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Normal Development of Refractive State and Ocular Component Dimensions in the Tree Shrew (*Tupaia belangeri*)

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The normal development of refractive state, ocular components and simple visually-guided behaviors was examined in maternally-reared tree shrews. Six groups consisting of 5 animals each were anesthetized and examined after 0, 15, 30, 45, 60 and 75 days of normal binocular visual exposure. Measures in the 75-day group provided values for an improved schematic eye of the tree shrew. Cycloplegic refraction showed a marked hyperopia (+25 D) at eye opening which decreased rapidly during the first 15 days of visual exposure and stabilized near the value (+5 D) expected in an eye of this axial length (approx. 7.8 mm). Corneal radius increased slightly during development. Anterior segment depth, measured by A-scan ultrasonography, seemed to complete most of its development at an earlier age (15–30 days of visual exposure) than did other ocular parameters. Lens thickness increased steadily throughout development. Vitreous chamber depth increased rapidly until 15 days of visual exposure, and then decreased because the lens thickness increased more rapidly than axial length. Crude orienting to, and following of, large objects developed shortly after eye opening (median age at onset, 5 and 6 days, respectively). Triggered visual placing responses developed at about the same time that the refractive state completed the rapid drop from highly hyperopic values. The slowed rate of ocular development after 15 days of visual exposure may be related to increased retinal activity that is permitted by neural maturation and by the presence of a relatively well-focussed retinal image. The increased activity may influence the final dimensions of the eye to coordinate the axial length with the focal length of the eye.

Animal myopia Visual optics Tree shrew Schematic eye Refractive development Vitreous chamber depth Ocular biometry

INTRODUCTION

The development of the eye, and in particular the tendency for eyes to develop so that they achieve emmetropia (Stenstrom, 1946; Sorsby, Benjamin, Davey, Sheridan & Tanner, 1957), has long been a topic of interest both in studies of humans and of animals (for recent reviews, see Goss & Criswell, 1981; McBrien & Barnes, 1984; Curtin, 1985). The tree shrew offers several advantages for studies of the mechanisms that regulate normal ocular development and that are disrupted by visual form deprivation to produce experimental myopia (Sherman, Norton & Casagrande, 1977; McKanna & Casgrande, 1978; Marsh-Tootle & Norton, 1989; Norton, 1990). First, it is a mammal, closely related to the primate line (Campbell, 1966; Cartmill, 1974; Luckett, 1980), increasing the likelihood that mechanisms governing eye development in this species will be shared with humans. Secondly, tree shrews breed readily, typically have 2–3 pups per litter and mature rapidly (sexual maturity at about 3.5–4 months postnatal), so that it is possible in a reasonably short period to study groups of animals of sufficient size that meaningful statistical analyses can be accomplished.

Although the ocular changes characteristic of the experimental myopia produced by long periods of visual deprivation have been defined (McKanna & Casagrande, 1978, 1981; Marsh-Tootle & Norton, 1989), there have been no studies on the normal development of the refractive and ocular component dimensions in tree shrew. The normal time course of ocular development provides a baseline against which data from experimentally myopic animals can be compared [as will be done in the succeeding paper (McBrien & Norton, 1992)] to pinpoint the manner and time at which the experimentally myopic eye departs from the normal developmental profile.

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Developmental measurements from the two eyes of normal tree shrews also define the range of interocular differences that occur during development and, hence, the sensitivity of our measurement techniques for detection of changes in one eye of an animal that result from an environmental manipulation, such as monocular visual deprivation. An additional consideration is that, in a monocularly deprived animal, the open, non-deprived "control" eye may not be completely normal. To allow examination of this issue, across-animal data from binocularly normal animals are needed to establish the variability of normal developing eyes.

Previous attempts to optically model the normal tree shrew eye (Schafer, 1969) have been hampered by an absence of normative data about the tree shrew eye, particularly regarding the curvature of the lens. The present paper uses new normative data to define an improved schematic eye and to assess the optical effects of the developmental ocular changes.

Finally, it has been suggested that retinal activity may be involved in the regulation of ocular development (Gottlieb & Wallman, 1987; Wallman, Gottlieb, Rajaram & Fugate-Wentzek, 1987). Because retinal activity is necessary, although not sufficient, for the development of visually-guided behaviors, the onset of these behaviors may offer clues to the level of retinal activity present during the early postnatal period. We thus examined the time of onset of simple visually-guided behaviors in normal tree shrews. A portion of this work has appeared in abbreviated form (McBrien & Norton, 1987; Norton, 1990).

METHODS

Subjects

Maternally-reared tree shrew pups (*Tupaia belangeri*) were used because we found that hand-reared pups gain weight more slowly and have smaller eyes than age-matched maternally-reared tree shrews. Animals were housed in large (61 × 61 × 61 cm) stainless steel cages containing 10 cm dia opaque tubes used as nest boxes. Luminance levels on the cage walls were 1–30 cd/m^2; on the walls and ceilings of the animal room, visible from the cages, they were 45–115 cd/m^2. The animals moved freely in and out of the nest boxes and thus self-regulated their exposure to light during the time the lights were on (16/8 hr light/dark).

