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EXPERIMENTAL MYOPIA IN A DIURNAL MAMMAL (*SCIURUS CAROLINENSIS*) WITH NO ACCOMMODATIVE ABILITY

BY NEVILLE A. McBRIEN, HADI O. MOGHADDAM, ROBERT NEW AND LESLIE R. WILLIAMS

From the Department of Optometry and Vision Sciences, University of Wales, College of Cardiff, Redwood Building, King Edward VII Avenue, Cardiff CF1 3XF

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SUMMARY

1. We examined the functional morphology of the intra-ocular muscles of the grey squirrel using pharmacological and histological methods. Using sympathomimetic (phenylephrine) and parasympathomimetic (carbachol) agents, administered by transcorneal iontophoresis, the response of the iris sphincter and dilator muscles and the ciliary muscle were recorded. Measurement techniques included both streak retinoscopy and coincidence optometry for measurement of ocular refraction and high resolution ultrasonography to monitor changes in the intra-ocular component dimensions.

2. The grey squirrel was found not to possess a functional accommodative system. No change in ocular refraction or intra-ocular dimensions could be induced with 40% carbachol. Marked changes in pupil diameter occurred with topical application of both phenylephrine (dilation) and carbachol (constriction). Histological findings were in agreement with pharmacological findings in showing well developed iris sphincter and dilator muscles but only a poorly developed ciliary muscle.

3. Calculation of the depth of focus of the grey squirrel eye reveals that this could be sufficient to account for the behavioural observations of near viewing habits.

4. We then determined whether we could induce axial elongation of the vitreous chamber and a consequent myopia by monocular deprivation (MD) of pattern vision.

5. Monocular deprivation of pattern vision produced a significant experimental myopia due to axial elongation of the vitreous chamber in the deprived eye.

6. The results demonstrate that a functional accommodative system is not necessary to induce experimental myopia in the grey squirrel eye.

INTRODUCTION

It has been demonstrated in several animal species that monocular deprivation (MD) of pattern vision in neonates produces a breakdown of the emmetropization process in the deprived eye, with increased axial elongation of the eye resulting in high myopia. These results have been observed in chicks (e.g. Wallman & Adams, 1987), cats (e.g. Ni & Smith, 1989), tree shrews (e.g. McBrien & Norton, 1992),

marmosets (Trolio, Judge, Ridley & Baker, 1990) and monkeys (e.g. Raviola & Wiesel, 1985). It has also been observed that deprivation of form vision in humans (e.g. haemangioma of the lid, ptosis, corneal opacification) results in axial elongation of the eye and high degrees of myopia (e.g. Hoyt, Stone, Fromer & Billson, 1981).

It has been proposed by several studies that ocular accommodation is a causative factor in both human myopia development and experimental myopia in animal models (Curtin, 1985). Accommodation is a likely candidate for the control of eye growth and refraction, as it can sense the refractive state of an eye and respond in an attempt to maintain a clear retinal image with consequential mechanical and innervational influences on the eye. Epidemiological studies reporting the strong association between near-work and myopia have been cited in support of a role for accommodation in myopia, although these studies merely indicate an association and not a cause and effect relationship. However, work on animal models of refractive development has provided more direct evidence that restricting vision to close viewing results in the development of myopia (e.g. Young, 1965). Studies have attempted to specifically implicate accommodation, as opposed to just near-work, by pharmacologically blocking the accommodative apparatus of the eye using muscarinic antagonists. In adolescent humans, daily administration of 1 % atropine was found to be effective in preventing the progression of juvenile myopia (e.g. Bedrossian, 1979). In mammalian animal models atropine administration has also been found to be effective in preventing or reducing experimentally induced myopia in tree shrews (McKanna & Casagrande, 1981) and monkeys (Raviola & Wiesel, 1985). The relative success of atropine administration in preventing myopia development has given strong support to the proposal that accommodation is a causative factor in this condition.

Recent investigations on animal models of myopia have, however, reported findings which question the importance of accommodation in myopia development. Studies have shown that blocking communication between the eye and higher centres, either by optic nerve section in chicks (Troilo, Gottlieb & Wallman, 1987; Wildsoet & Pettigrew, 1988) or monkeys (Raviola & Wiesel, 1985) or blockade of ganglion cell action potentials in tree shrew (Norton, Essinger & McBrien, 1989) and chick (Moghaddam & McBrien, 1993) does not prevent the development of axial elongation and experimentally induced myopia. Other studies have shown that deprivation of only part of the visual field results in local areas of elongation and myopia in the eye (e.g. Hodos & Kuenzel, 1984; Gottlieb, Fugate-Wentzek & Wallman, 1987). It has also been shown that myopia can be induced in chicks who have undergone bilateral lesioning of the Edinger–Westphal nucleus (Schaeffel, Troilo, Wallman & Howland, 1990). The above findings argue against a role for accommodation as a major causative factor in experimental myopia.

A more definitive way of determining the importance of accommodation in experimentally induced myopia would be to determine if excessive axial elongation and myopia could be induced in a diurnal animal that does not possess an effective accommodative system. This was the aim of the present investigation.

