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Refractive State of Tree Shrew Eyes Measured with Cortical Visual Evoked Potentials

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Abstract

Purpose—To determine the refractive state of tree shrew eyes using visual evoked potentials (VEP's) recorded from primary visual cortex and compare the values with those obtained with streak retinoscopy and with an autorefractor.

Methods—VEP's were recorded in seven normal tree shrews and three animals in which ~5 D of myopia (relative to control eye) was induced by monocular −5 D lens wear. While the animals were awake, refractive correction was measured with an autorefractor before and after cycloplegia (1% atropine and 2.5% phenylepherine). When anesthetized, cycloplegic refractive correction was measured with streak retinoscopy. Then VEP's were produced with square-wave counterphased (1 Hz) high-contrast checkerboard patterns near the animals' high spatial frequency cutoff. Spherical lenses (2 D steps) were placed before the eye, and the VEP (average of 128 sweeps) was measured to determine the lens that produced the largest first positive peak (P1).

Results—VEP's were obtained over a broad range of trial lenses. Tuning was narrower when check sizes were small. In normal and control eyes, the P1 amplitude was largest, on average, for a trial lens of (mean ± SD) −0.6 ± 1.6 D (corrected for working distance but not vertex distance). The mean streak retinoscopy value (spherical equivalent at the corneal plane) was 7.0 ± 0.8 D, and mean autorefractor values were 4.0 ± 1.1 D (cycloplegic) and 3.7 ± 1.2 D (noncycloplegic). In the eyes that compensated for a −5 D lens, the largest P1 values occurred with lenses with a power of −6.3 ± 3.2 D. Thus, the VEP measure showed a similar treated vs. control eye difference as did streak retinoscopy (treated eyes, 4.7 ± 0.4 D myopic) and the autorefractor (treated eyes, 4.8 ± 0.5 D myopic).

Conclusions—Normal tree shrew eyes are approximately emmetropic. The hyperopic values obtained with streak retinoscopy and the autorefractor are consistent with the presence of a “small-eye artifact” in tree shrews. Eyes that have compensated for a −5 D lens are myopic by approximately the value of the lens.

Keywords

animal models; refractive error; hyperopia; retinoscopy; tupidae

Cycloplegic streak retinoscopy is widely used to assess refractive state in human eyes (axial length ~24 mm). Refractive correction (the lens power needed to correct a refractive error) measured with streak retinoscopy corresponds well with the refractive correction determined subjectively.[1] When streak retinoscopy is used to measure the refractive correction of eyes with a short axial length (~6 to 8 mm), many appear to be hyperopic. In 1970, Glickstein and

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Millodot[2] proposed that the hyperopic values are due to a "small-eye artifact" that they hypothesized occurs because the retinoscopic reflex originates at the vitreous-retina boundary rather than from the photoreceptors located 150 to 200 m more posteriorly.[3] In large eyes, this error is small. However, in animals with short axial length, emmetropic eyes would appear to be substantially hyperopic. Such a small-eye artifact would need to be corrected to provide an accurate measure of refractive state.

Tree shrews (small mammals closely related to primates) have emerged as a useful model of refractive development and induced myopia.[4] When exposed to a monocular minus-power lens or to a translucent diffuser, the treated eye elongates and becomes myopic relative to the control eye. However, streak retinoscopy of normal tree shrew eyes (axial length ~8 mm) under atropine cycloplegia produces estimates that the eyes are 4 to 10 D hyperopic.[3, 5-8] Refractive measures made with a coincidence optometer,[3] an autorefractor,[9] and an infrared photoretinoscope (unpublished data) also yield hyperopic values of 2 to 6 D. It is important to learn whether these values are artifactually high for two reasons. First, for tree shrews to be a model for emmetropization, it is important that the eyes regulate their axial length relative to their focal plane to achieve emmetropia rather than a substantially hyperopic refractive state. Second, to be a model for induced myopia, it is important that the treated eyes of tree shrews actually become myopic, not just myopic relative to a hyperopic control eye.

Because it is not possible to obtain a refractive correction from tree shrews by a verbal answer to "which trial lens gives the clearest image?" the question of whether normal tree shrew eyes are emmetropic must be addressed another way. In humans, the amplitude of cortical visual evoked potentials (VEP's) produced by checkerboard patterns is greatest when the image is in focus and decreases as the image is defocused.[10-14] In cats[15] and rats,[16] the change in amplitude of cortical VEP's obtained with differing trial lenses has been used to determine the refractive state of the eyes. We thus recorded cortical VEP's from anesthetized tree shrews to assess the refractive state of their eyes. These results were compared with measures made with streak retinoscopy and with an autorefractor. The results of this study have appeared in abstract form.[17]

METHODS

Animals

Seven normal tree served as subjects along with three animals that wore a monocular −5 D lens to induce myopia in the treated eye. All were raised by their mothers in our breeding colony. At the time VEP's were recorded, the normal animals ranged in age from slightly <2 months to 3.7 years of age. Treated animals were all juveniles, ranging in age from slightly <2 months to nearly 4 months of age. The animal care and use in this study complied with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The project was approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham.

Electrode Implantation

The procedure for placing the chronically-implanted electrodes closely followed the method described by Siegwart and Norton[18] to attach a pedestal to the animal's skull. While each animal was anesthetized (17.5 mg ketamine HCl/1.2 mg xylazine HCl im with 1 to 3% halothane inhalant supplement), a stainless steel skull screw was placed extradurally near the *area centralis* representation of the primary visual cortex using the map of Kaas et al.[19] A reference and a ground electrode were placed over the frontal sinus. The screws were held in place with dental acrylic with the screw heads exposed so that a clip could be attached to connect them through shielded wire to a differential preamplifier. A vertically-oriented dental

acrylic tab (~1 cm tall) was attached the dental acrylic on the midline. This allowed the animal's head to be held firmly and painlessly during VEP recording sessions by a clamp attached to the tab. In the lens-treated animals, the vertical tab allowed a goggle frame containing a monocular −5 D lens to be clipped in place starting 24 days after natural eye opening.[18] After electrode implantation, the animals were returned to their home cage and recovered for at least 2 week before VEP's were recorded.

