Pergamon

0042-6989(94)00237-1

Vision Res. Vol. 35, No. 9, pp. 1141–1152, 1995

0042-6989/95 $9.50 + 0.00

The Effects of Blockade of Retinal Cell Action Potentials on Ocular Growth, Emmetropization and Form Deprivation Myopia in Young Chicks

NEVILLE A. MCBRIEN,*† HADI O. MOGHADDAM,* CHARLES L. COTTRIALL,* ELEANOR M. LEECH,* LYNN M. CORNELL*

Received 8 February 1994; in revised form 1 July 1994

To investigate the influence of brain mediated functions on control of ocular growth, young chicks were treated monocularly with intravitreally injected tetrodotoxin (TTX) to block retinal ganglion cell action potentials. TTX injections (0.7 μg in 7 μl) were given on day 6 after hatching in both binocularly open and monocularly deprived chicks. Injections were repeated every 48 hr for a period of 8 days (TTX-open; TTX-MD). Control groups of animals received intravitreally injected phosphate buffered saline (PBS-open; PBS-MD) to one eye on the same schedule. There was a minimum of eight animals in each group. Recovery from form-deprivation myopia during blockade of retinal cell action potentials was also investigated. Results demonstrate that blockade of retinal cell action potentials by TTX produces reduced growth of the anterior segment of the eye and crystalline lens in both binocularly open and MD chicks. Blockade of retinal cell action potentials does not prevent form-deprivation induced vitreous chamber elongation and myopia. Form deprived myopic eyes were found to emmetropize despite blockade of retinal ganglion cell action potentials giving further evidence for local ocular control of emmetropization. Blockade of retinal ganglion cell action potentials did not prevent changes in choroidal thickness in eyes developing axial myopia or eyes recovering from induced myopia.

Ocular development Hyperopia Myopia Tetrodotoxin Choroid Retina Emmetropization

INTRODUCTION

Despite the fact the eye continues to grow from birth to adult age, increasing in size considerably, the ocular components are usually so precisely regulated that the image of distant objects continue to be focused on the photoreceptor layer of the retina. This coordinated growth and precise spatial arrangement of the ocular components of the eye results in the majority of eyes having no appreciable refractive error (i.e. emmetropia). This process has been termed emmetropization and occurs in humans and animals (e.g. Van Alphen, 1961; Wallman, Adams & Trachtman, 1981). There is considerable evidence that this regulated growth of the eye is guided by the clarity of the retinal image. Deprivation of pattern vision in the developing eye of humans and animals results in a breakdown of the coordinated growth, with the eye undergoing axial elongation and developing myopia (e.g. Hoyt, Stone, Fromer & Bilson, 1981; Wiesel & Raviola, 1977; Wallman & Adams, 1987). In chicks it has recently been shown that altering the focal plane of the eye, using either positive or negative lenses, results in the eye adjusting its growth of the vitreous chamber in the appropriate direction to reduce defocus (Schaeffel, Glasser & Howland, 1988; Irving, Callender & Sivak, 1991).

Although the presence or absence of a clear image on the retina has been shown to be important in guiding the growth and refractive development of the eye, the mechanisms by which this is achieved are uncertain. Until recently it was assumed that visual information proceeded from the eye via central visual pathways to bring about changes in cortical or subcortical controlled functions, such as accommodation (e.g. McKanna & Casagrande, 1981; Schaeffel *et al.*, 1988). However, recent studies on animal models of form-deprivation myopia have reported findings which suggest that visual signals may proceed directly from the retina to the choroid and/or sclera without the need for central communication (Hodos & Kuenzel, 1984; Wallman,

*Department of Optometry and Vision Sciences, University of Wales—College of Cardiff, King Edward VII Avenue, Cardiff CF1 3XF, Wales [*Fax* 44 222 874859].
†To whom all correspondence should be addressed.

Gottlieb, Rajaram & Fugate Wentzek, 1987; Troilo, Gottlieb & Wallman, 1987; Wildsoet & Pettigrew, 1988; Schaeffel, Troilo, Wallman & Howland, 1990; Norton, Essinger & McBrien, 1994). These studies indicate that structures within the eye itself (i.e. the retina) can detect the degraded visual image without the need for central communication, and adjust its development.

What is not particularly clear from these studies is the role of central visual pathways on normal ocular growth and emmetropization. Troilo *et al.* (1987) found that in open eyes, sectioning of the optic nerve in chick resulted in reduced vitreous chamber development (measured by *in vivo* A-scan ultrasonography) and consequently high degrees of hypermetropia, which did not significantly remit with time. Wildsoet and Pettigrew (1988) also found an increase in hypermetropia in open eyes with optic nerve section, although they did not observe an obvious structural correlate using *in vitro* measures. It is uncertain whether the reduced vitreous chamber development noted by Troilo *et al.* (1987) was as a result of disrupting the brain's influence on eye growth and refractive development or due to the ganglion cell death that follows optic nerve section (Muchnick & Hibbard, 1980). In tree shrew eyes treated with tetrodotoxin (TTX), a voltage dependent sodium channel blocker, to prevent retinal ganglion cell action potentials, it was not possible to assess the effects of blocking central communication on normal ocular growth as the procedure of intravitreal injection itself produced a shorter vitreous chamber. By blocking afferent communication between the eye and higher visual processing pathways in chick, and efferent effects within the eye, with intravitreally injected TTX, the primary aim of the present study was to assess the role of centrally mediated mechanisms on normal ocular growth and emmetropization. This approach avoids the major surgical intervention of optic nerve section, thus avoiding possible complications of retinal ganglion cell death. It has been demonstrated in chicks that the technique of intravitreal injection itself, on an alternate day schedule, does not cause vitreous chamber reduction (McBrien, Moghaddam & Reeder, 1993). By observing the effects of retinal impulse blockade in both binocularly open and monocularly deprived chick eyes and also recovery from any induced changes, it is hoped to further understand the role of local and central mechanisms involved in the control of ocular growth and refractive development.

MATERIALS AND METHODS

Experimental subjects

Day-old chicks (Rhode-Island cross) were obtained from a local source. The chicks were maintained in a temperature controlled environment on 12 hr light/12 hr dark cycle. Illumination at food level was 250 lx. Food and water were supplied *ad libitum*. Animals were assigned to one of six groups on the basis of whether they were monocularly occluded (MD) and whether they received intravitreal injections of either TTX or phosphate buffered saline (PBS). Each group contained a minimum of eight subjects.

Experimental protocol

On day 6 after hatching, chicks were given an intravitreal injection of 7 μl of either TTX (0.1 μg/μl) or PBS in the left eye. The procedure employed for intravitreal injections in chicks has been described previously (McBrien *et al.*, 1993). All intravitreal injections were carried out under halothane (2–3.5%) anaesthesia, while the animal was maintained on a temperature controlled heating pad, the procedure taking approx. 10 min per animal. To assess the effect of blocking retinal action potentials on normal ocular growth two groups of animals received intravitreal injections of TTX or PBS but no occlusion of vision (TTX-open and PBS-open). To assess the effect of blocking retinal action potentials on induced myopia in the chick, two groups of animals (TTX-MD and PBS-MD) underwent MD in combination with intravitreal injection. Monocular deprivation of form vision was achieved by fixing a translucent occluder over the injected eye. The injection procedure was repeated every 48 hr over an 8-day period, resulting in four intravitreal injections per animal. The occluders were replaced immediately after every injection for MD animals.

To control for the effects of intravitreal injections in both open and deprived eyes a further two groups of chicks were included in the study. One group of chicks (sham-injected MD) underwent halothane anaesthesia, opening of the palpebral aperture and mechanical pressure from, but without insertion of, the needle on the sclera on exactly the same experimental procedure as MD chicks who underwent intravitreal injections. Another group of chicks were housed in identical conditions as experimental chicks, but underwent no experimental manipulations except a complete set of optical and structural measures at the same time as treated animals.