Pups were randomly assigned to one of 6 groups of 5 that were examined after 0, 15, 30, 45, 60 and 75 days of normal binocular visual exposure. Day 0 was the first day both eyes were open and occurred (mean age ± SD) 19.6 ± 2.2 days postnatal (range 16–24 days). To avoid potential genetic or gender bias, pups from the same parents were never used in the same group and there was an overall equal division by gender in the subject population with as equal a division as possible within groups of 5. Because repeated anesthesia and, particularly, repeated cycloplegia might influence the development of ocular growth, 26 animals underwent only one measurement session. Two animals tested after 0 days of visual exposure were retested, one as part of the 60-day group and the other in the 75-day group. Statistical analysis was performed both with and without second measurement of these animals with identical results.

Measurement procedures

During the measurement session, corneal curves, refractive measures (streak retinoscopy and coincidence optometry) and A-scan ultrasonographic measures were taken under anesthesia (90 mg/kg ketamine HCl with 10 mg/kg xylazine, i.m.). Booster doses were given as needed. The measurement procedures included numerous changes and improvements from those used by Marsh-Tootle and Norton (1989).

Head restraint. An individualized bite bar of dental impression material was inserted in the mouth and held firmly by tape wrapped around the long pointed nose and lower jaw of the tree shrew. The other end of the bite bar was secured in a bracket which could be locked in any orientation to painlessly and harmlessly hold the head with the corneal plane perpendicular to the various measuring instruments. Body temperature was maintained with a heating pad and heart rate was constantly monitored through an audiomonitor. The tear film was maintained by periodic application of artificial tears (Liquifilm Forte, Allergan). Excess fluid was removed before each measurement.

Corneal curve measurements

Corneal curvature was measured with a Bausch & Lomb one position keratometer modified with a +7 D extending lens, a brighter light source and a mire composed of thicker lines, to allow readings of the steeply curved cornea of the tree shrew eye. Four readings were taken in both the horizontal and vertical meridians of the cornea; then the keratometer was realigned and an additional set of four horizontal and vertical meridian measurements were taken. After conversion to corneal radius (mm) using a calibration equation derived by measuring ball bearings of known radii, the horizontal and vertical measures were averaged.

Ocular refraction measurements

Cycloplegia was produced with two drops of ophthalmic atropine sulfate 1% administered to each eye 1 hr prior to the commencement of the measurement procedure. Although not a direct measure of depth of cycloplegia, pupil reactions were always checked prior to refraction; no constriction was ever observed.

Streak retinoscopy. Refraction was measured to the nearest 0.5 D by streak retinoscopy on both the horizontal and vertical meridians at a working distance of 33 cm. Values were converted into measures of ocular refraction at the corneal plane by correcting for the working distance and the effectivity of the correcting lens, held 5 mm from the eye. Horizontal and vertical measures were averaged to obtain the spherical equivalent refraction.

Hartinger coincidence optometer. A second, objective, measure of refraction was obtained with a Hartinger coincidence optometer (Zeiss Jena) modified with a +20 D extending lens. The modified instrument was recalibrated as described by Wallman and Adams (1987). Four measurements were taken in the horizontal meridian of the eye and in the vertical meridian. The instrument was then realigned and another set of four horizontal and vertical measurements were taken. These all were averaged to provide the spherical equivalent refraction.

Effect of eye size. Refraction techniques involving reflections from the retina (e.g. retinoscopy; coincidence optometer) give increasingly hyperopic values in eyes of decreasing axial length (Glickstein & Millodot, 1970) when compared to other refraction techniques. This "small eye effect" was calculated for the tree shrew eye with the formula:

$$\text{small eye effect} = N_v \times T_{ret}/P' \times AL \times (AL - T_{ret}),$$

where N_v = refractive index of vitreous; T_{ret} = thickness of retina; P' = posterior focal length/AL; and AL = axial length (from cornea to photoreceptors).

The refractive index of the vitreous was assumed to be 1.336 (Schafer, 1969). From photographs of frozen sections of the tree shrew eye, retinal thickness was found to be approx. 150 μm. From schematic ray tracings using the mean ocular dimensions (in m) for each age group (see Table 1), the value for P' was found to be approx. 0.75. The ultrasound measurements for each age group provided the value for the axial length, allowing calculation of the small eye effect for each age group (Fig. 1).

Ultrasound measurements

Measurements of the axial dimensions of the structural components of the eye, along the optical axis, were obtained by A-scan ultrasonography. A 15 MHz 6.35 mm dia ultrasound transducer focussed at 20 mm and driven by a Panametrics 5052 pulser/receiver was coupled through a 0.9% saline-filled 14 mm plexiglass standoff positioned so that the saline column contacted the anesthetized cornea (0.5% Ophthaine) without applanation. Echoes from the pulser/receiver passed through a preamplifier (Accu-Tron Inc., Model 3080) into a Tektronix 7603 oscilloscope with a 7D20 digitizer plug-in. To enhance the signal-to-noise ratio, each waveform was the average of eight single waveforms produced by pulses presented within a period of a few msec. Six averaged waveforms from independent positionings of the transducer were collected for each eye and transferred to an IBM PC-XT computer for subsequent measurement with a waveform analysis package (TAMS, Tektronix Inc.). Time measurements between ocular surfaces could be made to a resolution of 25 nsec which converts (see below) to a theoretical linear accuracy of about 20 μm in locating an ocular surface. However, a better estimate of the practical limits of this technique in tree shrew will be provided by the variability of the normal measures presented in the Results.