The Eastern grey squirrel (*Sciurus carolinensis*) is a diurnal mammal belonging to the order Rodentia and the genus *Sciurus*. This small (500–700 g) arboreal rodent is extremely agile spending most of the daylight hours moving through a visually

complex habitat, running up and down trees, balancing on the flimsiest of twigs, as well as leaping up to seventeen body lengths from tree to tree (Shorten, 1954). These behavioural characteristics are indicative of a well-developed visual system and both anatomical and electrophysiological studies support this. While the squirrel's eyes are laterally placed there is a 60 deg binocular field and there is presumptive evidence from electrophysiological mapping for the presence of stereopsis (Hall, Kaas, Killackey & Diamond, 1971). Both electrophysiological evidence (e.g. Gouras, 1964) and histological evidence (e.g. West & Dowling, 1975) have shown that the grey squirrel has a duplex retina and is unusual among mammals in having more cones than rods, with approximately 40% rods and 60% cones. Behavioural measurements show a peak sensitivity to spatial frequencies of about 0·5 cycles/deg with a high frequency limit of between 2·2 cycles/deg to 3·9 cycles/deg depending on luminance (Jacobs, Birch & Blakeslee, 1982). Directionally selective ganglion cells of the grey squirrel eye have also been shown to be able to respond to moving grating patterns having spatial frequencies of up to 5 cycles/deg (Cooper & Robson, 1969). Although from behaviourial observations it has been suggested that the Eastern grey squirrel has 'exceptional powers of focusing' (Shorten, 1954), as a member of the rodent family it would seem likely that the grey squirrel would have either no accommodative ability or only a limited amount, as found in the ground squirrel (Gur & Sivak, 1979; McCourt & Jacobs, 1984).

The present study initially investigated the functional morphology of the intra-ocular muscles of the Eastern grey squirrel using both pharmacological and histological methods before determining if experimental myopia could be induced in this diurnal mammal.

METHODS

Animals

Grey squirrels were obtained from local woodlands with the assistance of the local forestry commission using approved live trapping methods. Only female squirrels that appeared pregnant or adolescent squirrels born the previous breeding season (< 6 months old) were taken into captivity. Animals were housed in large individual cages at a controlled temperature (18 °C). Animals experienced a natural dawn and dusk cycle which was supplemented by fluorescent strip lighting between 9.00 and 16.00 h. Food and water were available *ad libitum*. Studies on the intra-ocular muscles of the grey squirrel were conducted on adolescent or young adult squirrels (12–33 months). Studies on the development of experimentally induced myopia were conducted on pups born in the facility and on adolescent squirrels (≈ 6 months of age).

Optical and biometric measures

All measurements were performed with the animals under anaesthesia. Animals were anaesthetized (90 mg/kg ketamine HCl with 10 mg/kg xylazine, I.M.) and placed on a heating pad at 37 °C with the respiration rate constantly monitored. Supplemental doses of anaesthetic were given as required. The animal's head was positioned with the aid of individualized bite bar of dental impression material, the other end of which 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. Baseline measurements of the corneal curvature, ocular refraction (streak retinoscopy and coincidence optometry), and *in vivo* axial dimensions of the intra-ocular components using A-scan ultrasonography were taken. These measurement techniques have been described previously in detail (McBrien, Moghaddam & Reeder, 1993) and will be described only briefly. Corneal curvature was measured using a calibrated one position keratometer with a +4 dioptre (D) modifying lens to allow measurement of the steeply curved squirrel cornea. Retinoscopy

was performed using hand held trial lenses to an accuracy of 0·3 D in the horizontal and vertical meridians. A Hartinger coincidence refractometer (Zeiss, Jena, Germany) equipped with a +20 D modifying lens was also used; eight observations were recorded from both horizontal and vertical meridians. All readings of refractive error presented are the mean equivalent sphere effective at the corneal plane. No correction was made for the artifact of retinoscopy. Axial ocular dimensions were measured using A-scan ultrasonography; a 10 MHz focused transducer (Wells Krautkramer, UK) was employed with a Panametrics (USA) pulser/receiver, model 5052. For each eye six averaged waveforms (average of 20 single waveforms) were collected via a Lecroy 9400 (100 megasamples per second (Ms/s)) digital storage oscilloscope (Lecroy, Switzerland) connected superfluous to an Opus pcV (At) computer for storage and later measurement of waveforms. Conversion of time to distance employed previously published values for the conduction velocity of ultrasound in cornea, aqueous and vitreous humours as these have been shown to vary little between species (Coleman, Lizzi & Jack, 1977). Crystalline lens conduction velocity has been found to vary considerably between species (Schiffer, Rantanen, Leary & Bryan, 1982) and as the ultrasound velocity of the grey squirrel lens has not been reported previously we employed the method described by Coleman *et al.* (1977) to measure five lenses from four young adult squirrels. This yielded an average lens conduction velocity of 1768±47 m/s.

Pharmacological stimulation of intra-ocular muscles

In an attempt to pharmacologically stimulate accommodation in the grey squirrel eye the direct acting parasympathomimetic carbamylcholine chloride (carbachol), which has both muscarinic and nicotinic actions, was employed. Carbachol was administered topically via corneal iontophoresis using agar gel buttons (Koretz, Bertasso, Neider, True-Gabelt & Kaufman, 1987). It has previously been demonstrated that 40% carbachol is a supramaximal dose for inducing maximum accommodative amplitude in both monkey (Koretz *et al.* 1987) and raccoon (Rohen, Kaufman, Eichhorn, Goeckner & Bito, 1989). In squirrel 40% carbachol was found to cause maximum pupillary constriction (see Fig. 1*A*). However, due to the marked pupillary constriction produced by carbachol, it was necessary to pretreat the eye under measure with phenylephrine to enable further ocular measurements.