Duration of TTX effect

During the treatment period, an indication of the efficacy of the TTX effect was monitored by testing the chick's pupillary response to light and visual behaviours. Prior to each injection a comparison of both the resting pupil diameter and the direct pupil response to light in the treated and contralateral control eyes was made. This has been reported to give a reliable indication of the efficacy of effect (Wong-Riley, Tripathi, Trusk & Hoppe, 1989). In addition to pupil measures, startle and orientation tests (Troilo *et al.*, 1987) in the treated eye of TTX-open chicks were conducted prior to each injection. When the contralateral eye was covered no response could be elicited from the chick indicating functional blindness in the TTX treated eye.

To further evaluate the duration of effect of the TTX dose used a control study was conducted. Age matched chicks were given a single intravitreal injection of either TTX ($n = 5$) or PBS ($n = 3$) employing the same dose as above. Horizontal and vertical pupil diameters were

measured using an operating microscope with a measuring graticule eyepiece at 10× magnification with luminances of 250 and then 1250 lx for both injected and contralateral control eyes. Readings were taken before the injection, 20 min after the injection and then at intervals from 44 hr after injection until 76 hr. For these pupil measures the chick was gently restrained without anaesthesia. Results indicate that the duration of effect of the TTX, as measured by pupillary responses, is between 52 and 56 hr with full recovery between 68 and 72 hr (Fig. 1).

Optical and structural measures were taken on all chicks 8 days after the initial injection. Animals were anaesthetized with ketamine (50 mg/kg) and xylazine (3.5 mg/kg), supplementary doses of anaesthetic were given as required. All refractive and structural measures were taken under cycloplegic conditions. Due to the predominantly striated nature of chick intraocular muscles, the neuromuscular blocking agent vecuronium bromide (2 mg/ml) was applied topically to the cornea (5 × 25 μl drops) to produce cycloplegia. The cornea was pre-treated with topical proxymetacaine HCl (0.5%) to enhance penetration of the drug. Measurements of corneal curvature (keratometry), ocular refraction (retinoscopy) and intraocular dimensions (A-scan ultrasonography) were taken. Measurement procedures were identical to those described previously (McBrien *et al.*, 1993).

On completion of all *in vivo* optical and structural measures the animal was given an overdose of sodium pentobarbitol (45 mg/kg) and the eyes enucleated. Digital caliper measurements of the medial/lateral and dorsal/ventral equatorial diameters and axial length were taken to the nearest 0.01 mm. The eye was weighed to the nearest 10 μg. The eyes of some TTX and PBS treated animals then had an incision made through the sclera in the equatorial region and were placed in fixative (phosphate buffered 2.5% glutaraldehyde) for 24 hr before a 1.5 mm trephine was punched out at the posterior pole and at the nasal and temporal equatorial regions. This tissue was dehydrated and embedded in epoxy resin using standard procedures for histologic evaluation. Semi-thin sections were cut and examined at the light microscope level to assess the physical integrity of the retina.

Recovery from induced myopia

To determine whether the eye was able to emmetropize when communication between the eye and higher visual processing pathways was blocked, chicks ($n = 8$) were made myopic by monocular deprivation from day 5 after hatching for a period of 5 days. At this time the occluder was removed and a full set of optical and *in vivo* ocular component measures were taken on both eyes (without cycloplegia). On completion of the measures the previously form-deprived eye was treated with intravitreal TTX (0.7 μg in 7 μl) and then allowed to recover from the anaesthesia (ketamine/xylazine) and returned to the brooder without an occluder. The TTX injections were repeated on an alternate day schedule for 8 days as described previously, after which time a further set of optical and structural measures were taken to determine if the previously form-deprived myopic eye could emmetropize despite blockade of retinal ganglion cell action potentials.

To determine whether any changes in ocular component dimensions observed in TTX treated chick eyes show recovery after cessation of treatment a further two

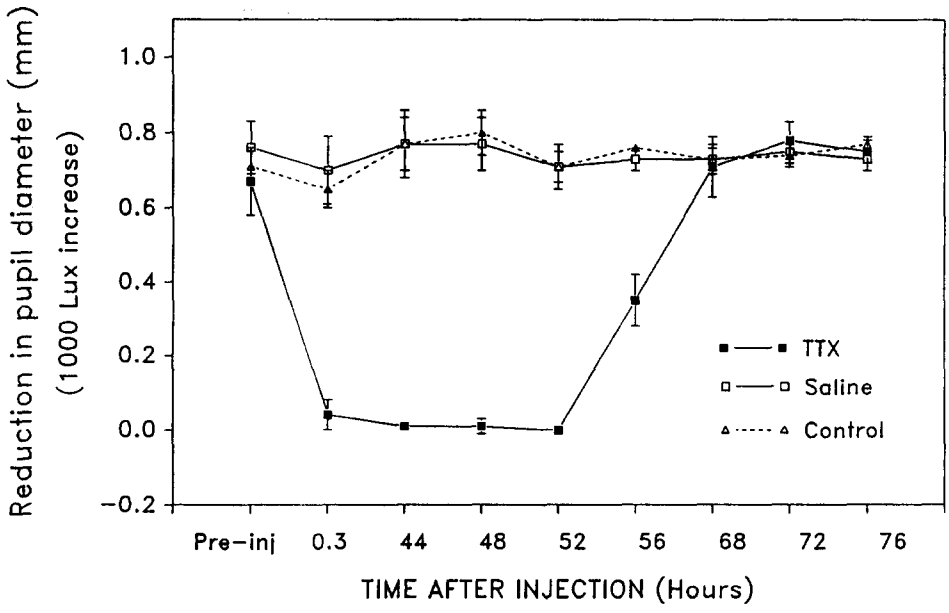

FIGURE 1. The reduction in average pupil diameter [(vertical + horizontal)/2] in the chick eye in response to an increase in light of 1000 lx (250 to 1250 lx). Measurements were recorded under a small operating microscope with a graticule eyepiece under 10× magnification. Measures were taken immediately prior to an intravitreal injection of TTX ($n = 5$) or phosphate buffered saline ($n = 3$) and then at the intervals indicated. Measurements were taken on the treated and contralateral control eye in each animal. Error bars = ±1 SEM.

groups of chicks were treated with TTX on exactly the same schedule as the main part of the study (start day 6, four injections over an 8 day period). One group (TTX-open-recovery, $n = 8$) with the treated eye left open and another group (TTX-MD recovery, $n = 8$) with the injected eye deprived. Optical and structural measures were taken at the end of the injection period and the animals were allowed to recover from anaesthesia and returned to the brooder. In the previously MD animals, the occluder was not replaced. After a further 14 days the animals underwent another full set of optical and structural measures to examine recovery from any TTX induced changes in ocular component dimensions.

Changes in choroidal thickness

Due to recent reports that have implicated choroidal thickness changes in both the development of induced myopia and particularly in the recovery from induced myopia (Wallman, Xu, Wildsoet, Krebs, Gottlieb, Marran & Nickla, 1992) it was of interest to determine if blockade of retinal ganglion cell action potentials prevented this choroidal mechanism. The eyes from chicks that were recovering from induced myopia while undergoing intravitreal TTX injections and also chicks who were monocularly deprived and receiving intravitreal TTX were frozen in liquid nitrogen immediately after enucleation. The eyes were sectioned on a cryostat until the maximum lens thickness was reached and then photographed and the choroidal thickness of TTX treated and contralateral control eyes were compared.

Data analysis

Data on structural component measures were entered into a spreadsheet and transferred to a statistical package (Minitab). Analysis of variance was used to examine overall effects and multiple comparison tests were used to assess individual group differences. Dependent or independent t statistics were used to examine specific differences within groups.