Conversion of time to distance used previously published values of 1557.5 m/sec for anterior segment (front of the cornea to front of the lens) (Marsh-Tootle & Norton, 1989), and 1540 m/sec for vitreous (Coleman, Lizzi & Jack, 1977). We used the method of Coleman *et al.* (1977) to measure the velocity of the ultrasound in 6 lenses from 3 young adult tree shrews (average age 8 months, range 6–11 months) that had been monocularly deprived for part of their developmental period. After dissection from the eye and removal of all extraneous vitreous gel, the lenses were gently placed into a beaker containing 0.9% saline maintained at 37°C throughout the 30 min measurement session. Eight measures were taken with and without the lens in place. Because there were no significant differences between values obtained for the control and deprived eyes, these were averaged, yielding a lens conduction velocity of 1723.3 ± 17.7 m/sec.

Schematic eye modeling

A schematic eye was derived with values obtained from the animals in the group with 75 days of visual exposure using a paraxial ray-tracing program obtained from O'Keefe (O'Keefe & Coile, 1988). Values for the anterior corneal radius, anterior and posterior lens radii, vitreous chamber depth and axial length were obtained from our experimental data (Table 1). Corneal thickness was taken as 0.3 mm (Schafer, 1969). Posterior corneal radius was assumed to be 10% steeper than the anterior radius. The refractive indices used were: cornea 1.378 (Schafer, 1969), aqueous 1.336 and vitreous 1.336 (Schafer, 1969). A value for the equivalent refractive index of the lens was found by trial and error that approximately yielded the refractions which were actually obtained with streak retinoscopy (after subtracting the small eye effect) in the 75-day group.

Development of visually-guided behaviors

After natural eye opening, the development of three simple visually-guided behaviors was monitored in 23 of the 28 tree shrews, usually on an every alternate day schedule: (1) orienting to a large object (a hand) presented in the visual periphery; (2) crudely following this object; and (3) giving "triggered" visual placing when lowered toward a surface (a high-contrast table edge) while supported only by the hindquarters (Hein & Held, 1967). The techniques used were similar to those previously applied to kittens (Norton, 1974). As expected in young animals, difficulty was encountered with some pups in taking the measurements because they tended to become disturbed when taken from the nest box for testing. Some that failed to show any visually-guided behaviors during formal testing clearly demonstrated the use of visual cues when they had the opportunity to run freely about the laboratory or the animal quarters. Thus, these measures may somewhat underestimate the ages at which these behaviors can first be demonstrated.

TABLE 1. Ocular refraction and axial dimensions of left and right eyes of normal tree shrews

Visual exposure (days)		0		15		30		45		60		75		Adult	
N		5		5		5		5		5		5		5	
Eye		R	L	R	L	R	L	R	L	R	L	R	L	R	L
Retinoscopy (D)	Mean	25.8	26.6	10.8	10.6	8.3	8.3	7.5	7.3	7.1	7.2	6.1	6.2	5.1‡	5.6‡
	SD	2.2	3.4	1.0	1.4	1.1	0.9	1.8	1.5	1.0	0.6	0.6	0.5		
	Median	25.6	25.2	10.9	11.2	8.4	8.4	8.1	7.8	7.5	7.0	6.4	6.4		
	Range	22.4–28.9	23.6–33.0	9.2–11.8	8.1–12.0	6.4–9.5	7.0–9.5	4.3–9.2	4.5–8.8	5.1–7.8	6.4–8.1	5.1–6.7	5.6–6.7		
Coincidence optometer (D)	Mean	23.0*	20.6*	10.2	9.0	6.2	5.7	6.0	6.2	6.0	6.8	5.7	6.4	4.8‡	1.4‡
	SD	0.3	0.0	1.3	2.0	1.4	1.8	1.5	1.3	1.2	0.7	1.3	0.9		
	Median	23.0	20.6	10.1	9.3	6.3	5.6	6.8	6.4	5.8	6.9	5.6	6.3		
	Range	22.7–23.4	20.6–20.6	8.8–12.4	5.2–11.2	3.7–7.6	3.2–8.4	3.2–7.4	4.0–8.0	4.8–8.1	5.6–7.6	3.8–7.4	5.3–8.0		
Corneal radius (mm)	Mean	3.47†	3.52†	3.34	3.39	3.45	3.46	3.50	3.54	3.53	3.57	3.60	3.57	3.76‡	3.65‡
	SD	0.07	0.09	0.11	0.17	0.12	0.12	0.10	0.07	0.10	0.13	0.08	0.04		
	Median	3.48	3.49	3.39	3.42	3.50	3.42	3.46	3.53	3.47	3.52	3.56	3.55		
	Range	3.37–3.54	3.42–3.66	3.16–4.46	3.16–3.62	3.21–3.52	3.27–3.60	3.40–3.68	3.44–3.66	3.40–3.67	3.42–3.73	3.52–3.74	3.53–3.62		
Anterior segment depth (mm)	Mean	0.78	0.76	1.05	1.06	1.14	1.13	1.13	1.14	1.12	1.11	1.10	1.10	1.14§	1.16§
	SD	0.04	0.08	0.05	0.03	0.02	0.01	0.04	0.03	0.04	0.03	0.04	0.04	0.02	0.05
	Median	0.80	0.79	1.03	1.07	1.14	1.13	1.12	1.15	1.12	1.10	1.10	1.08	1.14	1.18
	Range	0.71–0.81	0.60–0.82	0.99–1.12	1.00–1.10	1.11–1.17	1.12–1.14	1.07–1.19	1.08–1.18	1.08–1.20	1.09–1.18	1.04–1.16	1.06–1.16	1.12–1.16	1.09–1.21
Crystalline lens thickness (mm)	Mean	2.97	2.96	3.13	3.12	3.30	3.30	3.43	3.43	3.51	3.51	3.61	3.60	4.05§	3.97§
	SD	0.06	0.05	0.05	0.05	0.07	0.06	0.04	0.04	0.04	0.04	0.06	0.05	0.18	0.10
	Median	2.97	2.96	3.12	3.12	3.30	3.29	3.41	3.42	3.51	3.50	3.60	3.60	4.09	4.04
	Range	2.87–3.04	2.88–3.02	3.08–3.22	3.06–3.19	3.23–3.40	3.25–3.39	3.38–3.49	3.39–3.48	3.46–3.55	3.45–3.56	3.53–3.70	3.52–3.66	3.81–4.25	3.84–4.05
Vitreous chamber depth (mm)	Mean	2.96	2.93	3.17	3.18	3.20	3.20	3.14	3.15	3.13	3.13	3.10	3.11	2.89§	2.92§
	SD	0.08	0.07	0.05	0.05	0.03	0.03	0.07	0.07	0.05	0.06	0.04	0.03	0.14	0.10
	Median	2.96	2.91	3.15	3.18	3.21	3.20	3.09	3.10	3.12	3.10	3.08	3.09	2.81	2.90
	Range	2.89–3.10	2.85–3.05	3.12–3.25	3.12–3.24	3.16–3.26	3.16–3.24	3.07–3.24	3.08–3.24	3.07–3.21	3.08–3.23	3.06–3.17	3.08–3.17	2.78–3.08	2.82–3.05
Axial length (mm)	Mean	6.71	6.65	7.35	7.36	7.64	7.63	7.69	7.71	7.77	7.75	7.80	7.80	8.08§	8.06§
	SD	0.12	0.16	0.09	0.12	0.10	0.08	0.09	0.09	0.07	0.08	0.10	0.09	0.07	0.06
	Median	6.74	6.71	7.37	7.40	7.62	7.63	7.67	7.71	7.75	7.75	7.79	7.80	8.06	8.05
	Range	6.48–6.84	6.33–6.80	7.23–7.49	7.20–7.53	7.52–7.83	7.54–7.77	7.54–7.78	7.59–7.84	7.67–7.89	7.62–7.88	7.70–7.97	7.69–7.95	8.01–8.17	7.98–8.14