Five young adult grey squirrels, weighing between 490 and 586 g were utilized for assessment of accommodative ability. After baseline optical and structural measurements were taken on both eyes of an animal, baseline horizontal and vertical pupil diameters for right and left eyes were measured through a calibrated measuring eyepiece of a binocular microscope at 10× magnification under constant illumination (100 lx). Readings were taken every minute for 10 min. Then two 15 μl drops of 2% phenylephrine, spaced 1 min apart, were topically applied to the left eye. This pretreatment maintained a sufficiently large pupil diameter to enable measurement of refractive state and axial dimensions of the intra-ocular components (see Fig. 1*B*). Previous studies have shown that the predominantly α-receptor sympathomimetic phenylephrine has only a very limited effect on accommodative amplitude while producing good pupil dilatation (e.g. Zetterström, 1988). Pupil measurements were then taken every minute for 10 min and then every 5 min until 25 min after instillation. To confirm that 2% phenylephrine did not alter the ocular parameters under investigation all structural and refractive measurements, as described above, were repeated on both eyes of the animal.

On completion of postphenylephrine structural measurements, pupil diameter readings (precarbachol) were again taken before transcorneal iontophoresis of 40% carbachol was performed on the experimental eye. One drop of the local anaesthetic, 0·5% proxymetacaine was then applied topically to the cornea of the eye being measured to enhance penetration of the carbachol. Topical application of either 2% phenylephrine or 40% carbachol using the method described, would not be expected to cause any pain when applied to the eye. For transcorneal iontophoresis the negative electrode was connected to the ipsilateral ear of the eye under investigation, a freshly made 2·5% 5 mm agar button was placed on the central cornea and the positive electrode was gently applied to the agar button for 20 s with a current of 200 μA. Postcarbachol pupil measurements were recorded at 2 min intervals for the first 10 min and then at 5 min intervals until 25 min post-iontophoresis of carbachol. Refractive and structural measurements were then recorded, firstly on the experimental eye (30–60 min postcarbachol) and then on the contralateral control eye. In a separate control experiment three squirrels had their refraction measured after 5, 10 and 20 min postcarbachol administration to confirm that no changes were of short duration. A small, systemic dose of atropine sulphate (10 μg/kg) was given prior to topical application of 40% carbachol. This

was to prevent drug-induced reduction in systemic blood pressure and ciliary muscle blood flow that may occur with large systemic and topical doses of carbachol (Rohen *et al.* 1989). The left eye of each animal was used as the experimental eye, with comparison made between pre- and postdrug measures. The right eye of each animal was used as a control to monitor for any systemic effects of the topical application of drugs to the left eye.

Histological studies

The eyes of three grey squirrels were enucleated under deep anaesthesia (sodium pentobarbitone 80 mg/kg, I.P.). A small window was made in the vitreous chamber and the eyes were then immediately placed into 2·5% glutaraldehyde in 0·1 M phosphate buffer (pH 7·4). After overnight fixation and under a dissecting microscope the eyes were hemisectioned into anterior and posterior sections, the cornea removed and the lens dissected out using fine forceps and Vannas scissors. The remaining anterior sclera, with the ciliary body and iris attached, was then dissected into eight sectors. Some of these specimens were then dehydrated in graded alcohols and embedded in paraffin wax. Paraffin sections were cut at 6 μm and stained with Haematoxylin and Eosin, van Geison's or Mallory's trichrome stain. The other sectors were prepared for transmission electron microscopy by postfixation in two changes of 1% osmium tetroxide in 0·1 M sodium cacodylate buffer for a period of 15 min in each. Sectors were then dehydrated in ascending concentrations of ethanol and embedded in epoxy araldite. Sections were stained with uranyl acetate and lead citrate.

Monocular deprivation of pattern vision

On the day of natural eye opening ($35 \pm 1{\cdot}0$ days) four squirrel pups born in the facility had one eye deprived of pattern vision. Animals were anaesthetized with 2–4% halothane and placed on a heating pad at 37 °C. Baseline measures of refraction and axial ocular dimensions were taken using retinoscopy and A-scan ultrasonography respectively. Then the lids of one eye were surgically trimmed and apposed again with three mattress sutures using sterile 6–0 silk. The lids were fused together within the next week to produce a translucent membrane over the eye which prevented pattern vision. A small nasal opening was left for drainage. After this short surgical procedure the pups were returned to their mother's cage. To gain some insight into the developmental time course of any induced changes in structural dimensions the period of deprivation was varied (20, 30, 40, and 90 days) in the four pups. Three adolescent grey squirrels were also deprived of pattern vision by monocular lid suture for periods of 7, 12 and 16 months.

When the animals completed the allocated period of monocular visual deprivation, a full set of refractive and structural measures were taken on both eyes. Atropine sulphate (1%) was topically applied to the cornea of both eyes 1 h before general anaesthesia (90 mg/kg ketamine HCl with 10 mg/kg xylazine, I.M.). Measurements of corneal curvature, refraction, and ocular dimensions were recorded using the techniques described above. On completion of all *in vivo* measurements, animals were killed by an intraperitoneal injection of sodium pentobarbitone (100 mg/kg).