RESULTS

Eyes treated with TTX had significantly reduced anterior segment and lens development. This induced significant hyperopia in TTX treated open eyes and reduced the observed myopia in TTX treated MD eyes. Monocular deprivation of pattern vision in all experimental groups was associated with significant elongation of the vitreous chamber in the deprived eye (both axially and equatorially) when compared to the contralateral control eye. These results indicate that TTX blockade of retinal cell action potentials altered normal ocular growth but did not prevent form-deprivation induced elongation of the eye in chick.

Effect of retinal impulse blockade on normal eye growth

Although important structural and refractive changes occurred in MD chicks, it is the changes in ocular development in binocularly open animals which is of primary concern in this study.

Open eyes treated with TTX had significantly flatter corneal curves than their contralateral control eye ($P < 0.01$), whereas neither PBS treated or normal binocular chicks had significant interocular differences in corneal curvature (Table 1). TTX injected open eyes developed a significantly shallower anterior segment depth compared to the contralateral control eye ($P < 0.001$). No significant differences in anterior segment depth between eyes was found for PBS-open or untreated binocular chicks [Fig. 2(A)]. Significant interocular differences in lens thickness were also observed between binocularly open groups ($F = 13.9$, $P < 0.001$). This difference was due to TTX injected open eyes having thinner lenses than their contralateral control eyes ($P < 0.001$), a finding not observed in PBS-open ($P = 0.25$) or normal ($P = 0.69$) chicks [see Fig. 2(B)]. In contrast to the reduction in ocular component dimensions in the anterior eye, TTX treated open eyes had significantly longer vitreous chamber depths than their contralateral control eye (5.66 ± 0.14 mm vs 5.45 ± 0.15 mm, $P < 0.01$). No differences were observed in interocular vitreous chamber depth in PBS-open or normal binocular chicks [Fig. 2(C)]. The effect of reductions in anterior segment depth and lens thickness outweighed the increase in vitreous chamber depth in TTX injected open eyes, producing an eye with a smaller axial length compared to its contralateral control eye [-0.17 ± 0.03 mm, $P < 0.001$; see Fig. 2(D)].

TTX-injected open eyes were significantly more hyperopic than their contralateral control eyes ($+6.6 \pm 1.1$ D vs $+3.2 \pm 0.4$ D). PBS-open (0.1 ± 0.4 D) and untreated chicks (-0.1 ± 0.2 D) had no significant interocular difference in ocular refraction [see Fig. 2(E)].

TTX-injected open eyes also had increased equatorial diameters when compared to the uninjected contralateral eyes [$P < 0.002$; see Fig. 2(F), Table 1]. A significant increase in whole eye weight of TTX-injected open eyes was also observed when compared with non-injected contralateral eyes ($P < 0.01$).

Ocular dimensions and refraction in MD chicks

All three MD groups (TTX-MD, PBS-MD and sham-injected MD) showed a similar relative elongation of the vitreous chamber in the deprived eye of 0.96 ± 0.07, 0.89 ± 0.05 and 0.86 ± 0.10 mm respectively when compared to the contralateral control eye [$F = 0.37$, $P = 0.7$; see Fig. 2(C)].

In contrast to the similar changes found in the posterior segment due to monocular deprivation, significant differences were noted in the anterior segment dimensions of deprived eyes of TTX-MD chicks when compared to control MD groups. The TTX treated deprived eye of TTX-MD chicks had significantly flatter corneal curves ($P < 0.02$), shallower anterior segments ($P < 0.001$), thinner crystalline lenses ($P < 0.001$) and consequently developed significantly less myopia ($P < 0.01$) than deprived eyes of PBS-MD and sham-injected-MD chicks (see Table 1 and Fig. 2).

TABLE 1. Ocular refraction and axial dimensions of all treated (LE) and control eyes in binocularly open and monocularly deprived chicks

		TTX-open ($n = 10$)		PBS-open ($n = 12$)		Normal ($n = 11$)		TTX-MD ($n = 9$)		PBS-MD ($n = 10$)		Sham-injected MD ($n = 8$)	
		RE	LE	RE	LE	RE	LE	Open	Dep	Open	Dep	Open	Dep
Retinoscopy (D)	Mean	3.2	6.6	3.0	3.1	3.0	2.9	4.4	−3.2	3.5	−17.0	3.2	−15.4
	SEM	0.4	1.1	0.2	0.4	0.1	0.3	0.5	1.2	0.7	1.2	0.6	2.6
	T − C	3.4†		0.1		−0.1		−7.6†		20.5†		−18.5†	
	SEM	±1.1		±0.4		±0.2		±1.1		±1.1		±2.9	
Corneal radius (mm)	Mean	3.17	3.28	3.17	3.17	3.22	3.22	3.17	3.23	3.23	3.22	3.18	3.15
	SEM	0.04	0.04	0.02	0.03	0.02	0.03	0.04	0.04	0.03	0.03	0.02	0.04
	T − C	0.11†		0.00		0.00		0.06*		−0.01		−0.03	
	SEM	±0.03		±0.02		±0.01		±0.02		±0.01		±0.04	
Anterior segment (mm)	Mean	1.46	1.15	1.53	1.49	1.52	1.50	1.59	1.36	1.54	1.61	1.57	1.70
	SEM	0.04	0.04	0.00	0.02	0.01	0.01	0.03	0.03	0.02	0.04	0.04	0.08
	T − C	−0.31†		−0.04		−0.02		−0.23†		0.07		0.13†	
	SEM	±0.03		±0.01		±0.01		±0.02		±0.04		±0.06	
Lens thickness (mm)	Mean	2.30	2.23	2.20	2.22	2.19	2.19	2.38	2.26	2.25	2.25	2.33	2.34
	SEM	0.07	0.06	0.03	0.04	0.02	0.02	0.07	0.06	0.06	0.06	0.07	0.08
	T − C	−0.07†		0.02		−0.01		−0.11†		0.01		0.01	
	SEM	±0.01		±0.01		±0.01		±0.01		±0.02		±0.02	
Vitreous chamber (mm)	Mean	5.45	5.66	5.24	5.22	5.30	5.29	5.48	6.44	5.45	6.34	5.50	6.39
	SEM	0.15	0.14	0.08	0.09	0.04	0.04	0.11	0.16	0.11	0.15	0.14	0.17
	T − C	0.21†		−0.02		−0.01		0.96†		0.89†		0.86†	
	SEM	±0.05		±0.02		±0.02		±0.07		±0.05		±0.10	
Axial length (mm)	Mean	9.20	9.04	8.97	8.93	9.01	8.98	9.45	10.05	9.24	10.21	9.40	10.40
	SEM	0.24	0.23	0.12	0.12	0.05	0.05	0.19	0.24	0.17	0.19	0.23	0.27
	T − C	−0.17†		−0.04		−0.03		0.61†		0.97†		1.00†	
	SEM	±0.03		±0.02		±0.02		±0.07		±0.07		±0.14	
Equatorial dimensions (mm)	Mean	11.96	12.27	12.06	12.09	12.33	12.27	12.03	12.72	12.25	12.81	12.11	12.56
	SEM	0.10	0.10	0.08	0.08	0.07	0.08	0.15	0.21	0.10	0.14	0.09	0.15
	T − C	0.31†		0.03		−0.06		0.70†		0.56†		0.45†	
	SEM	±0.05		±0.06		±0.02		±0.10		±0.06		±0.08	
Eye weight (g)	Mean	0.61	0.66	0.63	0.63	0.67	0.67	0.62	0.74	0.65	0.80	0.64	0.76
	SEM	0.01	0.01	0.01	0.01	0.01	0.01	0.02	0.03	0.02	0.02	0.01	0.03
	T − C	0.05†		0.00		0.00		0.12†		0.15†		0.12†	
	SEM	±0.01		±0.02		±0.01		±0.03		±0.03		±0.02	

T − C, treated − control. *$P < 0.05$; †$P < 0.01$.