*$N = 2$; †$N = 4$; ‡$N = 1$; §$N = 3$.

Statistical analysis

Changes in refractive and ocular parameters with age were assessed with an analysis of variance (SAS, general linear model). Changes in across-animal and within-animal variability were assessed with a variance ratio test. In these tests of developmental changes, the data from either the right or left eye were dropped from each animal because of the close correlation between eyes (Ederer, 1973; Ray & O'Day, 1985).

RESULTS

The primary data of interest in this study were the changes in refractive and ocular parameters with age, the derivation of an improved schematic eye and measurement of the onset of simple visual behaviors. Figure 1 and Table 1 present data separately for the left eyes and the right eyes of the normal animals. Although the data appeared to justify the use of parametric statistics, the median values and ranges are also presented in Table 1.

Also of interest in this study was: how different from the normal development pattern an eye must be to be considered outside the normal range. The average of the left and right eye standard deviations (Table 2) provided an estimate of the across-animal variability within each age group and formed the basis for the 95% confidence intervals presented in Table 2. An estimate of the ability of our measurement techniques to detect differences between two eyes within an animal is provided by the within-animal standard deviation of the two eyes in each animal around the mean value for that animal (Table 2).

Refractive development

At eye opening, a marked hyperopia (approx. +25 D) was observed in the eyes of the normal tree shrew pups [Fig. 1(A) and (B)]. This decreased rapidly during the first 2 weeks of visual exposure, followed by a more gradual decline up to 75 days of visual exposure, which appeared to be close to the adult refractive state. Overall, the decrease in refraction from eye opening to 75 days of visual exposure was highly significant ($P < 0.001$). When the small eye effect (Glickstein & Millodot, 1970) is taken into account, the tree shrew eyes were nearly emmetropic by 75 days of visual exposure.