RESULTS

Pharmacologically induced changes in ocular component dimensions

Pupil size changes

The time course of mean pupil diameter changes in response to varying concentrations of carbachol are shown in Fig. 1. The maximum miotic effect usually occurred 10–12 min after corneal iontophoresis of all except the weakest concentration of carbachol (0·02%). There was a clear ascending dose–response relationship between carbachol concentration and pupil constriction up to 7·5% carbachol. It was found that 7·5% carbachol produced the same magnitude and rate of constriction of the pupil as 40% carbachol (see Fig. 1*A*).

Phenylephrine (2%) caused a significant dilatation of the pupil 25 min after administration in the treated eye ($4{\cdot}47 \pm 0{\cdot}43$ mm *vs.* $6{\cdot}45 \pm 0{\cdot}15$ mm, $P < 0{\cdot}01$); no change was observed in the fellow control eye ($4{\cdot}58 \pm 0{\cdot}37$ mm *vs.* $4{\cdot}41 \pm 0{\cdot}37$ mm).

Pretreatment with 2% phenylephrine markedly reduced carbachol (40%)-induced pupil constriction (see Fig. 1*B*), thus enabling refractive and structural measurements to be recorded.

Refractive and intraocular component measurements

Non-cycloplegic ocular refraction was found on average to be more hyperopic (+1·4±0·4 D) with retinoscopy than with Hartinger refractometry, a finding that

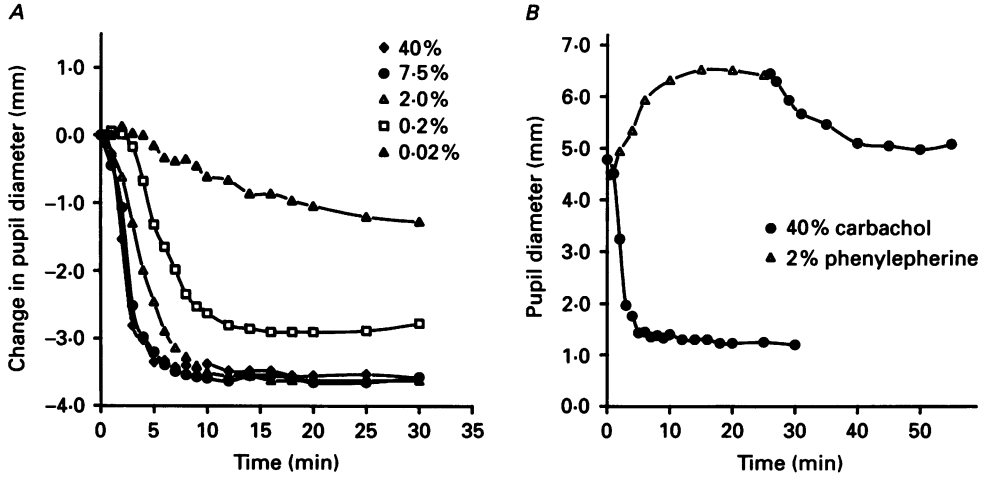

Fig. 1. *A*, dose–response curves for the effect of carbachol on pupil diameter. Carbachol was topically applied by incorporating it in a 2·5% agar button placed on the central cornea and a 200 μA current applied for 20 s (transcorneal iontophoresis). Each curve is the mean response from the left eye of two animals. *B*, the effect of 40% carbachol on pupil diameter prior to and after pretreatment with two 15 μl drops of 2% phenylephrine.

has been reported previously (McBrien & Norton, 1992). This was a consistent finding for all animals. Differences in refraction between animals were similar for both refraction techniques. Due to the similarity of the two refractive measurement techniques, only retinoscopy findings will be discussed as there was less variability of repeat measurements between the two observers (N.McB. and H.O.M.) with this technique.

Phenylephrine (2%) was found to cause no significant change from pretreatment baseline values in refraction as measured 30–60 min after administration (+1·9±0·4 D *vs.* +1·7±0·5 D, $P = 0·9$). Administration of 40% carbachol also produced no significant change in ocular refraction in all five animals (+1·7±0·5 D *vs.* +1·9±0·6 D, $P = 0·9$). Three animals showed no measurable change in refraction (< 0·3 D), one animal showed a decrease in refractive power of the eye of 0·8 D and the other animal an increase of 0·4 D in refractive power. No change greater than 0·3 D was observed in the contralateral control eyes of these animals across all conditions. Also no changes in refraction were observed in the three squirrels where retinoscopy measurements were taken at 5, 10 and 20 min postcarbachol stimulation.

Concordant with this lack of any induced change in accommodation with 40% carbachol, was the finding that none of the squirrels showed a significant change in intra-ocular component dimensions or corneal curvature after administration of 40% carbachol (see Fig. 2). This indicates that there was no change in either crystalline lens curvature or position with carbachol stimulation.