Changes in crystalline lens curvature

A schematic eye programme (O'Keefe & Coile, 1988) was used to evaluate the extent to which changes in ocular component dimensions in TTX treated eyes accounted for the refractive errors observed. Some discrepancy between observed and predicted refractive errors was found. In TTX-MD eyes the changes in corneal curvature, anterior segment depth and lens thickness reduced the myopic effect of vitreous chamber elongation and predicted a relative myopic refractive error in TTX-MD chicks of −12.0 D, instead of the observed error of -7.6 ± 1.2 D. In TTX treated open eyes there was found to be less hyperopia predicted than was observed, although the discrepancy was less. Thus, in deprived and open TTX treated eyes the schematic modelling predicted more myopia or less hyperopia respectively than was actually measured. To address the possibility that these differences may be accounted for by changes in crystalline lens curvature that may have accompanied changes in lens thickness (especially in light of the observed equatorial enlargement in TTX treated open eyes), *in vitro* measures of lens curvature were taken on three TTX treated chicks using a previously described technique (McBrien & Norton, 1992). Findings revealed that the TTX treated eyes not only had thinner lenses (0.14 ± 0.02 mm, $n = 3$) but also lenses with flatter anterior (3.96 ± 0.3 mm vs 3.50 ± 0.2 mm, $n = 3$) and posterior curvatures (2.5 ± 0.3 mm vs 2.1 ± 0.1 mm, $n = 3$) when compared to the contralateral control eye lenses. When the measured values for crystalline lens curvature for the contralateral control eyes and TTX treated eyes were input into the schematic model, on average a further 5.5 D of hyperopia was predicted. Thus when changes in crystalline lens curvature are also incorporated into the model the observed refractive changes closely match predicted values.

Time–course of structural changes in TTX treated animals

A group of age matched chicks ($n = 6$) were given intravitreal injections of TTX on the same alternate day schedule as the main study, but had ocular dimensions measured using A-scan ultrasonography prior to each injection in order to determine the sequence of structural

changes occurring in TTX treated open eyes. It was found that just prior to the second TTX injection (i.e. 48 hr after first injection) the reduction in lens thickness was already 71% (−0.05 mm) of that observed after the full treatment period of four injections (−0.07 mm), whereas the vitreous chamber elongation was 56% (+0.13 mm vs +0.24 mm) of that observed at the end of the treatment period. The reduction in anterior segment depth was only 10% of the final reduction (−0.03 mm vs −0.3 mm, $n = 6$). The findings indicate

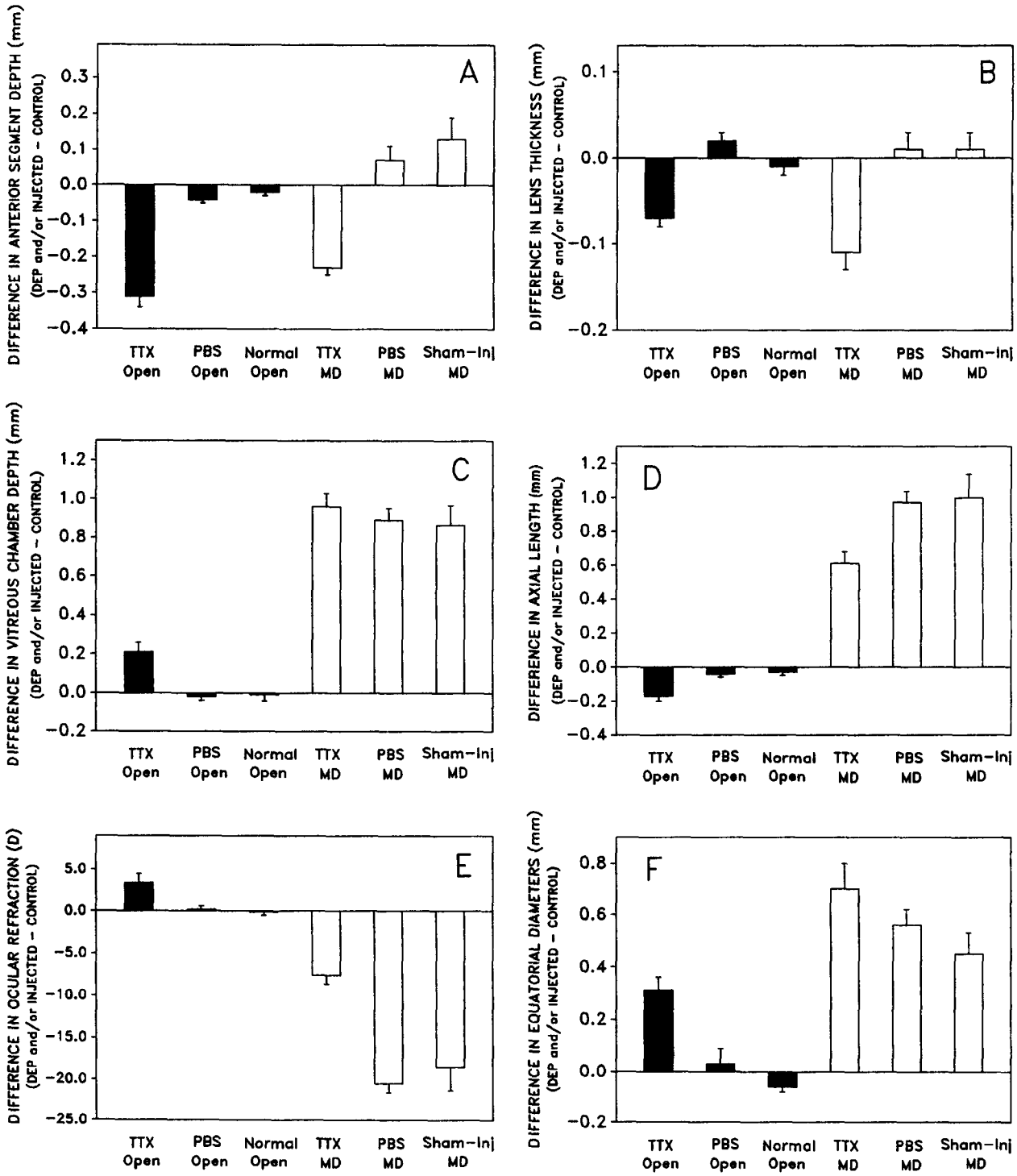

FIGURE 2. Differences in ocular dimensions (A-scan ultrasonography) and ocular refraction (cycloplegic retinoscopy) between treated and contralateral control eyes. (A) Differences in anterior segment depth (anterior chamber depth + corneal thickness). Eyes treated with TTX have significantly shallower anterior segment depths ($P < 0.01$). (B) Differences in crystalline lens thickness. Eyes treated with TTX had thinner lenses than their contralateral control eye ($P < 0.01$). (C) Differences in vitreous chamber depth. Eyes undergoing form-deprivation showed significant vitreous chamber elongation. Open eyes treated with TTX also underwent significant elongation of the vitreous chamber ($P < 0.01$). (D) Differences in axial length. (E) Differences in ocular refraction. Open eyes treated with TTX were relatively hyperopic and deprived eyes treated with TTX developed significantly less myopia than MD control chicks ($P < 0.001$). (F) Differences in equatorial diameter. Open eyes treated with TTX had significantly enlarged equatorial diameters than their contralateral control eyes ($P < 0.001$). Error bars = 1 SEM.