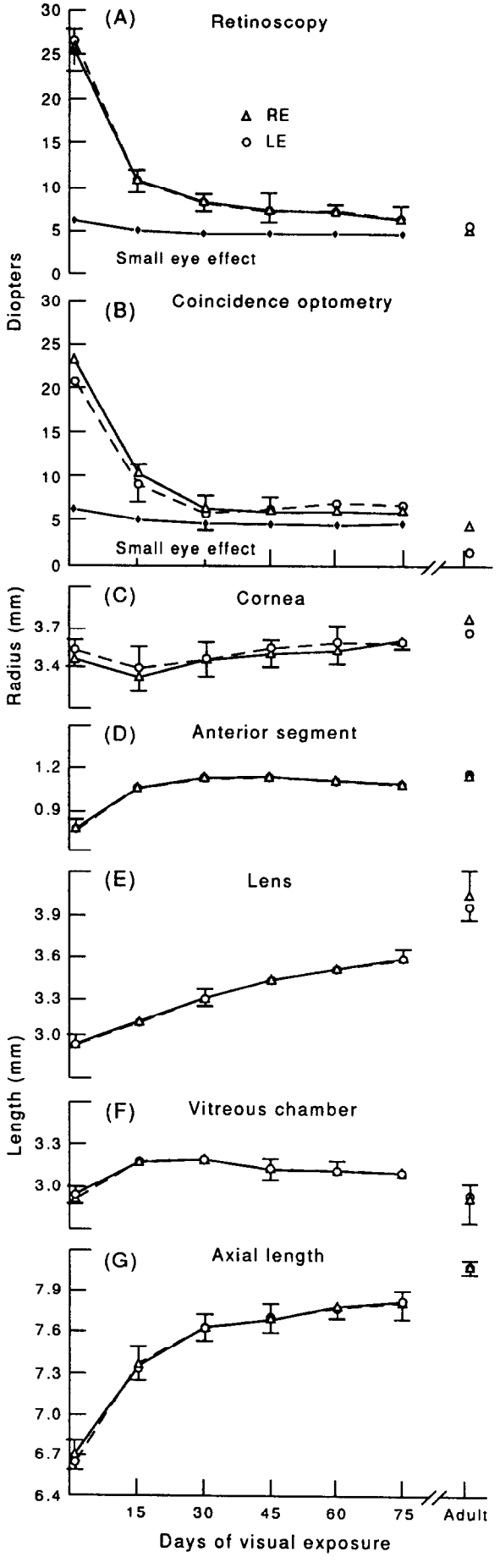

FIG. 1

FIGURE 1. Development of refractive state and ocular component dimensions in normal tree shrews. In each age group, mean values (and SDs) are plotted separately for the right eyes (open triangles, solid line) and left eyes (open circles, broken line). In (A) and (B), the small eye effect (solid diamonds, solid line) is the artifact of retinoscopy expected in eyes of this size (Glickstein & Millodot, 1970). Data from adult animals [$N = 1$, (A)–(C); $N = 3$, (D)–(G)] are included for comparison. (C)–(G) have the same y-axis scale to facilitate comparisons between each ocular component. Standard deviations for the left eye are plotted downward in (A), (B), (E) and upward in (C), (D), (F) and (G).

TABLE 2. Across-animal and within-animal standard deviations

Days of visual exposure	Retinoscopy (D)		Coincidence optometer (D)		Corneal radius (mm)		Anterior segment (mm)		Crystalline lens (mm)		Vitreous chamber (mm)		Axial length (mm)	
	Across-animal*	Within-animal	Across-animal*	Within-animal	Across-animal*	Within-animal	Across-animal*	Within-animal	Across-animal*	Within-animal	Across-animal*	Within-animal	Across-animal*	Within-animal
0	2.8	1.7	0.2†	1.2†	0.08‡	0.05‡	0.06	0.02	0.05	0.01	0.07	0.02	0.14	0.03
15	1.2	0.4	1.7	0.6	0.14	0.03	0.04	0.01	0.05	0.02	0.05	0.01	0.10	0.02
30	1.0	0.4	1.6	0.6	0.12	0.04	0.01	0.01	0.06	0.01	0.03	0.01	0.09	0.01
45	1.6	0.2	1.4	0.3	0.09	0.02	0.03	0.01	0.04	0.01	0.07	0.01	0.09	0.02
60	0.8	0.3	0.9	0.5	0.12	0.02	0.04	0.01	0.04	0.01	0.05	0.01	0.08	0.01
75	0.6	0.2	1.1	0.4	0.06	0.03	0.04	0.01	0.06	0.01	0.04	0.01	0.09	0.02
Average	1.3	0.5	1.2	0.6	0.10	0.03	0.04	0.01	0.05	0.01	0.05	0.01	0.10	0.02
95% Int.§ (mean ±)	1.8	0.8	1.5	1.1	0.13	0.04	0.05	0.02	0.06	0.02	0.06	0.02	0.12	0.04

*Average of left and right eye standard deviations from Table 1.
†$N = 2$.
‡$N = 4$.
§For $N = 5$, t, d.f.(4) = 2.78; 95% interval = $\pm SD \times 2.78/\sqrt{N}$; value is average of values for each age group.

The across-animal standard deviation across all age groups was 1.3 D with retinoscopy (average of left and right eye values) and 1.2 D using the coincidence optometer. The across-animal variability did not change significantly with age for these two refractive measures.

As expected, the difference between right and left eye refractions in these normal animals was very small. The within-animal standard deviation for retinoscopy in the 0-day group (1.7 D) was significantly larger than in the 75-day animals (0.2 D) (variance ratio test, $P < 0.01$), indicating that the retinoscopic values in the two eyes became more closely coordinated in the older animals. Because of missing values (only 2 animals measured in the 0-day age group) a similar statistical comparison was not attempted for the coincidence optometer refractions.

Comparison of Fig. 1(A) and (B) shows that the two refractive techniques were in good agreement, both in the overall magnitude of refractive error measured and the observed variability. The Pearson correlation between the two measures had a slope of 0.87 and a correlation coefficient of 0.95. On average, retinoscopic measures were 1.2 D hyperopic in comparison with the Hartinger values.