VEP Procedures

On 1 to 3 occasions, each animal was transported from the animal colony to the lab for the recording sessions reported here. The animal was weighed before each session to ensure that repeated recording sessions (at least 2 weeks apart) did not produce weight loss. Refractive measures were made in the awake animal on the pupillary axis with a Nidek ARK-700 autorefractor following the procedures outlined by Norton et al.[9] The spherical equivalent refraction at the corneal plane was calculated from the first five measures with a confidence value of ≥7 (on a scale of 1 to 9). This value is provided by the autorefractor as an index of the validity of the measures. Two drops of 1% ophthalmic atropine sulfate were then administered along with 2 drops of 2.5% phenylepherine HCl. On two occasions, no phenylepherine was administered. At least 1 hour later, the autorefractor measures were repeated.

The animal was then anesthetized with 17.5 mg ketamine hydrochloride/1.2 mg xylazine im. Up to three booster injections (5 mg ketamine/0.34 mg xylazine) were given at approximately 30 min intervals to maintain an anesthetic plane. Atropine sulfate (0.04 mg ip) was administered to prevent mucus secretions. The animal was placed on a temperature-regulated heading pad to maintain body temperature. Heart rate was recorded through skin leads and monitored through a loudspeaker.

The animal's head was held by the pedestal tab in an upright position approximating normal posture, and the upper eyelid of the right eye (except, in two lens-treated animals, both eyes) was held open with a single strand of ophthalmic 5-0 suture. Streak retinoscopy on the pupillary axis was always performed on the right eye and on both eyes in the lens-treated animals. Black electrical tape was placed in front of the nonstudied eye to prevent visual stimulation through that eye. In recording sessions in which only one eye was examined, the nonstudied eye was treated with 0.3% gentamicin sulfate ophthalmic ointment to prevent corneal drying. The corneal surface of the studied eye(s) was maintained by frequent (~ every 2 to 3 min) rewetting with artificial tears (Tears Naturale, Alcon, Ft. Worth, TX). Streak retinoscopy measures were repeated at the end of the recording session (typically about 2 h) and did not differ significantly from the initial measures. Inspection through an indirect ophthalmoscope at the end of the session confirmed the continued clarity of the cornea and ocular media. Observation of the eye through a CCD camera using infrared illumination ensured that excess fluid was removed during VEP recording and provided assurance that the position of the eye did not drift under anesthesia.

Recording

As in previous studies of tree shrew,[20] differential recording of VEP's was made between the cortical electrode and the reference electrode. The other anterior skull screw was connected to ground. A Grass P-15 amplifier (1000× gain, 0.1-Hz low-frequency, 100-Hz high-frequency filter settings) was connected to a Tektronix 7806 digital oscilloscope. A signal with known voltage was used to verify the gain of the amplification system.

Visual Stimuli

A checkerboard stimulus was generated by a Vision Research Graphics system on an Eizo monitor located 50 cm from the tree shrew. The stimulus area was ~38 × 26 cm, subtending

~41° W × 29° H at the animal's eye. The checkerboard pattern was composed of high-contrast (~99%) black and white nearly square rectangles with a mean luminance of 34 cd/m^2. The checkerboard was temporally square-wave counterphased (2 reversals/s). The checkerboard pattern was presented only while VEP waveforms were being collected. Otherwise, the screen was illuminated with a 10.7 cd/m^2 homogeneous gray background. The check sizes used ranged between ~0.5° to ~0.2° (1 to 2.5 cpd).

Using a large check size that produced a large VEP, the monitor position was adjusted to place the response field (about 15° to 20° in diameter, always smaller than the monitor screen) in the center of the monitor screen so that small drifts of the eye would not affect the VEP. For all animals, the response field was located directly in front of the animal, confirming that the *area centralis* region of the retina, with the greatest ganglion cell density,[21] was stimulated. The check size was then decreased to determine the smallest checks that produced a VEP with no lens in front of the animal. A check size near the smallest that produced a VEP response was selected. In each case, the VEP amplitudes with the selected check size were smaller than with larger checks, eliminating any concern of response saturation. Each VEP was the response to 128 phase reversals of the stimulus averaged by the digital oscilloscope. The VEP's were transferred to a PC and stored for later analysis.

Trial Lenses

A phoropter-like frame containing a set of spherical lenses from −12 D to +12 D (in 2 D steps) was positioned in front of the eye at a vertex distance of approximately 10 mm. VEP's were recorded with various lenses in place to determine the lens that yielded the strongest response from the primary visual cortex. Because anesthetic level, and thus VEP amplitude, often varied through the course of the recording session (iv administration was not possible in chronic tree shrews), VEP's were collected in sets of six, each set containing one VEP taken with a 0-power (plano) lens. This provided a measure of altered responsiveness unrelated to lens power. From 30 to 80 VEP's were collected during a recording session. The animal was then allowed to recover from anesthetic and was returned to its home cage. After the final recording session, the recording electrode position was verified as being over primary visual cortex (V1) by direct examination after an overdose of pentobarbital sodium and transcardiac perfusion with normal saline followed by 10% formalin.