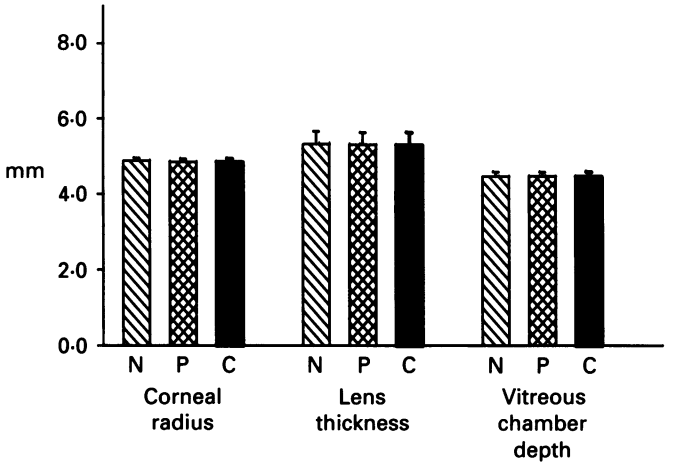

Fig. 2. Corneal radius, axial lens thickness and vitreous chamber depth measures of the grey squirrel eye before and after treatment with both 2% phenylephrine and 40% carbachol. Corneal radius was measured by a modified keratometer and axial ocular dimensions by A-scan ultrasonography. No significant changes were found in any of the ocular dimensions measured. $n = 5$. Error bars ± 1 S.E.M. N, predrug; P, post-phenylephrine (2%); C, postcarbachol (40%).

Histological findings

The morphology of the anterior segment of the grey squirrel eye shows many of the characteristics found in diurnal mammals. A trabecular-like meshwork is found at the junction of the iris root and corneoscleral transition zone, although a true canal of Schlemm is not differentiated. The ciliary processes possessed typical unpigmented and pigmented epithelia and extend a considerable way forward beneath the iris root. In micro-anatomical terms the iris of the grey squirrel is typical in possessing an anterior border layer, collagenous stroma containing small capillaries, the sphincter pupillae, the dilator pupillae and the posterior pigment epithelium (see Fig. 3*A*). A well-developed sphincter pupillae muscle could be observed; the smooth muscle cells (fusiform) are numerous and densely packed within a loosely arranged connective tissue matrix. The myo-epithelial portion of the anterior epithelium (dilator pupillae) is clearly visible and consists of longitudinally arranged spindle like cells, the nuclei of which are clearly defined. The dilator pupillae muscle can be seen to end behind the sphincter pupillae muscle (Fig. 3*A*).

There is evidence of smooth muscle cells surrounded by a collagenous stroma in the posterior portion of the ciliary body of the grey squirrel (see Fig. 3*B* and *C*). However, this relatively sparse population of smooth muscle cells forms what could

be termed a poorly developed ciliary muscle. Observations using transmission electron microscopy confirmed findings at the light microscope level. These histological findings are concordant with pharmacological results showing large responses to parasympathomimetic and sympathomimetic stimulation of both the