Corneal radius

There was a small but statistically significant increase in the corneal radius from eye opening to 75 days of visual exposure [Fig. 1(C)]. The corneal radius in the 0-day group was approx. 2.5 D more powerful (total corneal power) than in the 75-day group. This group, in turn, was about 3 D more powerful than the values obtained in one adult animal. There was a slight (approx. 3 D) corneal steepening noted in the 15-day animals that was not statistically significant because of the high variability in the measures. Given the finding (Marsh-Tootle & Norton, 1989; McBrien & Norton, 1992) that eyelid closure flattens the cornea, it is possible that the cornea steepens slightly after normal eye opening and then flattens with the continued growth of the eye.

The large standard deviations show that the variability of the corneal measures was large in comparison to both the refractive measures and to the A-scan ultrasound measures. Because this was the case both within individual animals and across animals (Table 2) it probably reflects the practical difficulties of obtaining accurate corneal measures in anesthetized animals.

Anterior segment

Unlike the cornea, but similar to the refractive measures, the depth of the anterior segment underwent a rapid increase during the first 15 days of visual exposure [Fig. 1(D)] and a much slower increase during the second 15-day period. By 30 days of visual exposure, it stabilized at a value close to the mean of the 3 adult animals. The relative contribution of changes in corneal thickness and anterior chamber to the anterior segment measure was not determined. However, based

on examination of the ultrasonograms, changes in corneal thickness did not appear to contribute in a major way.

Anterior segment depth was extremely consistent both across and within animals (Table 2). Neither the across-animal nor the within-animal standard deviation changed consistently with age although there was an interesting, significant (variance ratio test, $P < 0.01$) decrease in variability in the 30-day group.

Lens thickness

Lens thickness [Fig. 1(E)] increased at a relatively constant rate throughout the ages that were examined and was considerably larger in the 3 adult animals. Variability in lens thickness across and within animals was generally quite small and showed no change with age.

Vitreous chamber depth

The depth of the vitreous chamber [Fig. 1(F)] followed an interesting pattern. There was a rapid increase in vitreous chamber depth during the first 15 days after eye opening, followed by relatively constant values during the next 15 days and then a slight decrease in older animals. The decrease appeared concordant with the steady increase in lens thickness against a background of small increases in axial length. The markedly smaller vitreous chamber depths in the adult animals suggests that this process continues into adulthood. The small across- and within-animal variability (Table 2) did not change as a function of age.

Axial length

As described in the Methods, axial length was the sum of the anterior segment depth, lens thickness and vitreous chamber depth. During the first 15 days of visual exposure, as expected from the rapid increases in these three components, the axial length also rapidly increased [Fig. 1(G)]. The increased axial length beyond this age primarily reflected increases in lens thickness. As may be noted in Table 2, the 30-day and the 60-day within-animal standard deviations were significantly smaller than the 0-day value (variance ratio test, $P < 0.05$). Thus, axial length, like refraction, was somewhat more closely coordinated between the left and right eyes in the older animals.

Schematic eye

Figure 2 shows the values for the schematic eye that was derived using the average values from the 10 eyes of the animals in the group measured after 75 days of visual exposure. The derived lens index value (1.576) seems reasonable in comparison with the values used in other schematic models of small eyes (Hughes, 1979a,b; Coile & O'Keefe, 1988). It is also interesting to note that calculation of the small eye effect with this schematic eye,