Data Analysis

The stored VEP waveforms were examined with a Matlab program. For each check size used in a recording session, the program averaged all the VEP's obtained with a particular trial lens power and displayed the mean value ±1 SD. It then calculated the average baseline value of the averaged waveform during the first 29 ms, which was always a period shorter than the onset latency of the VEP. The amplitude of the first positive VEP peak (P1) was computed by subtracting the baseline from the positive peak value. The P1 values for each trial lens power were imported into Excel and normalized to the amplitude of the largest amplitude VEP obtained with that check size (given a value of 100). The normalized values were plotted vs. trial lens power after correction for the 50-cm working distance. The VEP measures were made at the spectacle plane because the trial lens values were not corrected for vertex distance. However this did not impact the results.

Statistics

For each animal, the VEP data across all sessions and check sizes were examined. When there was variability in the trial lens giving the largest P1 value, the investigators selected the trial lens value that best characterized the response pattern, giving heavier weight to VEP's taken using the smaller check sizes. The streak retinos-copy value and autorefractor value for each eye was the average of measures made across VEP recording sessions. Spearman rank order

correlations were used to determine whether streak retinoscopy and autorefractor measures were significantly correlated with VEP values. A Wilcoxon signed rank test (one-tailed, $\alpha = 0.05$) was used to determine whether the streak retinoscopy and autorefractor values were significantly more hyperopic than the VEP values.

RESULTS

Normal Eyes

Large-amplitude visual evoked potentials were consistently produced by stimulation with larger check sizes (~0.5° × 0.5°). As shown in Fig. 1, when large checks were used, there usually was little change in VEP amplitude as a function of trial lens power.

As shown in Fig. 2, when smaller checks were used, the P1 amplitude varied as a function of trial lens power. Checks that produced tuning generally produced smaller-amplitude VEP's and were close to the smallest checks that would produce a detectable VEP in a particular animal. These check sizes generally were close to the acuity limit of tree shrews, which was found to be 2 to 3 cpd by Petry et al.[22]

Fig. 3 shows the P1 amplitude vs. trial lens power in the second and third recording sessions in the same animal using three check sizes. When small check sizes were used, the largest amplitude VEP's occurred with a −4 D lens in one session and a −2 D lens in another session. However, as may be seen in Fig. 3, the P1 amplitude was near a maximum at a 4 to 6 D range, centered at about −1 D across these two sessions. Data from this and an additional session led us to record the best trial lens power as −1.5 D. The average cycloplegic autorefractor and streak retinoscopy values for the three sessions are shown in Fig. 3.

Fig. 4 shows the tuning in another normal tree shrew in two recording sessions using small check sizes. Similar tuning curves were obtained in each session, estimating the refractive correction at −1 D.

Table 1 shows the average refractive correction values obtained for each eye in the study using VEP's, streak retinoscopy, and the autorefractor. The Table also shows the noncycloplegic autorefractor values that were slightly (−0.5 ± 0.6 D) but significantly ($t = 2.9$, $p < 0.01$) less hyperopic than the values obtained with cycloplegia, consistent with the presence of a small amount of accommodation in the noncycloplegic measurements.

Eyes Treated with a −5 D Lens

In animals that wore a monocular −5 D lens, the VEP values for the control eyes were similar to normal eyes (Table 1). In the lens-treated eyes, the largest VEP's through the treated eye occurred when lenses with a power of −6.3 ± 1.6 D were placed before the eyes. This differed from the refraction in the normal and control eyes (−0.6 D) by 5.7 D. Fig. 5 shows the tuning profile for the lens-treated and control eyes of tree shrew 0065. In this session, a trial lens of −2 D produced the largest P1 amplitude in the control eye. In another session, the best response occurred with a +2 lens, leading to the estimate for this eye of 0 D. The tuning profile for the lens-treated eye was considerably shifted toward more negative trial lens powers (−10 D), the largest shift found in the study. Also shown in Fig. 5 are the streak retinoscopic and autorefractor values for the treated and the control eyes. In the three lens-treated animals, the treated eyes, measured with streak retinoscopy, were 4.7 ± 0.4 D myopic compared with the control eyes. The difference with the autorefractor was −4.8 ± 0.5 D. Thus, both techniques found a similar treated-eye vs. control-eye difference as did the VEP measure. However, the streak retinoscopy and autorefractor values indicated that the lens-treated eyes were slightly hyperopic (2.0 ± 1.4 D and 0.9 ± 1.3 D, respectively), whereas the VEP measure indicated the eyes were, in fact, myopic.

Comparison of VEP Measures with Streak Retinoscopy and Autorefractor Measures

Fig. 6 plots the streak retinoscopy and autorefractor values against the VEP values for normal, control, and −5 D lens-treated eyes. The VEP values for normal, control, and lens-treated eyes were significantly correlated with both the streak retinoscopy (Spearman $r_s = 0.73$, $p < 0.01$) and autorefractor values (Spearman $r_s = 0.76$, $p < 0.01$). As shown in Table 1, the estimate of refractive correction using VEP's was −0.6 ± 1.6 D. The autorefractor value in the same eyes was 4.0 ± 1.1 D, a difference of 4.7 ± 1.5 D. The streak retinoscopy value was 7.0 ± 0.8 D, which was higher than the VEP estimate by 7.6 ± 1.6 D. For every eye, the VEP estimate was lowest, the autorefractor estimate was intermediate, and the streak retinoscopy measure was the highest value. A similar pattern was found with the VEP, autorefractor, and streak retinoscopy measures in the lens-treated eyes. Both the streak retinoscopy and autorefractor values were significantly more hyperopic than the VEP values (Wilcoxon $p < 0.001$ and $p < 0.005$).

DISCUSSION

Are Normal Tree Shrew Eyes Emmetropic?

This study found that the amplitude of the first positive peak (P1) of the VEP recorded from tree shrew primary visual cortex was, on average, greatest with trial lenses of close to 0 D power for normal eyes and the control eyes of the lens-treated animals. Although it would be of interest to know the refractive state of these eyes with precision, the VEP technique had limited precision to detect small deviations from emmetropia. Factors that limited the precision include the variability of the responses within and across recording sessions, the breadth of tuning of the VEP amplitude vs. trial lens power, and the size of the steps between trial lens powers.