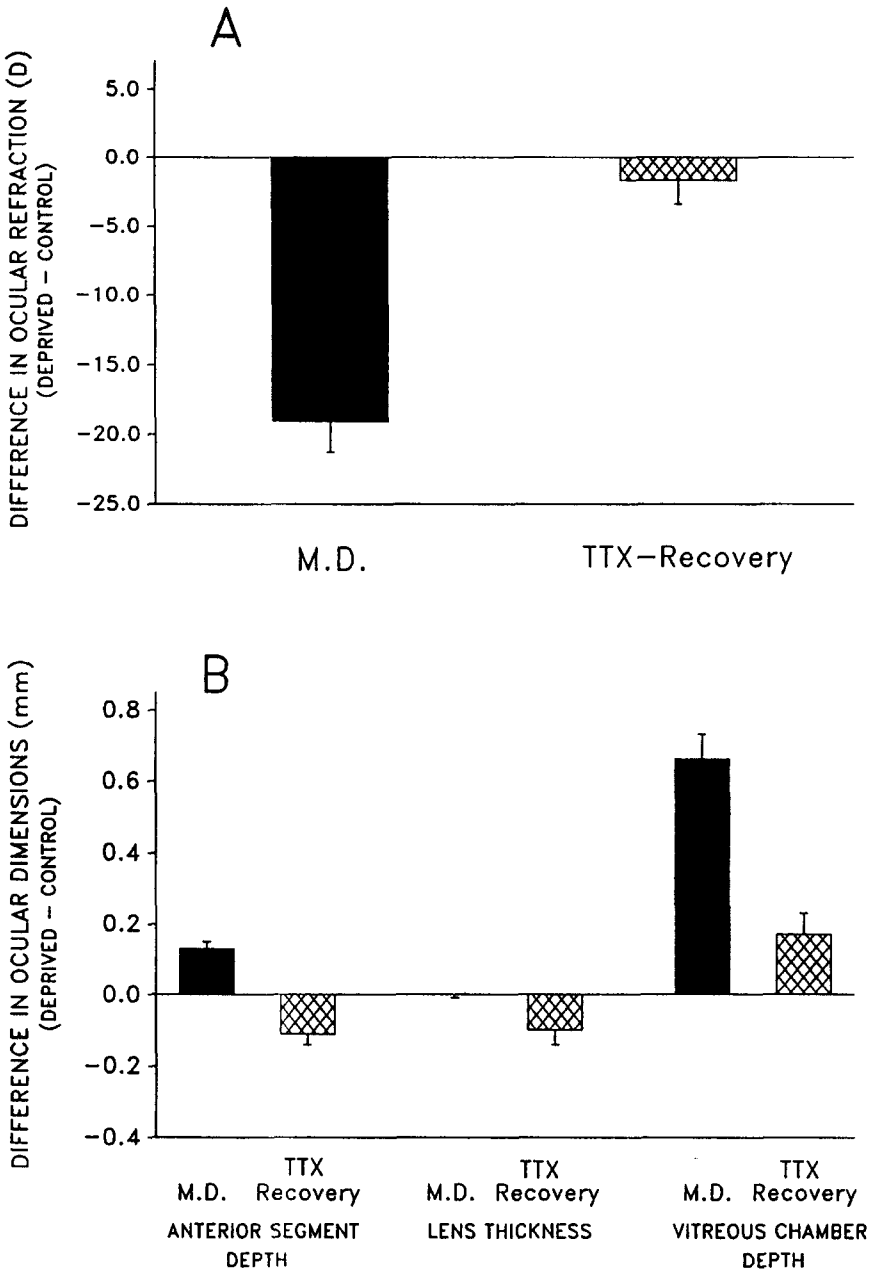

FIGURE 3. The effect of retinal impulse blockade on recovery from induced myopia and axial elongation in chicks. Chicks ($n = 8$) were deprived of form vision for 5 days and then the occluder was removed and ocular and refractive measures taken. Chicks were then intravitreally injected with TTX on an alternate day schedule over the 8-day recovery period. (A) Differences in ocular refraction (retinoscopy) immediately after removal of the occluder (MD—solid bars) and after a further 8 days during which time retinal impulses were blocked (recovery—hatched bars). There is almost complete recovery from induced myopia despite blockade of central communication. (B) Differences in ocular component dimensions (A-scan ultrasonography). Previously deprived eyes recovered from deeper anterior segments and developed shallower anterior segments and thinner lenses due to TTX treatment. There is a significant reduction in vitreous chamber differences ($P < 0.01$), but complete recovery does not occur presumably in order to offset the hyperopia induced by anterior segment changes and thus maintain emmetropia. Error bars = 1 SEM.

that lenticular changes precede the reduction in anterior segment depth, but it is not proven that the lenticular changes precede (and thus possibly initiate) the elongation of the vitreous chamber depth. Ultrasonography measures taken at 12 hr after the first injection demonstrated no consistent changes in ocular dimensions, arguing against the possibility that lenticular changes were due to the alteration of tonic accommodation produced by intraocular TTX, as this would occur within 1 or 2 hr after the injection.

Recovery from ocular component changes

Animals made myopic by MD were found to show recovery from vitreous chamber elongation (difference between treated eye and contralateral eye: $+0.66 \pm 0.07$ mm vs $+0.17 \pm 0.06$ mm, $n = 8$) and induced myopia (-19.1 ± 2.2 D vs -1.7 ± 1.7 D) even though retinal cell action potentials were blocked (Fig. 3). It should be noted that although emmetropization was nearly complete there was still a significant relative elongation of the vitreous chamber

($+0.17 \pm 0.06$ mm) in the previously myopic eye which offset the hyperopia induced by the shallower anterior segment depth and thinner and flatter lens (see Table 2 and Fig. 3).

It was also found that chick eyes treated with TTX for 8 days were able to recover from the induced hyperopia (TTX-open) and myopia (TTX-MD) indicating that no functional damage resulted from TTX treatment. In TTX-open animals the recovery from hyperopia was achieved predominantly by a relative deepening of the anterior segment depth and thickening of the lens in the previously treated eye (Table 2). In TTX-MD animals recovery was achieved by a slowing of vitreous chamber growth in the previously myopic eye, accompanied by a relative deepening of the anterior segment depth, thickening of the lens and choroidal thickening (Table 2).

Retinal histology

Further evidence indicating that at the dose used TTX did not cause any toxic damage to the retina is given by histological evaluation of retina (posterior pole) at the light microscope level. Findings revealed no observable toxic effects to the retina of TTX treated eyes when compared to control eyes at the dose used (Fig. 4).

Choroidal thickness changes

It was found that the choroid of eyes recovering from induced myopia were thicker (both posteriorly and equatorially) than the choroid in the contralateral control eye, irrespective of whether the eye had received TTX during the recovery period or not (see Fig. 5). Although the myopia induced from 5 days MD was similar in both standard recovery animals (-25 D, $n = 2$) and TTX treated recovery animals (-20 D, $n = 8$), it should be noted that the relative thickening of the choroid was less in recovering eyes treated with TTX (154 μm posteriorly; 206 μm equatorially) than eyes not treated with TTX (375 μm posteriorly; 357 μm equatorially). This could be due to either inter-animal variability or the fact that the TTX induced anterior segment changes, which reduced

TABLE 2. Ocular refraction and axial dimension of all deprived and open control eyes of monocularly deprived chicks and right and left eyes of binocularly open chicks (in all cases it was the left eye that received the intravitreal injection and/or deprivation)