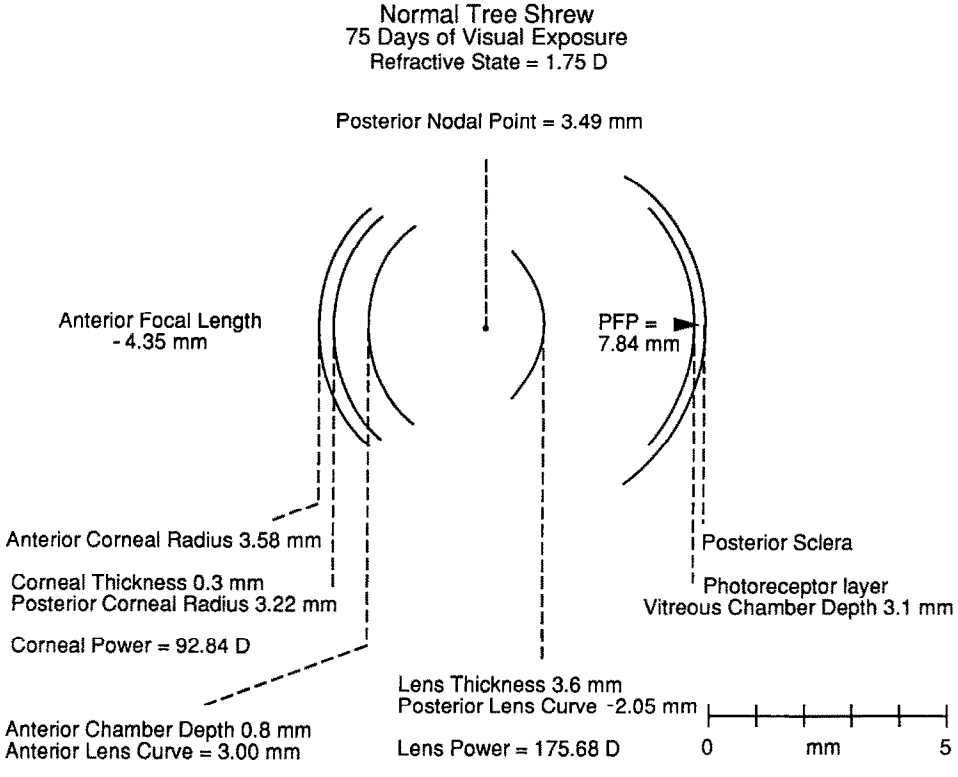

FIGURE 2. A schematic eye of the tree shrew based on values from animals exposed to 75 days of normal binocular vision. The lens refractive index, for which there was no direct experimental evidence, was adjusted in the model until a value (1.576) was found that yielded a refraction (1.75 D) close to that obtained in this age group (1.61 D) after subtraction of the small eye effect. PFP = posterior focal point. The posterior focal length was 5.81 mm. This value, divided by the axial length (7.8 mm), yields a P' value close to 0.75. This was used in the calculation of the small eye effect on refractive measures [Fig. 1(A) and 1(B)]. For cornea, the anterior surface power was 105.59 D and the posterior surface power was −13.04 D. Comparable values for lens were 80.0 and 117.07 D. Total power for the entire eye was 229.9 D.

assuming a retinal thickness of 150 μm, yielded a value (5.4 D) that was within 1 D of the value we derived from the small eye effect equation (4.5 D). Although this schematic eye is not as elegant as some that have been derived for small animals eyes (Hughes, 1979a,b; Campbell & Hughes, 1981), it represents an incremental improvement over the previous schematic eye for tree shrew (Schafer, 1969).

Development of visually-guided behaviors

Orienting and following behaviors were detected at a considerably younger age than was visual placing, and orienting preceded following by a short interval (see Fig. 3). The median age for the onset of visual orienting was 4 days of visual exposure. For following, the median onset was 5 days and for placing it was 15 days.

DISCUSSION

Ocular and refractive development

At the time of eye opening and beginning of visual exposure, tree shrew eyes have completed a substantial fraction of their development. For example, the axial length in the group with 0 days of visual exposure (nearly 20 days after birth) is 85% of the axial length of the eyes in animals after 75 days of visual exposure and 82% of the axial length in the 3 adult animals. Thus, the period when the visual environment may influence eye development begins relatively late in the developmental process.

During the first 15 days of visual exposure, the eyes move rapidly toward their adult dimensions. In the 15-day animals, the development of the anterior segment depth was nearly complete (within 5% of its 75-day value). This component also matures relatively early in cats (Thorn, Gollender & Erickson, 1976) and in humans (Sorsby, Benjamin, Sheridan, Stone & Leary, 1961). The axial length in the 15-day animals had increased rapidly to within 6% of its value in the 75-day group. The vitreous chamber depth by day 15 exceeded the 75-day value. Both of these components then slowed their rate of development and gradually approached their adult dimensions.

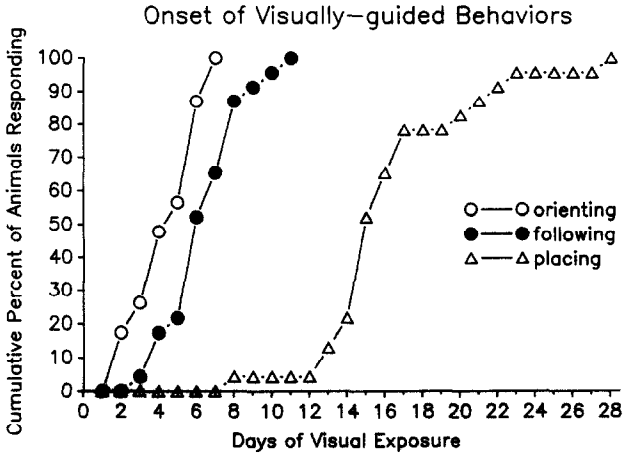

FIGURE 3. Development of simple visually-guided behaviors. The cumulative percent of animals responding positively to tests for visually-guided orienting, following and elicited visual placing is plotted as a function of the days of visual exposure of the animals.

In contrast with the relatively mature structural dimensions, the tree shrew eye is extremely hyperopic at the time of eye opening. Because of the rapid changes in the ocular components during the first 15 days of visual exposure, the initial hyperopia dropped dramatically from about 20 D to just over 5 D (both values corrected for artifact of retinoscopy). The rate of change in ocular refraction then slowed, commensurate with the reduced rate of development of the ocular components. Because there is only a small developmental change in corneal radius and because the optical effect of the increased anterior chamber depth is to decrease the hyperopia by a small amount (<4 D determined with the schematic eye model), these components are not large contributors to the refractive change. The increase in lens thickness, assuming no change in lens curvature, would tend to increase the hyperopia. Thus, most of the decrease in ocular refraction is due to the increase in vitreous chamber depth.

A rapid decrease from an initial hyperopia also has been found in chicken, occurring in the first few days after hatching (Wallman & Adams, 1987; Pickett-Seltner, Sivak & Pasternak, 1988). Since chickens hatch with their eyes open, in both species the rapid decrease from initial hyperopia coincides with the start of visual exposure. Humans also show a progression from initial hyperopia toward emmetropia (Sorsby *et al.*, 1961; Larsen, 1971) although its time course is more protracted. Indeed, the refractive and ocular development in tree shrew seems to naturally divide itself into stages at least roughly comparable to the infantile and juvenile stages in humans (Sorsby *et al.*, 1961). Based on parallels in the development of the eye and on the age of puberty (about 3.7 months of age in the tree shrews in this laboratory), it would appear that a tree shrew after 75 days of visual exposure (just over 3 months of age) is roughly equivalent to a young teenage human in terms of ocular development.