Response Variability

Within each recording session, there was variability in the amplitude of responses that occurred with any particular trial lens power. The variability appeared to be related to anesthetic state, such that the VEP amplitude seemed to wax and wane over time as the anesthetic state varied. Although efforts were made to use an intravenous route for anesthetic administration, it was not feasible to do so in chronic animals because of the small vein size in general and, in particular, the small size of the tail vein in tree shrews relative to rats. The effect of this variability on the response vs. trial lens function was minimized by taking repeated VEP's with the trial lenses and then averaging the responses as shown in Fig. 2. However, in some cases, the presence of dips and peaks in the lens power vs. VEP amplitude tuning function made it difficult to determine which trial lens produced the largest P1 response.

A benefit of using chronically implanted electrodes was that it allowed repeated measures so that results in a single animal could be compared across more than one session. For example, in Fig. 3, the overall similar shape of the functions and their similar peak values increased our confidence in the VEP estimate of refractive state of the eye.

Breadth of Tuning

As expected from previous studies in animals[15] and humans,[10-12, 16] the visual evoked potentials were not well tuned when large checkerboard patterns were used. Even when the smallest check sizes that would produce a response in an individual animal were used, the tuning was rather broad (e.g., the tuning for the control eye in Fig. 5). In part, this must be due to the depth of focus of the tree shrew eye, which was calculated[23] by Norton and McBrien[3] to be approximately ±1.5 to 2 D, or a range of 3 to 4 D for a normal pupil diameter of 3 mm. “Narrow” tuning functions where the response amplitude fell to 50% of maximum for lenses

2 D either side of the peak (as in session 2 in Fig. 5) were occasionally found. The breadth of tuning coupled with response variability made it difficult in some sessions to determine which one lens gave the “best” response.

The breadth of tuning of the human VEP's[10-12] is approximately one-fourth of that found in tree shrews. To the extent that the human tuning function is related to the depth of focus (approximately ±0.25 D), the breadth of the tree shrew tuning function would suggest that the depth of focus in tree shrews is ±1.0 D, somewhat narrower than was suggested by Norton and McBrien.[3]

Possible Effects of Pupil Diameter on the Tuning Function

Although opening the pupil with atropine and phenylepherine might have the effect of reducing the depth of focus and, hence, narrowing the tuning function, larger pupils also allow more spherical and chromatic aberration, which could increase the breadth of tuning. Central rays might focus best with one trial lens power and peripheral rays at another trial lens power, providing a large response over several trial lens powers. Although no direct assessment was made of the effect of pupil diameter on our measures, two factors suggest that the very large pupil did not produce a large distortion in the refractive estimates. First, on two sessions, phenylepherine was not used. The pupil diameter was still large due to the atropine, but smaller than with phenylepherine. In Fig. 3, no phenylepherine was used in session 3, yet the tuning function was similar to that in session 2 when phenylepherine was used. Second, autorefractor measures were taken both before and (>1 h) after atropine and phenylepherine were instilled. As reported previously,[9] there was a slight (0.5 ± 0.6 D) hyperopic shift (across all eyes in the study) when the autorefractor measures were obtained with cycloplegia and mydriasis. The noncycloplegic pupil diameter was still rather large (∼3 mm) in the awake tree shrews (which allowed the autorefractor to be used) possibly because they were agitated by restraint used for the measurements. The similarity of the autorefractor measures under these two conditions suggest that the large pupil size probably did not affect VEP and streak retinoscopy estimates to a greater extent than the autorefractor values. The relatively slight difference between the cycloplegic and noncycloplegic autorefractor measures also suggests that restraining the animal did not produce a sizable shift in refractive state as was found in shark.[24]

Two-Diopter Trial Lens Steps

To differentiate whether the VEP amplitude would be greatest at values predicted by streak retinoscopy (∼8 D of hyperopia) or whether a plano lens would provide the largest response as predicted by the analysis of Glickstein and Millodot[2], it was necessary to have a wide range of trial lens powers available. In addition, the number of VEP's that could be recorded in a session was limited. To collect VEP's with a wide range of trial lenses and to repeat measures to compensate for response variability, it was necessary to use 2 D steps between trial lens powers. This obviously limited the precision with which the best lens could be estimated. For instance, on occasions (Fig. 4) when relatively narrow tuning was found, it could not be determined whether larger VEP amplitudes would occur, for instance, with a trial lens power of −1 D or +1 D.

In summary, the VEP technique as applied in this study is consistent with the conclusion that normal tree shrew eyes are approximately emmetropic. Taken with data showing that tree shrew eyes actively regulate their axial length during the juvenile period, this suggests that the eyes are emmetropizing, rather than regulating their axial length to achieve a substantially hyperopic refractive state. To the extent that tree shrew eyes are approximately emmetropic, the optical effects of minus-power and plus-power lenses[25] can be understood as inducing hyperopic and myopic defocus, respectively, in situations where the viewing distance is controlled. This would not be the case if the eyes started from a substantially hyperopic refractive state.

Tuning Shift in Eyes with Induced Myopia

In two treated animals, VEP's were recorded both from the control eyes and from the eyes that developed myopia induced by wearing a −5 D lens. Because tree shrews have binocular vision and their cortical neurons are driven binocularly,[26] these VEP's were obtained from the same cortical electrodes by switching the opaque covering from one eye to the other and switching the phoropter position. Finding that the tuning function shifted to myopic values when VEP's were produced through the treated eyes showed that this measurement tool is capable of detecting large differences in refractive state. In addition, based on the VEP data, eyes that have compensated for a −5 D lens are, in fact, myopic. As described in the Results, the difference between the lens-treated and control eye values was similar with all three techniques and was close to the 5 D myopic shift expected from compensating a monocular −5 D lens. In a study with a larger number of animals with induced myopia,[9] similarly good agreement was found between streak retinoscopy and the Nidek autorefractor at measuring the difference in refraction between two eyes.

