigment that is significantly shifted toward longer wavelengths compared to the human S pigment. Both pigments of the horse (a Perissodactylid) differ from those of the Artiodactylids that were measured earlier; the horse S-cone pigment is shifted 20 to 25 nm shorter, and its M/L pigment is shifted closer to the human M-cone pigment.

In an earlier study in which ERG measurements were used to estimate the peak of the S cone in humans, it was estimated at 430 nm (Crognale, Jacobs, & Neitz, 1991), approximately 10 to15 nm longer than microspectrophotometric (MSP) and in vitro measurements (Dartnall, Bowmaker, & Mollon, 1983; Merbs & Nathans, 1992; Fasick et al., 1999). Considering this, one might wonder if a similar discrepancy would be seen if other estimates were available for the S cone in the horse. Much of the difference may be attributable to the fact that the earlier human estimate was obtained using a hybrid photopigment template that is less accurate than single templates, which have been used more recently. To our knowledge, the only more recent published examples where ERG and in vitro results can be compared for short wavelength pigments are UV pigment measurements from mice and rats (Jacobs et al, 1991; Yokoyama, Radlwimmer, & Kawamura, 1998), which show close agreement. We have used a newly derived template curve designed to accurately represent all A_1-based pigments (Carroll et al., 2000). Theoretically, this template should provide a valid estimate of S-cone spectral peaks. As a test, we measured human S-cone spectral sensitivity using our ERG system and estimated λ_{max} using the same procedure described here for the horse. We obtained an average value of 414 nm (for two human trichromats), which is in line with the MSP and in vitro estimates.

Finally, for the horse we have corrected for lens absorbance using data obtained from the same species. Not using any correction gives approximately a 3-nm *shorter* estimate of the S-cone peak. Thus, any uncertainty in the lens measurement would not produce large errors in the λ_{max} estimate. In conclusion, although there may be some uncertainty in the exact value of the peak for the S-cone pigment, we can say with confidence that its peak is considerably shorter than that of other ungulates studied to date.

Prospects for Color Vision in the Horse

How does the color vision of the horse compare to that of other mammals? Behavioral experiments have demonstrated color discrimination ability in the horse (Macuda & Timney, 1999). Evidence from immunohistochemistry (Sandmann et al., 1996), together with the data presented here, indicates that the horse has two cone types. This dictates that at photopic light levels the color vision capabilities must be limited to dichromacy, the only form of color vision that has been observed in nonprimate mammals (Jacobs, 1993). People often wonder what the visual world is like for an animal whose eyes and nervous system are different from our own. Because of its special relationship with humans as a companion and a form of transportation, as well as a beast of burden and source of recreation, there are probably few animals that have more often been the subject of curiosity about alternate sensory worlds than the horse.

Information about the ocular transmittance of the horse and the estimates of the spectral sensitivities of the cones presented here can be used to derive a sense of what the daytime color experience of the horse might be like. Normal human trichromats see four basic unique hues: red, green, blue, and yellow (Hurvich, 1981). They also see a continuum of intermediate hues that can be conceived as the simultaneous sensation of pairs of the unique hues in a range of proportions. Examples are blue-green and yellow-green as well as single-term colors like orange, which can be thought of as yellowish-red and violet, a reddish-blue. In total, a normal trichromat can distinguish about 100 different subtle variations of hue. Experience with human dichromats who have inherited red-green color vision defects indicates that instead of having four basic colors, they only have two hues, the ones most analogous to blue and yellow (Neitz, Carroll, & Neitz, 2001). One of the most dramatic differences believed to differentiate the visual world of the dichromat from the trichromat is that for dichromats there are no intermediate hues. For a dichromat, when colors from the two ends of the spectrum are mixed, rather than getting an intermediate hue, the result is either achromatic (white or gray) or a desaturated version of one of the two basic hues (ie, a pastel blue or yellow).

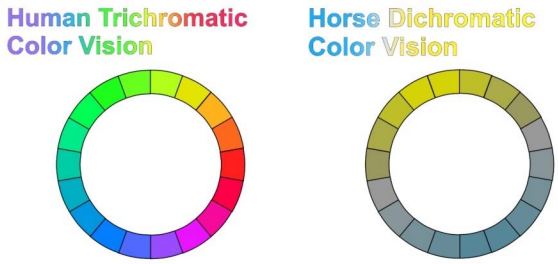

Figure 3. Difference between dichromatic color vision of the horse and normal human color vision. Left. Color wheel representing the spectrum of colors perceived by the trichromatic human visual system. Right. Reducing the number of types of cone from three to two results in dichromatic color vision, and an enormous reduction in the number of different colors seen.

For dichromats, there is a region, the spectral neutral point, in the middle of the spectrum that appears achromatic. Which wavelengths of light match an achromatic white depends on the positions of the spectral peaks of the two cone types and on preretinal absorption by the lens. Based on the results obtained here, Figure 3 simulates how the full gamut of colors might look to a horse. It is predicted to be similar to the experience of a red-green color blind human except the neutral (gray) regions are rotated (counterclockwise in Figure 3) compared to the human protanope or deuteranope. In Figure 4, we used methods similar to those described by Vienot et al to illustrate the color vision distinctions a horse might be able to make in a natural daytime scene (Vienot, Brettel, Ott, M'Barek, & Mollon, 1995; Brettel, Vienot, & Mollon, 1997). Gaussian blur has been added to the horse's view to roughly approximate the lower visual acuity of the horse compared to that of humans (Timney & Keil, 1992) (J.N.V.H., E. Bentley, R. Scagliotti, J.N., C.J.M., unpublished data, 2001). These illustrations give only a sense of the color world of the horse. There are many differences between the horse and human visual systems, including the positioning and optics of the eye and the anatomy and physiology of the retina (and higher visual centers), that contribute to differences between the visual capacity and experience of the horse and that of humans. These differences are not captured in such a simulation.

Figure 4. Real life implications of dichromatic color vision for the horse. Two unaltered digital images (A,B) and digitally altered (C,D) forms of the same pictures simulate the dichromatic color vision of the horse. A computer algorithm was used to simulate how each color in the original picture would appear to a dichromatic horse possessing visual pigments with the spectra determined in this study. To more closely approximate the horse's visual experience, the images were also adjusted to take into account the decreased spatial acuity of the horse.

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