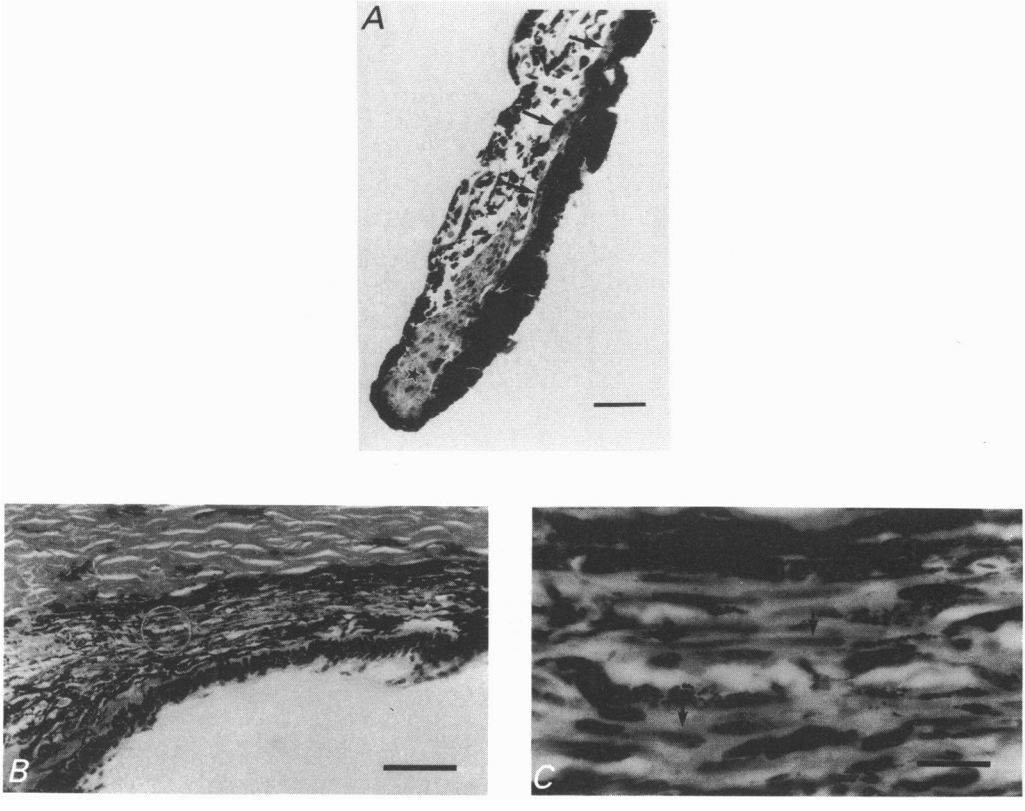

Fig. 3. *A*, sagittal section through the pupillary portion of the iris of the Eastern grey squirrel. In micro-anatomical terms the iris of the grey squirrel is typical in possessing an anterior border layer, collagenous stroma containing small capillaries, sphincter pupillae and dilator pupillae muscles bounded by the posterior pigment epithelium. The large and well-developed sphincter pupillae muscle (★) contained numerous smooth muscle cells (fusiform) within a loosely arranged connective tissue matrix. The myoepithelial portion of the anterior epithelium (dilator pupillae) is clearly visible (arrows) and consists of longitudinally arranged spindle like cells, the nuclei of which are clearly defined. The dilator pupillae muscle can be seen to end behind the sphincter pupillae muscle. Van Geison stain, 400 ×. Scale bar = 50 μm. *B*, section through the posterior portion of the ciliary body and anterior sclera of the eastern grey squirrel (Van Geison stain, 200 ×, scale bar = 100 μm). There is evidence of a relatively meagre ciliary muscle within the stroma of the ciliary body, which is confirmed by viewing under higher magnification (circled area). *C*, under higher magnification a relatively sparse population of spindle-like smooth muscle cells (arrows) can be clearly detected. Van Geison stain, 1000 ×, scale bar = 20 μm.

iris sphincter and dilator muscles respectively but a lack of response and thus accommodative ability, on stimulation of the ciliary muscle.

Effects of monocular deprivation

The baseline predeprivation measurements of refractive state and axial dimensions between the right and left eyes of experimental animals showed no significant differences ($P > 0{\cdot}10$) for any of the ocular components.

Corneal radius

All deprived eyes had a significantly flatter corneal radius than their contralateral open eye (mean $\pm$ S.E.M.; $5{\cdot}10 \pm 0{\cdot}14$ mm *vs.* $4{\cdot}70 \pm 0{\cdot}14$ mm; $P < 0{\cdot}01$, see Fig. 4). This finding is similar to results obtained in other mammalian models of refractive development, such as tree shrew (McBrien & Norton, 1992), where eyes have been deprived via lid-suture techniques. Recent studies (Siegwart & Norton, 1990; McBrien & Norton, 1992) have found that this flattening is due to the mechanical effects of eyelid closure, rather than to the visual deprivation itself. Substantial corneal flattening occurred even for the shortest deprivation period (20 days MD produced 0·36 mm relative flattening) suggesting that this mechanical effect occurs rapidly after eyelid closure.

The refractive effect of the observed corneal flattening was to induce a hyperopic shift in the refraction of the deprived eye relative to the contralateral open eye. The value for the real refractive index of the cornea of the grey squirrel eye was assumed to be the same as that found for the tree shrew (1·378; Schafer, 1969) another diurnal mammal with similar eye size. However, to model the change in refraction due to corneal flattening, with only anterior corneal curvature measures available, an 'effective' corneal refractive index was determined using schematic modelling, producing a value of 1·335. Using this effective refractive index of 1·335 and taking the amount of flattening as the difference between the corneal curvature of the deprived eye and the contralateral open eye (binocularly normal animals show no significant differences in corneal curvature between eyes), it was possible to determine the actual refractive effect of corneal changes for each animal. The mean refractive effect of the observed corneal flattening was $5{\cdot}7$ D $\pm 0{\cdot}8$ D. Therefore, as in a previous study (McBrien & Norton, 1988) we calculated a 'compensated refraction' value for the deprived eyes which accounted for this corneal flattening effect.

Refractive measurements

Comparison of retinoscopic values for deprived eyes (uncompensated for corneal flattening) and their contralateral control eyes revealed no significant differences ($+4{\cdot}7 \pm 2{\cdot}0$ D *vs.* $+4{\cdot}9 \pm 1{\cdot}0$ D; $P = 0{\cdot}87$). When refractive measurements were 'compensated' for the mechanical effects of corneal flattening in the MD eyes a significant difference in refractive state between deprived and control eyes was found ($-1{\cdot}0 \pm 1{\cdot}5$ D *vs.* $+4{\cdot}9 \pm 1{\cdot}0$ D; $P < 0{\cdot}001$). This produced a relative myopia of $-5{\cdot}9$ D $\pm 0{\cdot}7$ D. Thus, despite lacking a functional accommodative system it is possible to induce a significant degree of experimental myopia in the grey squirrel. For the neonatal squirrels which were monocularly deprived at eye opening, only 20 days of MD was sufficient to induce an experimental myopia of $-4{\cdot}8$ D (compensated

refraction differences). The largest induced myopia found in the neonatal animals was −7·2 D and was for the pup deprived for the longest period (90 days). However, evidence of intersubject variability was apparent as evidenced by the fact that the smallest degree of experimental myopia induced in neonatal squirrels was for the