Refractive State Measured with the Autorefractor and Streak Retinoscopy

The autorefractor and streak retinoscopy yielded estimates that were significantly hyperopic compared with the eyes' refractive state measured with VEP's, suggesting that there is a substantial small-eye artifact for measures made with these two methods. The values (4.0 ± 1.1 D) obtained with the autorefractor in the current study are similar to those obtained in normal and control tree shrew eyes using a coincidence optometer (4 to 6 D).[3, 5, 27] These values also are close to that expected if, as suggested by Glickstein and Millodot[2] the light is reflected from the retina-vitreous boundary. Using a tree shrew schematic eye and the formula of Glickstein and Millodot, Norton and McBrien[3] calculated that the small-eye artifact would be approximately 4.5 D for a 7.8-mm normal tree shrew eye with a 150-m retinal thickness. That is, an emmetropic eye would appear 4.5 D hyperopic. Using the axial lengths of the normal and control eyes in this study (7.8 ± 0.2 mm), the estimated value would be 4.4 ± 0.3 D. This is very close to the values obtained with the Nidek autorefractor.

The nerve-fiber layer in tree shrew appears highly reflective when viewed with incandescent light[28] (Fig. 7) and, when measures were made with the coincidence optometer,[3] the image of the optometer mire was clearly seen on the nerve fiber layer. These observations suggest that visible wavelengths are strongly reflected by the nerve fiber layer. The similar values obtained with the infrared autorefractor suggests that infrared wavelengths also are reflected at the retina-vitreous boundary in this species. Recently, measurements were made of tree shrew eyes with a Shack-Hart-mann wavefront sensor[29] using an infrared light source. The wavefront sensor detected twin images, a deep one that appeared to come from the photoreceptors and a superficial one that appeared to come from the inner limiting membrane. The spherical equivalent value of the superficial image pattern was approximately 4 D, similar to the autorefractor values in the present study and to the values predicted by Glickstein and Millodot. Thus, a variety of measures appear to assess the refractive state of the retina-vitreous boundary in tree shrew eyes rather than the refractive state of the photoreceptors.

The streak retinoscopy values (7.0 ± 0.8 D for the normal and control eyes) were significantly more hyperopic than the autorefractor values. The reason for this is not known, but may be related to the large amount of “scissoring” in the retinoscopic reflex under cycloplegia, which may bias the values toward hyperopia if the retinoscopists attend to the central “with” reflex. To examine whether this scissoring was caused by the large pupil diameter in eyes with atropine cycloplegia, streak retinoscopy was performed on two additional tree shrews with and without a 3-mm artificial pupil. The estimate of refractive state was unaffected.

Comparison Across Species

Comparison of more than one refractive measure has rarely been made in any species except humans. In adolescent macaque monkeys (axial length ~17 mm), Ramamirtham et al.[30] compared streak retinoscopy with behaviorally measured subjective refraction and concluded that streak retinoscopy in young monkeys is approximately 2 D hyperopic relative to the subjective refraction, which is in reasonable agreement with the predicted artifact of Glickstein and Millodot[2] of retinoscopy for an eye of this axial length.

In rats, a variety of estimates of refractive state have been obtained over many years.[31] Two studies have used somewhat similar methods to the present study. Hughes[31] recorded from single ganglion cell axons in the rat optic nerve and examined their responses when a range of trial lenses were in place.[31] He found that there was very broad tuning that he concluded was consistent with the rat eye being emmetropic when the pupil is small. Because retinoscopy in rat yielded a strongly hyperopic value (refractive correction approximately +9 D), Hughes concluded there is a large small-eye artifact as suggested by Glickstein and Millodot.[2] More recently, Mutti et al.[16] measured the refractive state of pigmented and albino rats, comparing streak retinoscopy with estimates obtained with cortical VEP's. They reported that the largest-amplitude VEP responses occurred when plus lenses were in place before the eye and that the trial lens yielding the largest VEP corresponded rather well with the highly hyperopic estimate of refractive state provided by streak retinoscopy, They concluded that the small-eye artifact in rat was very small (~2 D).[16]

The differing estimates of the refractive state of the rat eye complicate comparisons between the tree shrew and rat. If the rat eye is emmetropic, there is good agreement between species. If the rat eye is strongly hyperopic, the source of the differences between the present results and those of Mutti et al.[16] are of interest. One possibility is that rat and tree shrew eyes may really be different such that the light used for streak retinoscopy reflects in rats from a location other than the retina-vitreous boundary. As a nocturnal animal, rats may be relatively indifferent to optical quality[32] and may accept significant hyperopia.

The evidence from VEP measures suggesting that tree shrew eyes are approximately emmetropic is consistent with the role of vision in this species. Tree shrews are highly vision-dependent animals with cone-dominated retinas.[33, 34] They have a relatively high ganglion cell density throughout the retina with an increased density at the *area centralis*.[21] Their behaviorally measured high spatial-frequency cutoff of approximately 2 to 3 cpd[22] is about half of that of cats and dogs despite a much smaller axial length. Achieving an eye with clearly focused images may have been of considerable survival value to tree shrews, leading to the development or preservation of a mechanism that uses visual input to guide the axial elongation of the eye to produce an eye that is approximately emmetropic.