Comparison of the data from the 75-day animals with the small group of adult animals indicates that ocular development does not cease at 75 days of visual exposure. By day 75, however, the eyes have entered a fairly stable phase where ocular refraction has plateaued and where the reduction in vitreous chamber depth seems related to, and may be produced by, continued expansion of the lens. As in other species (Scammon & Hesdorffer, 1937; Sorsby *et al.*, 1961; Larsen, 1971; Thorn *et al.*, 1976), the lens appears to continue its growth at a steady rate into adulthood.

In summary, tree shrew eyes appear to progress through two relatively distinct phases after the eyes open. The first phase, approx. 2 weeks in duration, is characterized by rapid changes in refractive state and the ocular components. The longer second phase is characterized by slower rates of change in the ocular components and refractive state which gradually approach the adult levels. Throughout this second stage images are relatively well focussed on the retina so that changes in

the visual environment, such as defocus, have ample opportunity to influence the final stages of ocular development. The visual environment need not have a large effect on ocular development to have a large impact on the refractive state in tree shrew. A vitreous chamber depth change of 0.05 mm would produce approx. a 2 D change in refraction. The depth of focus has not been measured in tree shrew. However, calculations based on Green, Powers and Banks's (1980) model [1–2 c/deg grating acuity (Petry, Fox & Casagrande, 1984), 3 mm pupil] suggest it may be as small as ± 1.2–± 2.5 D. Thus, small changes in ocular dimensions may be optically significant.

Behavioral development

In comparison with visually precocious animals, such as chicken, the more altricial tree shrew develops visually-guided behavior slowly. When tree shrews open their eyes the photoreceptor outer segments are present (Casagrande, personal communication) and ocular media are relatively clear in comparison to kittens (Thorn *et al.*, 1976; Bonds & Freeman, 1978). The anterior lenticular vasculature has nearly cleared, only a few tufts remain of the posterior lenticular tunic and, although the ocular media are slightly cloudy in comparison with older pups, the retinal landmarks may be visualized through an ophthalmoscope.

Despite the relatively clear optical media at eye opening, visually-guided behaviors are not immediately present; responsiveness to visual stimuli matures over a period of about 2 weeks, following the same pattern found in kittens (Norton, 1974, 1981) and in humans (Bronson, 1974). Orienting responses develop slightly before following responses, and both develop earlier than visual placing. As in these other species, simple orienting and following responses to large objects in tree shrew can be achieved with limited visual acuity, do not require movement of the animal through its environment and may depend more strongly on pathways through the superior colliculus. In contrast, to achieve visual placing the animals must be able to utilize depth information, detect edges and move their paws in a coordinated manner. This type of behavior may require a substantially higher level of response from cortical cells than do orienting and following which may explain its later development. It also may require the development of motor skills by the animals.

By the time elicited visual placing develops at about 2 weeks of age, sufficient retinal activity must be present to support this and other visual behaviors. Thus, as the animals enter the stage where the rate of maturation of refraction and ocular components slows, retinal activity is available, if needed, to modulate the final development of the eye in tree shrew.

Across-animal variability

The across-animal variability and the 95% confidence limits presented in Table 2 are useful as a guide to how different from normal an eye must be before it can be considered to be outside the normal range (see McBrien & Norton, 1992). The across-animal variability remained relatively unchanged throughout the ages examined in this study. This may have been because the eyes in the different animals were simply developing toward different end-point dimensions and refractive state. However, variability in maturational rate may also have contributed importantly to the across-animal variability. This is particularly likely to happen when maturation is occurring rapidly and an animal whose individual maturational rate is only a day or two ahead or behind that of another animal would have substantially different values.

Within-animal variability

The within-animal variability and confidence limits presented in Table 2 provide a mechanism for determining the amount of interocular difference that must occur between a control eye and an experimental eye in order to detect an effect of the experimental manipulation.

There are two possible sources of this variability: actual variations between the eyes and measurement error. It is at least theoretically possible that the two eyes in any animal are identical. If so, then the observed variability reflects errors in measurement for each technique. To the extent that there actually are variations between the eyes, so that the within-animal variability reflects both true interocular differences and measurement error, the measurement error may be lower than the within-animal variability. Thus, within-animal variability (Table 2) sets an upper limit on the errors of measurement using the present techniques.

In the cases of the A-scan ultrasound measures, the within-animal variability values (Table 2) are remarkably close to the theoretical limits of the technique (about 20 μm). The refractive measures, made both with the retinoscope and the coincidence optometer, had a greater measurement error. As in the previous study of Marsh-Tootle and Norton (1989) there was a strong correlation between the values obtained with the two measures.

The reduction with age of the within-animal variability for retinoscopy (75-day group < the 0-day) and for axial length (30-day and 60-day values < 0-day values), along with the progression of the normal eyes toward emmetropia during development, are both consonant with the notion that axial length may be regulated developmentally to match the focal length of the eye. They are not definitive evidence, however, and further studies are needed to determine whether such a developmental regulatory mechanism exists in the tree shrew.

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