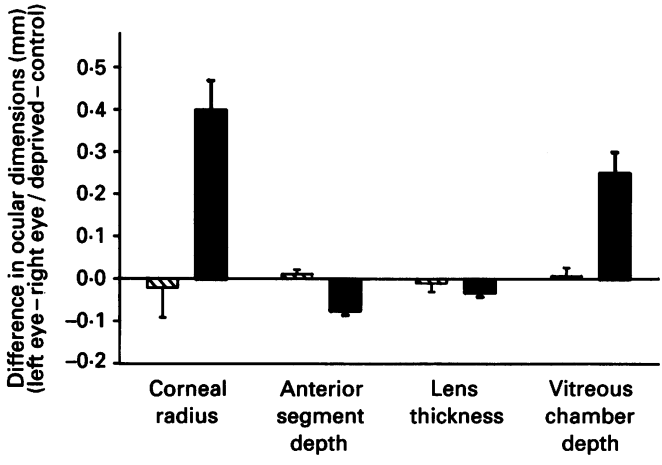

Fig. 4. Differences in corneal radius, anterior segment depth, lens thickness and vitreous chamber depth between deprived and contralateral control eyes, before (▨) and after (■) monocular deprivation of pattern vision. Monocular deprivation produces significant elongation of the vitreous chamber depth and marked flattening of the corneal radius in deprived eyes. $n = 7$. Error bars ± 1 S.E.M.

animal monocularly deprived for 40 days. The degree of experimental myopia induced in the three squirrels monocularly deprived in young adulthood was similar in magnitude to that found for the 90 day pup, although deprivation was for markedly longer time periods (7, 12 and 16 months).

Anterior segment

A consistently shallower anterior segment depth (anterior chamber depth + corneal thickness) was found in monocularly deprived eyes when compared to contralateral open control eyes ($P < 0{\cdot}01$) as shown in Fig. 4. This reduction in anterior segment depth has been previously shown to be related to the mechanical effects of lid suture (McBrien & Norton, 1992). The refractive effect of this change in anterior segment depth between deprived and open eyes is only 0·3 D, as determined by schematic modelling assuming that the lens thickness and vitreous chamber depth remain constant.

Crystalline lens thickness

The axial thickness of the crystalline lens was slightly thinner in the deprived eyes of all MD animals (Fig. 4). This finding has been noted in other mammalian models of experimental myopia (McBrien & Norton, 1992) although not in avian models (Wallman & Adams, 1987; McBrien *et al.* 1993). In tree shrew this thinning of the lens

has been found to be associated with a lens lighter in weight but of the same curvature (McKanna & Casagrande, 1978; McBrien & Norton, 1992). A similar finding of a thinner lens in experimentally myopic squirrel eyes argues against the role of accommodation in this structural change.

Vitreous chamber depth

Figure 4 shows that the vitreous chamber depth of deprived eyes increased significantly in comparison to control eyes ($4{\cdot}54 \pm 0{\cdot}11$ mm *vs.* $4{\cdot}29 \pm 0{\cdot}11$ mm, $P < 0{\cdot}01$). Thus, the lack of a functional accommodative system in the grey squirrel did not prevent the development of deprivation-induced vitreous chamber elongation. Vitreous chamber elongation was the major structural cause of the experimentally induced myopia. There was a significant correlation of $r = 0{\cdot}77$ between vitreous chamber depth differences and compensated refractive error differences ($P < 0{\cdot}05$). The greatest vitreous chamber elongation observed in the four neonatally deprived squirrels was for the 90-day-deprived pup (0·34 mm) which also developed the largest myopia. The three squirrels monocularly deprived of pattern vision in adolescence developed similar degrees of vitreous chamber elongation (0·26, 0·31 and 0·37 mm) as the 90-day-deprived pup. This similarity in vitreous chamber elongation between squirrels deprived in adolescence for periods up to 16 months and a neonatal squirrel deprived of pattern vision at eye opening for 90 days indicates a reduction in the susceptibility to induce vitreous chamber elongation and myopia with age, as found previously with other animal models of myopia.

The vitreous chamber elongation accounted for the increase in axial length of deprived eyes compared to control eyes (10·67 mm *vs.* 10·53 mm; $P < 0{\cdot}05$).

Recovery from vitreous chamber elongation

After 7 months of monocular deprivation in an adolescent squirrel there was an elongation of 0·37 mm in the vitreous chamber depth of the deprived eye relative to the control eye, resulting in a myopia of −8·6 D (compensated for corneal flattening). Monocular deprivation ceased at 12 months of age and the animal was remeasured after a further 5 months of binocular viewing. There was a significant reduction in relative vitreous chamber differences between the two eyes of (mean ± S.D.) $0{\cdot}14 \pm 0{\cdot}05$ mm ($P < 0{\cdot}01$) with the difference of $0{\cdot}37 \pm 0{\cdot}04$ mm at the end of the deprivation period reducing to $0{\cdot}23 \pm 0{\cdot}04$ mm at the end of the recovery period. This reduction in vitreous chamber differences contributed to the observed reduction in myopia (−8·6 to −2·9 D). Also contributing to this reduction in myopia was a steepening of the corneal curvature in the previously deprived eye, with differences between the two eyes reduced from −0·53 to −0·17 mm, with a concordant reduction in anterior segment differences.

DISCUSSION

The main findings of this investigation are that the grey squirrel does not possess a functional accommodative system and that despite this it is still possible to experimentally induce vitreous chamber elongation and myopia via monocular deprivation of pattern vision.