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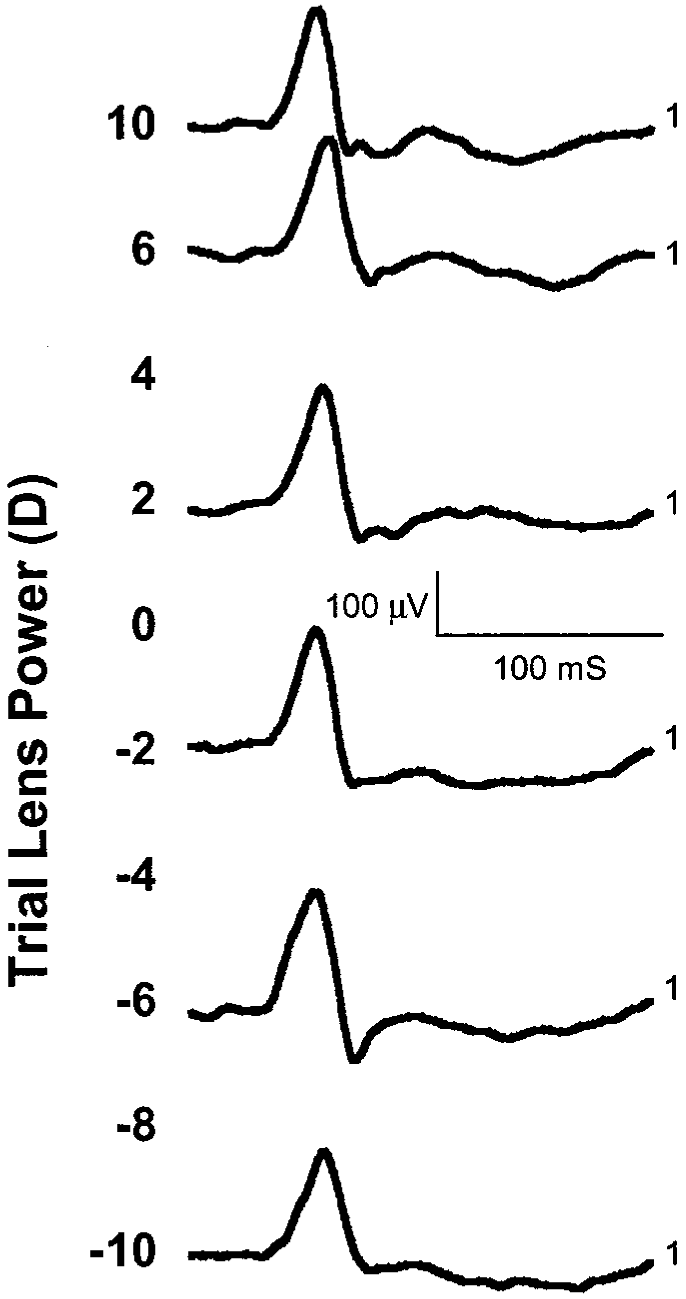

FIGURE 1.

Visual evoked potentials (VEP's) recorded from primary visual cortex in a normal tree shrew (0028) using a checkerboard pattern with each check having a width of 0.37° and a height of 0.41° (approximately 1.3 cpd). Large first positive peak (P1) amplitudes were obtained over a 20 D range of trial lenses (+10 D to −10 D). This broad tuning is shown graphically in Fig. 3. Numbers to the right of each VEP waveform indicate that a single VEP was taken with each trial lens in the recording session. The scale marker indicates time and response amplitude.

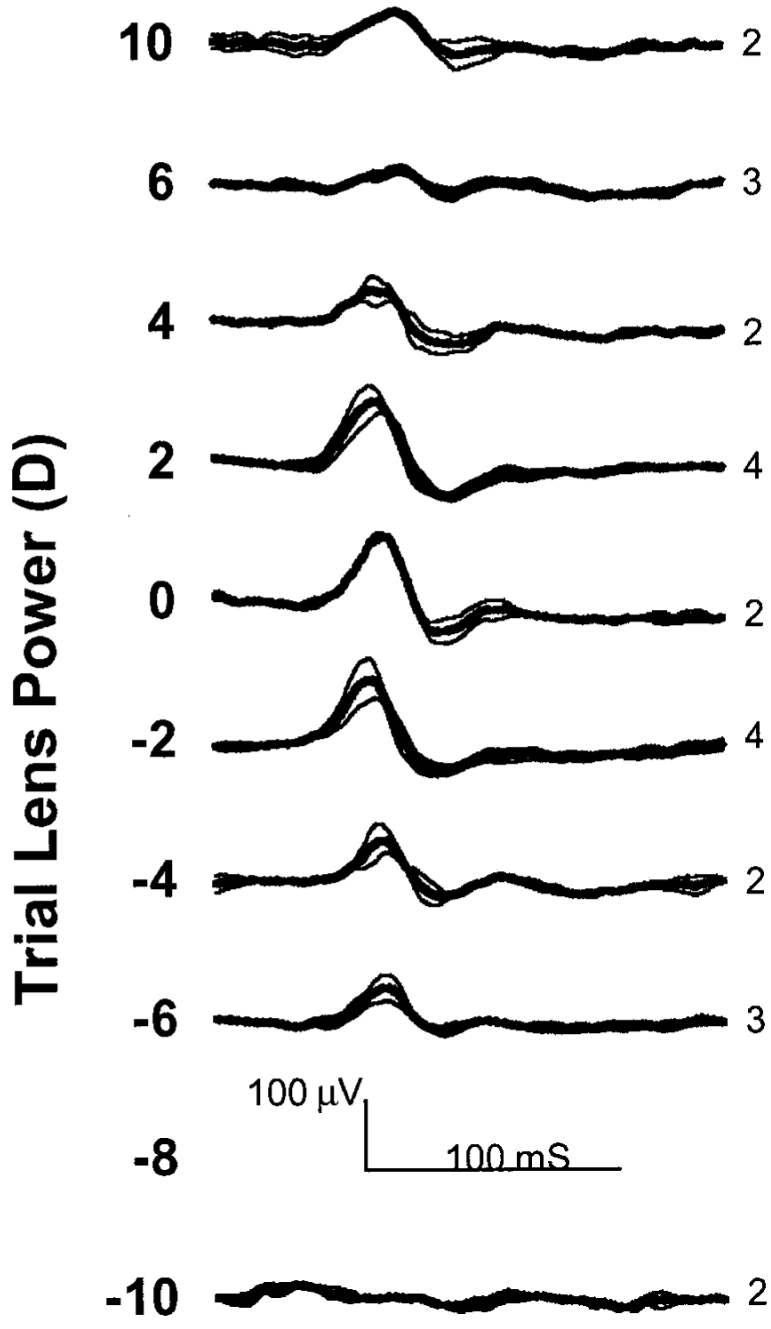

FIGURE 2.