		MD + recovery (TTX) ($n = 8$)				TTX-MD + recovery ($n = 8$)				TTX-open + recovery ($n = 8$)			
		Treatment period		Recovery period		Treatment period		Recovery period		Treatment period		Recovery period	
		RE	LE	RE	LE	RE	LE	RE	LE	RE	LE	RE	LE
Retinoscopy (D)	Mean	2.5	−16.6	1.8	0.1	2.2	−7.4	2.0	1.4	2.0	6.2	2.4	1.7
	SEM	0.2	2.1	0.2	1.7	0.3	1.7	0.1	0.4	0.1	1.4	0.4	0.3
	T − C	−19.1†		−1.7		−9.6†		−0.6		4.2*		−0.7	
	SEM	±2.2		±1.7		±1.6		±0.4		±1.4		±0.3	
Corneal radius (mm)	Mean	3.04	3.01	3.30	3.26	3.05	3.08	3.66	3.70	3.07	3.11	3.65	3.68
	SEM	0.04	0.04	0.05	0.06	0.02	0.02	0.06	0.06	0.04	0.03	0.05	0.04
	T − C	−0.03*		−0.04†		0.03		0.04†		0.03		0.03†	
	SEM	±0.01		±0.01		±0.03		±0.01		±0.04		±0.01	
Anterior segment (mm)	Mean	1.36	1.49	1.52	1.41	1.41	1.25	1.70	1.68	1.39	1.18	1.68	1.63
	SEM	0.01	0.02	0.02	0.03	0.02	0.03	0.03	0.02	0.01	0.02	0.02	0.03
	T − C	0.13†		−0.11†		−0.16†		−0.02		−0.21†		−0.05	
	SEM	±0.02		±0.03		±0.03		±0.01		±0.02		±0.02	
Lens thickness (mm)	Mean	2.14	2.14	2.40	2.30	2.26	2.14	2.61	2.57	2.24	2.12	2.61	2.58
	SEM	0.01	0.02	0.06	0.06	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.02
	T − C	0.00		−0.10†		−0.12†		−0.04*		−0.12†		−0.03	
	SEM	±0.01		±0.04		±0.02		±0.02		±0.02		±0.02	
Vitreous chamber (mm)	Mean	4.99	5.65	5.36	5.53	5.01	5.72	5.84	6.07	4.99	5.31	5.75	6.01
	SEM	0.07	0.10	0.09	0.12	0.07	0.11	0.14	0.14	0.07	0.12	0.12	0.07
	T − C	0.66†		0.17*		0.71†		0.23†		0.32†		0.26†	
	SEM	±0.07		±0.06		±0.08		±0.06		±0.07		±0.07	
Axial length (mm)	Mean	8.48	9.28	9.28	9.24	8.68	9.10	10.20	10.32	8.63	8.62	10.05	10.23
	SEM	0.08	0.12	0.10	0.12	0.09	0.13	0.17	0.15	0.09	0.14	0.13	0.14
	T − C	0.80†		−0.04		0.42†		0.12*		−0.01		0.18*	
	SEM	±0.08		±0.07		±0.08		±0.04		±0.07		±0.05	
Equatorial dimensions (mm)	Mean							13.85	13.89			13.77	13.88
	SEM							0.17	0.15			0.18	0.17
	T − C							0.04				0.11†	
	SEM							±0.06				±0.02	
Eye weight (g)	Mean							0.94	0.98			0.93	0.98
	SEM							0.03	0.03			0.11	0.23
	T − C							0.04				0.05	
	SEM							±0.08				±0.09	

T − C, treated − control. *$P < 0.05$; †$P < 0.01$.

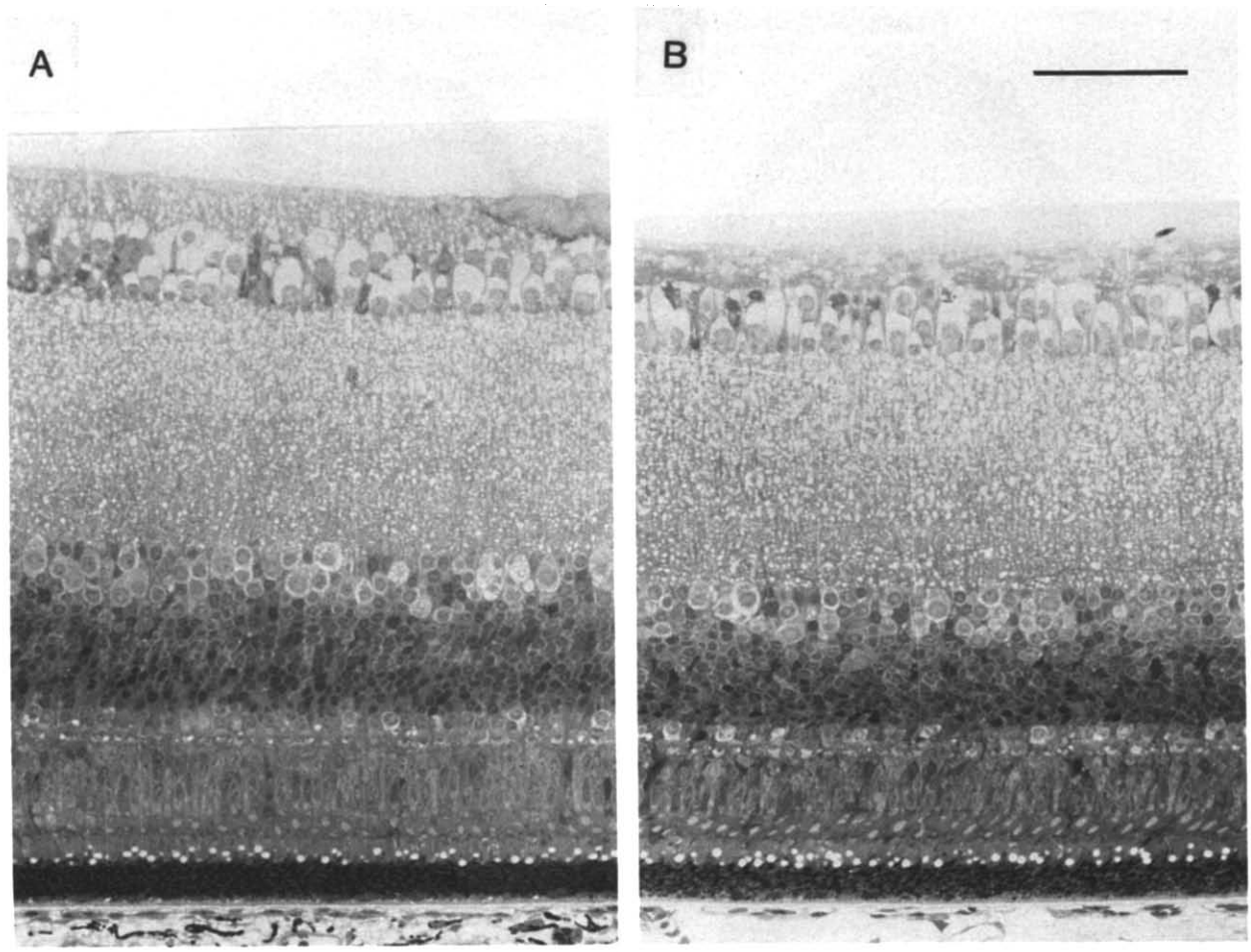

FIGURE 4. Histological appearance of the retina at the posterior pole from (A) a control untreated eye and (B) a TTX treated MD chick eye. No signs could be observed, at the light microscope level, of toxic damage to the retina of the TTX treated eye when compared to the untreated control eye. The thinner retina in the TTX-MD eye is due to the axial elongation and myopia in this eye. Stained with toluidine blue 1%. Scale bar = 50 μm.

the amount of myopia, may have also reduced the amount of choroidal thickening required. It was also found that a form-deprived eye treated with TTX during deprivation had a thinner choroid both posteriorly ($-92\,\mu$m) and equatorially [$-61\,\mu$m; see Fig. 5(C)].

DISCUSSION

The primary objectives of this study were to determine if blockade of action potentials from the retina interfered with either normal ocular growth and/or form deprivation induced vitreous chamber elongation in chick. The findings indicate that ocular development of the anterior segment of the eye is altered in all TTX treated chicks but form-deprivation induced vitreous chamber elongation still occurs despite blockade of retinal cell action potentials to higher visual processing pathways.

Effects of TTX on normal ocular growth

There were significant changes in anterior ocular dimensions in all TTX treated eyes. These changes resulted in TTX treated open eyes developing significant degrees of hyperopia when compared to their contralateral untreated eye. Although it has previously been reported that open chick eyes that undergo optic nerve section also develop significant degrees of hyperopia (Troilo *et al.*, 1987), the structural cause of the hyperopia in such cases was due to reduced vitreous chamber depth. The fact that blockade of ganglion cell output to central visual pathways by TTX does not cause reduced vitreous chamber depth, but optic nerve section does, supports the suggestion that this reduction in vitreous chamber depth is related to the ganglion cell degeneration associated with optic nerve section and not due to a lack of the brain's influence on ocular growth (Troilo, 1989, 1990). The finding that chick eyes treated with TTX showed recovery from refractive and structural changes indicates that prior treatment with TTX did not interfere with the normal recovery process. Of greater importance was the finding that the eye was able to emmetropize and recover from the induced myopia while communication with higher visual pathways was blocked by TTX. This supports previous reports (Schaeffel *et al.*, 1990; Troilo & Wallman, 1991) indicating that emmetropization can occur under local ocular control.