From an evolutionary standpoint it is not surprising to find that the grey squirrel lacks a developed accommodative system. It is known that the majority of animals belonging to the order Rodentia are nocturnal and do not possess an accommodative ability. It is interesting to note the difference between the present findings and those

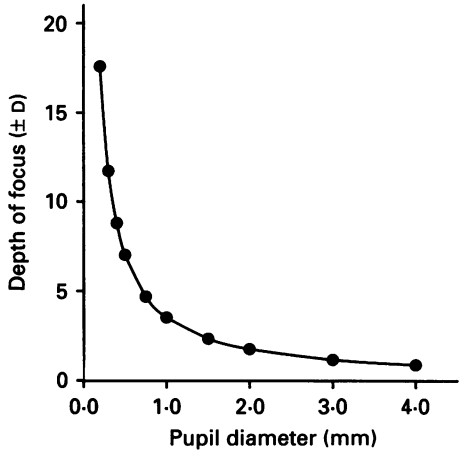

Fig. 5. Depth of focus of the Eastern grey squirrel eye. Calculations were based on the structural measures made in the present study for pupil and ocular dimensions and employing the equations of Green *et al.* (1980) and behavioural measures of visual acuity (Jacobs *et al.* 1982). The grey squirrel has a depth of focus of at least ± 5 D at small pupil diameters ($< 1{\cdot}0$ mm). ($D = 7{\cdot}03/p \times v$, where p is pupil diameter (in mm) and v is visual acuity (in cycles/deg).)

for another diurnal squirrel, the ground squirrel, which has been found to possess a limited accommodative ability of 2–4 D (Gur & Sivak, 1979; McCourt & Jacobs, 1984). However, tree- and ground-dwelling squirrels have marked differences in their crystalline lens dimensions with ground squirrels reported to have a much thinner lens (Gur & Sivak, 1979) than that found in the grey squirrel, whose intra-ocular dimensions with its large lens, are more closely proportioned to those of the nocturnal rat. It is feasible that the relatively underdeveloped ciliary muscle of the squirrel eye is unable to cause any deformation or movement of the thicker and denser lens of the grey squirrel. Even histological evidence of a well-developed ciliary muscle in itself is not sufficient proof of a functional accommodative system, as other mammals such as bovine and rabbit have been found to have a relatively well-developed ciliary muscle with little or no accommodative ability (e.g. Prince, Diesem, Eglitis & Ruskell, 1960).

Although the grey squirrel has evolved several characteristics to adapt to its chosen arboreal habitat and in many ways resembles the tree shrew, which has a functional accommodative apparatus (N. A. McBrien, unpublished observations), it may be that there is no requirement for it to have a functional accommodative system. In the rat, a nocturnal rodent which has been described as possessing no discernible ciliary muscular apparatus (Lashley, 1932), it has been suggested that depth of focus can serve its near-field requirements. Hughes (1977) has calculated

that due to the small size and relatively poor acuity of the rat eye it should possess a depth of focus as great as 30 D for a 0·3 mm pupil diameter. From the biometric information gained in the present study and using previously reported behavioural data on visual acuity (Jacobs *et al.* 1982) it is possible to approximately determine the depth of focus of the Eastern grey squirrel eye. While the resting pupil diameter of the grey squirrel under light anaesthesia is in the order of 4 mm, under pharmacological stimulation it can reduce to less than 1 mm and it is reasonable to assume that in bright photopic conditions it could naturally constrict to a similar diameter, which of course considerably increases the depth of focus. Using the equations and assumptions of Green, Powers & Banks (1980), which give a conservative estimate, the depth of focus of the squirrel eye for several pupil diameters is shown in Fig. 5. It can be seen that at the smaller pupil diameters ($< 1{\cdot}0$ mm) the Eastern grey squirrel eye can have a depth of focus of at least ± 5 D. A similar depth of focus has been reported for the ground squirrel (McCourt & Jacobs, 1984). This range of depth of focus may well be sufficient to account for the behaviourial observations of the near-viewing habits of the grey squirrel.

The present investigation was able to address the role of accommodation in experimentally induced myopia in eyes with an intact nervous innervation, thus avoiding the possibly confounding effects on other ocular functions that can occur using surgical or pharmacological intervention techniques. Results indicate that a functional accommodative system is not necessary to induce experimental axial myopia in this mammalian model. Although previous studies have proposed accommodation as a major causative factor in the development of experimentally induced axial elongation and myopia (e.g. McKanna & Casagrande, 1981), the present findings argue against this hypothesis. McKanna & Casagrande (1981) proposed an eloquent negative feedback model incorporating accommodation based on lenticular changes in myopic tree shrew eyes (lighter lenses and zonular fibre changes) and the effect of atropine in preventing experimental myopia. Similar reductions in lens thickness were noted in the myopic squirrel eye as that found in tree shrew and recent studies indicate that muscarinic antagonist effects on myopia development may have their effect via a non-accommodative mechanism (Stone, Lin & Laties, 1991; McBrien, Moghaddam & Reeder, 1991, 1993).

The present study does not have sufficient neonatal subjects to address the question of whether the process of emmetropization, that is found in many species (e.g. Wallman *et al.* 1978), can occur in the absence of a functional accommodative system. Although studies on both avian and mammalian models of refractive development give support to local ocular control of eye growth (Troilo *et al.* 1987; Norton *et al.* 1989), recent evidence (e.g. Troilo & Wallman, 1991) suggests that the role of brain-mediated functions, other than accommodation, may be involved in the visual control of eye growth and is worthy of further investigation.

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