Visual evoked potentials recorded the same tree shrew (0028) as in Fig. 1 but using smaller check sizes (0.25° × 0.29°, ~1.9 cpd). The tuning (change in first positive peak [P1] amplitude with trial lens power) is shown graphically in Fig. 3. Numbers to the right of each line indicate the number of waveforms that were averaged with this check size and trial lens power in this recording session. The thin lines indicate ±1 SD in response amplitude.

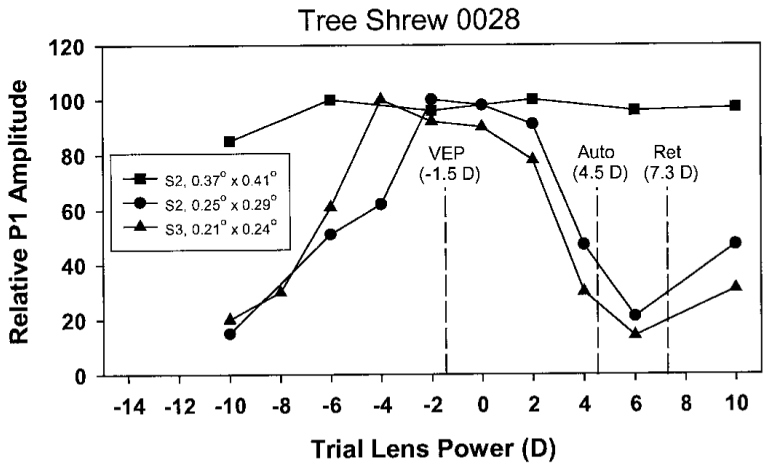

FIGURE 3.

Plots of first positive peak (P1) amplitude (normalized to the largest P1) vs. trial lens power measured in visual evoked potential (VEP) recording sessions 2 (S2) and 3 (S3) in animal 0028. In session 2, the first positive peak (P1) amplitude of the VEP's was high for all lenses when a large checkerboard pattern was used (Fig. 1). When a smaller check size was used (Fig. 2), the largest P1 amplitudes occurred with lenses near 0 D(−2 D to +2 D). Measures made several weeks later (session 3) with a small check size showed a similar tuning of the P1 amplitude (−4 D to +2 D). Combined with tuning obtained in session 1, the best trial lens power was determined as −1.5 D (indicated by the vertical dashed line). The average measures obtained with streak retinoscopy (Ret) and the autorefractor (Auto) were more hyperopic. The check sizes used (W × H in degrees) are indicated in the box.

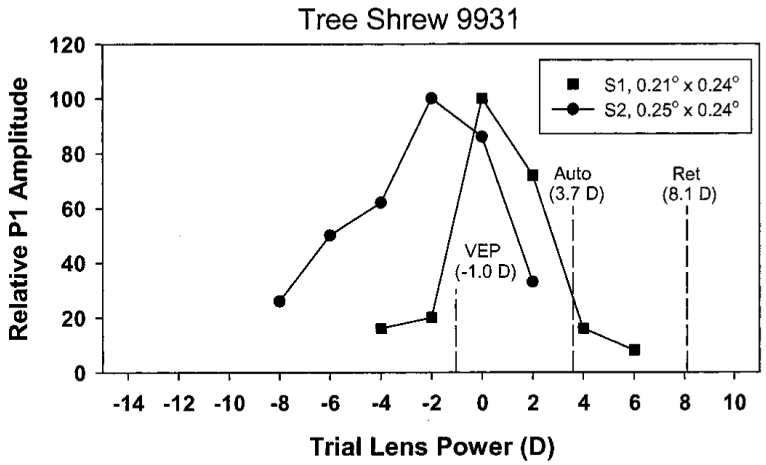

FIGURE 4.

Plots of normalized first positive peak (P1) amplitude vs. trial lens power measured in two recording sessions using small check sizes in animal 9931. The best responses in the two sessions (with lenses of −2 D and 0 D) led to the estimate of best trial lens of −1.0 D. As shown, the average value obtained with streak retinoscopy (Ret) and the autorefractor (Auto) were more hyperopic. S1, session 1; S2, session 2; VEP, visual evoked potential.

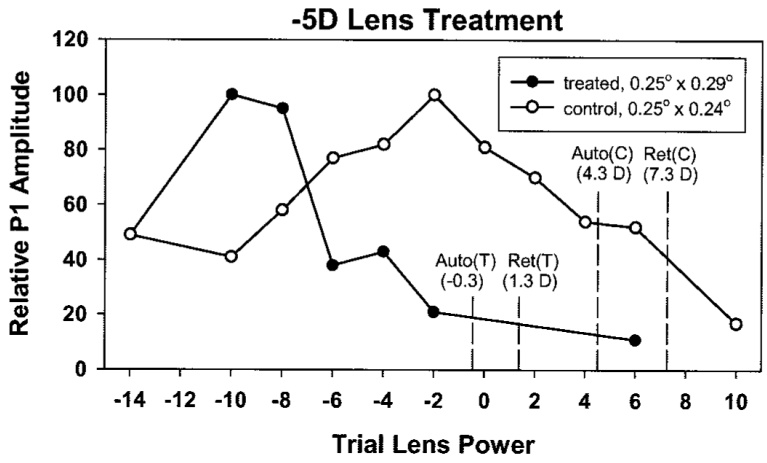

FIGURE 5.