The consistent finding of reduced development of the anterior segment in TTX treated eyes is of considerable interest. As TTX blocks accommodation it could be argued that sustained blockade of accommodation results in reduced anterior segment and lens development. However, ablation of the Edinger–Westphal nucleus in chick eyes does not result in reduction of anterior

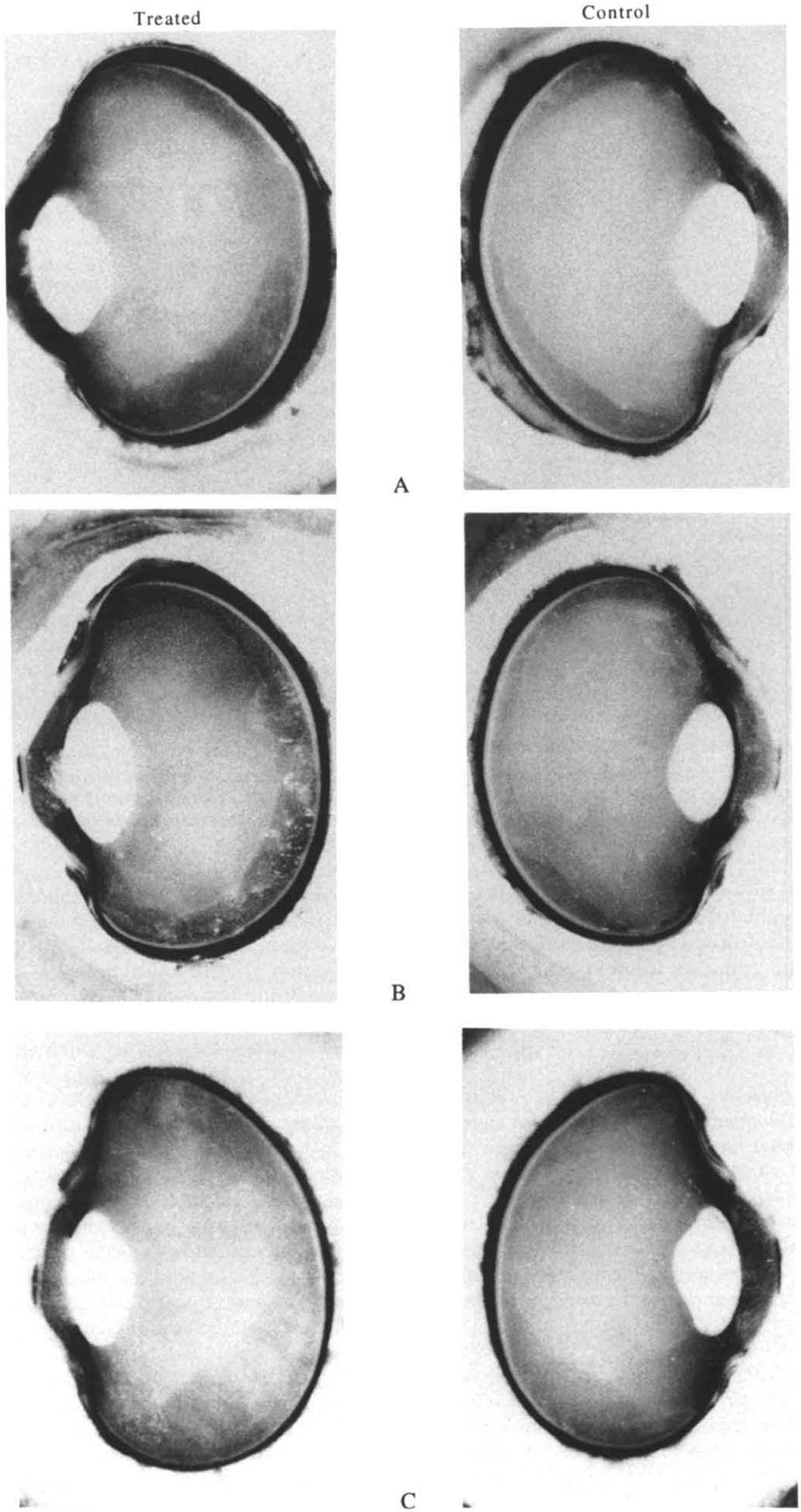

FIGURE 5. *Caption on facing page.*

segment or lens dimensions (Schaeffel *et al.*, 1990). Similarly it is difficult to reconcile the possibility that some other brain mediated function could be responsible for the observed anterior eye changes as again no such changes are reported in ONS treated chick eyes. Another possible mechanism could be that TTX blockade of retinal impulses alters the release or production of retinal derived growth factors involved in development of the anterior part of the eye. It has been known for some time that the retina contains factors (e.g. FGF, IGF and EDGF) which can promote or inhibit the growth and development of corneal and lens cells (e.g. Chamberlain & McAvoy, 1987; Richardson, Chamberlain & McAvoy, 1993). It is feasible that while TTX blockade of retinal impulses does not block the retinal signals involved in form-deprivation induced vitreous chamber elongation it may alter release of other retinal factors which are involved in the development of the anterior segment of the eye.

The structural changes in the anterior segment of the eye due to TTX treatment in chick differ from that found in tree shrew (Norton *et al.*, 1994). In TTX treated open tree shrew eyes there was only a small reduction in anterior segment depth (-0.03 ± 0.01 mm) and no change in corneal radius. However, as the procedure of intravitreal injection in tree shrew eyes produced a significantly shallower vitreous chamber depth (-0.07 ± 0.01 mm), the anterior segment changes may have been modified in an attempt to offset the potential hyperopia of the shallower vitreous chamber.

Effects of TTX on MD-induced ocular changes

Despite the blockade of action potentials from the eye to central visual pathways by injection of TTX, chick eyes deprived of form vision still underwent vitreous chamber elongation of a similar magnitude as PBS treated MD chicks. Thus the retinal signals that detect form-deprived images and induce vitreous chamber elongation are not appreciably altered by blockade of retinal cell action potentials which suggests that these signals proceed directly from the retina to the choroid and/or sclera by some, as yet, undetermined route. These results are in general agreement with previous studies that have investigated the role of communication between the eye and central visual pathways on the development of form-deprivation myopia (e.g. Troilo *et al.*, 1987; Raviola & Wiesel, 1985; Wildsoet & Pettigrew, 1988; Norton, *et al.*, 1994), and give further support to a role for local control of ocular growth and direct retino-scleral communication.

Local control of emmetropization?

An important finding in TTX treated open eyes was that not only was there a reduction in anterior segment depth, lens thickness and curvature, there was also a small but significant elongation (0.21 ± 0.05 mm) of the vitreous chamber when compared to the contralateral control eye. A possible explanation is that this vitreous chamber elongation occurred in an attempt by the eye to maintain emmetropia by offsetting the hyperopia induced by the reductions in anterior segment depth, lens thickness and curvature and corneal power. Further evidence is given by the finding that eyes made axially myopic by form deprivation are able to emmetropize despite blockade of ganglion cell action potentials. This recovery process in TTX treated eyes was found to be, in part at least, produced by choroidal thickening which resulted in pushing the retina forward. This emmetropizing mechanism has previously been observed in both chicks with functional central communication and in chicks with optic nerve section (Wallman *et al.*, 1992; Wildsoet & Wallman, 1992). It was also found that in chick eyes recovering from induced myopia while retinal impulses were blocked with TTX, that although at the end of the recovery period the previously myopic eyes had significantly shallower anterior segment depths and thinner lenses, the eyes emmetropized by maintaining a slightly longer vitreous chamber depth (Fig. 3). These findings give strong support for local ocular control of an active emmetropization mechanism.