Plots of normalized first positive peak (P1) amplitude vs. trial lens power measured in an eye that had compensated for a −5 D lens (treated eye [T]) and the untreated fellow eye (control [C]) in animal 0065. As indicated in the box, small check sizes were used for both eyes. The average streak retinoscopy and autorefractor measures are also indicated in the Figure.

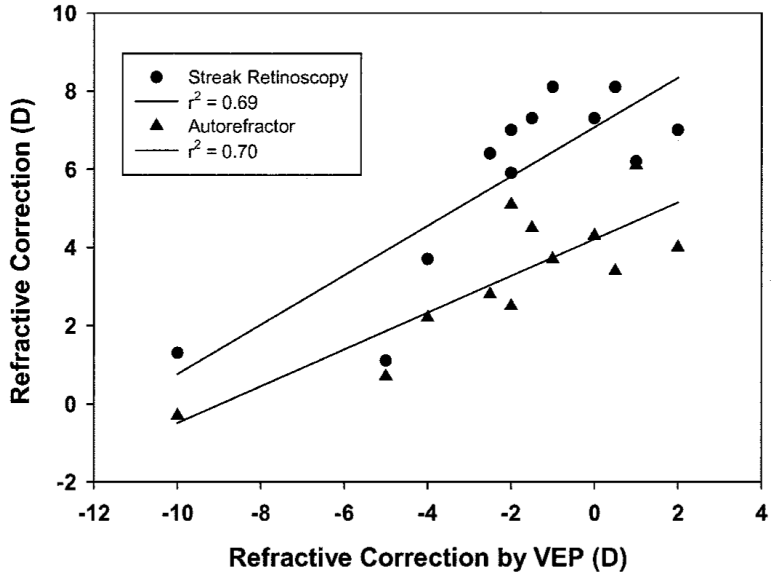

FIGURE 6.

Plot of refractive values measured with streak retinoscopy and the autorefractor vs. values measured with visual evoked potentials (VEP's) for all eyes. The regressions were VEP × 0.47 + 4.2 D for the autorefractor and VEP × 0.63 + 7.1 D for streak retinoscopy.

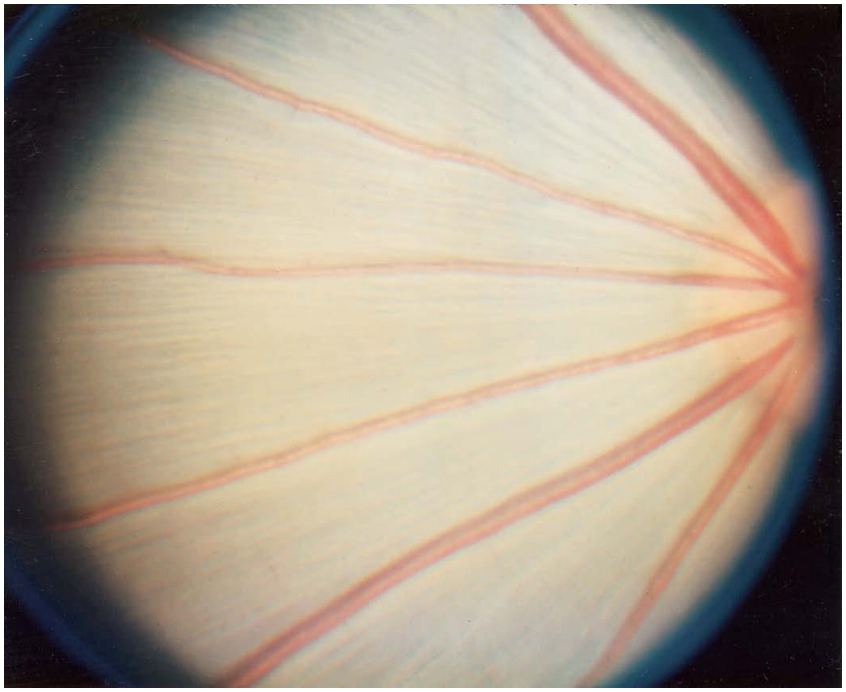

FIGURE 7.
Fundus photograph of the posterior pole of the left eye of a tree shrew. Nasal is to the left. Axons in the highly reflective optic nerve fiber layer may be seen coursing toward the optic nerve head.

TABLE 1

Comparison of refractive correction values.

Animal	No. of VEP Sessions[a]	Cycloplegic VEP		Cycloplegic Streak Retinoscopy		Cycloplegic Autorefractor		Noncycloplegic Autorefractor	
		Normal and Control Eye	−5 D Lens Eye	Normal and Control Eye	−5 D Lens Eye	Normal and Control Eye	−5 D Lens Eye	Normal and Control Eye	−5 D Lens Eye
9692	2	−2.5		6.4		2.8		3.3	
9806	1	−2.0		7.0		2.5		1.9	
9931	2	−1.0		8.1		3.7		3.0	
0012	3	0.5		8.1		3.4		4.0	
0016	2	−2.0		5.9		5.1		3.9	
0028	3	−1.5		7.3		4.5		4.0	
0054	2	2.0		7.0		4.0		2.8	
0063	1		−4.0		3.7		2.2		0.7
0065	3	0.0	−10.0	7.3	1.3	4.3	−0.3	3.9	−1.1
0074	2	1.0	−5.0	6.2	1.1	6.1	0.7	6.1	0.0
	Mean	−0.6	−6.3	7.0	2.0	4.0	0.9	3.7	−0.1
	SD	1.6	3.2	0.8	1.4	1.1	1.3	1.2	0.9

[a]VEP, visual evoked potential.