In summary the findings show that blockade of retinal cell action potentials does interfere with normal ocular growth, in particular the anterior segment of the eye. The possibility that this is due to blocking the brain's influence on ocular growth is not supported by previous findings utilizing optic nerve section, in which no changes in anterior segment were noted. Evidence is presented for local ocular control of emmetropization, based on recovery from induced refractive errors in the absence of central communication, choroidal thickness

FIGURE 5 (*opposite*). Frozen section photographs of chicks eyes taken at the maximum crystalline lens thickness. (A) Treated and control eyes from a chick that had been monocularly deprived of form vision for 5 days and then the occluder was removed and the animal was returned to the brooder for a further 8 days. After optical and *in vivo* structural measures the eyes were enucleated, frozen and then sectioned on a cryostat. The treated eye has a markedly thicker choroid than its contralateral open control eye both posteriorly (560 μm vs 185 μm) and equatorially (640 μm vs 283 μm). (B) Treated and control eyes from a chick who had undergone MD for 5 days and then the occluder was removed and the previously deprived eye treated with TTX on an alternate day schedule for 8 days. Again the choroid in the treated (recovery) eye was considerably thicker than the contralateral open control eye in both the posterior (308 μm vs 154 μm) and equatorial (403 μm vs 197 μm) regions. It should be noted that the choroidal thickening in the TTX treated animal was less than the standard MD recovery chick even though both animals had similar degrees of induced myopia at the start of the recovery period ($\approx$25 D). This could be due to inter-animal variation or the fact that the structural changes to the anterior segment of the TTX treated eye reduced the need for as much choroidal thickening to aid emmetropization. (C) Treated and control eyes of a chick undergoing 8 days of MD during which time the deprived eye received TTX on an alternate day schedule. Thinning of the choroid in the myopic (-6 D) eye was observed both posteriorly (154 μm vs 246 μm) and equatorially (170 μm vs 231 μm) despite blockade of ganglion cell action potentials.

changes and is also supported by vitreous chamber elongation in TTX-open eyes to offset the refractive effect of anterior segment changes.

REFERENCES

Chamberlain, C. G. & McAvoy, J. W. (1987). Evidence that fibroblast growth factor promotes lens fibre differentiation. *Current Eye Research, 6*, 1165–1168.

Hodos, W. & Kuenzel, W. J. (1984). Retinal-image degradation produces ocular enlargement in chicks. *Investigative Ophthalmology & Visual Science, 25*, 652–659.

Hoyt, C. S., Stone, R. D., Fromer, C. & Billson, F. A. (1981). Monocular axial myopia associated with neonatal eyelid closure in human infants. *American Journal of Ophthalmology, 91*, 197–200.

Irving, E. L., Callender, M. G. & Sivak, J. G. (1991). Inducing myopia, hyperopia, and astigmatism in chicks. *Optometry and Vision Sciences, 68*, 364–368.

McBrien, N. A. & Norton, T. T. (1992). The development of experimental myopia and ocular component dimensions in monocularly lid-sutured tree shrews (*Tupaia belangeri*). *Vision Research, 32*, 843–852.

McBrien, N. A., Moghaddam, H. O. & Reeder, A. P. (1993). Atropine reduces experimental myopia and eye enlargement via a nonaccommodative mechanism. *Investigative Ophthalmology & Visual Science, 34*, 205–215.

McKanna, J. A. & Casagrande, V. A. (1981). Atropine affects lid-suture myopia development. *Documenta Ophthalmologica Proceedings Series, 28*, 187–192.

Muchnick, N. & Hibbard, E. (1980). Avian retinal ganglion cells resistant to degeneration after optic nerve section lesion. *Experimental Neurology, 68*, 205–216.

Norton, T. T., Essinger, J. A. & McBrien, N. A. (1994). Lid-suture myopia in tree shrews with retinal ganglion cell blockade. *Visual Neuroscience, 11*, 143–153.

O'Keefe, L. P. & Coile, D. C. (1988). A BASIC computer program for schematic and reduced eye construction. *Ophthalmic and Physiological Optics, 8*, 97–100.

Raviola, E. & Wiesel, T. N. (1985). An animal model of myopia. *New England Journal of Medicine, 312*, 1609–1615.

Richardson, N. A., Chamberlain, C. G. & McAvoy, J. W. (1993). IGF-1 enhancement of FGF-induced lens fiber differentiation in rats of different ages. *Investigative Ophthalmology & Visual Science, 34*, 3303–3312.

Schaeffel, F., Glasser, A. & Howland, H. C. (1988). Accommodation, refractive error and eye growth in chickens. *Vision Research, 28*, 639–657.

Schaeffel, F., Troilo, D., Wallman, J. & Howland, H. C. (1990). Developing eyes that lack accommodation grow to compensate for imposed defocus. *Visual Neuroscience, 4*, 177–183.

Troilo, D. (1989). The visual control of eye growth in chicks. Doctoral dissertation, City University of New York, N.Y.

Troilo, D. (1990). Experimental studies of emmetropization in the chick. In Bock, G. & Widows, K. (Eds), *Myopia and the control of eye growth, Ciba Foundation Symposium 155* (pp. 89–114). Chichester: Wiley.

Troilo, D. & Wallman, J. (1991). The regulation of eye growth and refractive state: An experimental study of emmetropization. *Vision Research, 31*, 1237–1250.

Troilo, D., Gottlieb, M. D. & Wallman, J. (1987). Visual deprivation causes myopia in chicks with optic nerve section. *Current Eye Research, 6*, 993–999.

Van Alphen, G. W. H. M. (1961). On emmetropia and ametropia. *Ophthalmoligica, Basel (Suppl.)*, 142.

Wallman, J. & Adams, J. I. (1987). Developmental aspects of experimental myopia in chicks: Susceptibility, recovery and relation to emmetropization. *Vision Research, 27*, 1139–1163.

Wallman, J., Adams, J. I. & Trachtman, J. (1981). The eyes of young chicks grow toward emmetropia. *Investigative Ophthalmology & Visual Science, 20*, 557–561.

Wallman, J., Gottlieb, M. D., Rajaram, V. & Fugate-Wentzek, L. A. (1987). Local retinal regions control local eye growth and myopia. *Science, 237*, 73–77.

Wallman, J., Xu, A., Wildsoet, C., Krebs, W., Gottlieb, M., Marran, L. & Nickla, D. (1992). Moving the retina: A third mechanism of focusing the eye. *Investigative Ophthalmology & Visual Science (Suppl.), 33*, 1053.

Wiesel, T. N. & Raviola, E. (1977). Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature, 266*, 66–68.

Wildsoet, C. F. & Pettigrew, J. D. (1988). Experimental myopia and anomalous eye growth patterns unaffected by optic nerve section in chickens: Evidence for local control of eye growth. *Clinical Vision Sciences, 3*, 99–107.

Wildsoet, C. F. & Wallman, J. (1992). Optic nerve section affects ocular compensation for spectacle lenses. *Investigative Ophthalmology & Visual Science (Suppl.), 33*, 1053.

Wong-Riley, M. T. T., Tripathi, S. C., Trusk, T. C. & Hoppe, D. A. (1989). Effect of retinal impulse blockage on cytochrome oxidase-rich zones in the macaque striate cortex: I. Quantitative electron-microscopic (EM) analysis of neurons. *Visual Neuroscience, 2*, 483–497.

Acknowledgements—The authors gratefully acknowledge the technical assistance of Patrick Lawlor. Thanks are due to Drs David Troilo, John Phillips and two anonymous reviewers for commenting on earlier versions of the manuscript. Supported in part by grants from the Biotechnology and Biological Sciences Research Council (BBSRC J33265) and The Wellcome Trust.