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The effects of spectacle wear in infancy on eye growth and refractive error in the marmoset (*Callithrix jacchus*)

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Abstract

We made a comprehensive study, involving observations on 45 marmosets, of the effects on ocular growth and refraction of wearing spectacles from the ages of 4–8 weeks. This period was within the period early in life when the eye grows rapidly and refraction changes from hyperopia to its adult value of modest myopia. In one series of experiments we studied the effect of lenses of powers −8, −4, +4 and +8D fitted monocularly. In another series of experiments we studied the effect of lenses of equal and opposite powers fitted binocularly, with the two eyes alternately occluded, so as to give an incentive to use both eyes, and in particular to accommodate, for at least part of each day, through the negative lens. The vitreous chamber of eyes that wore negative lenses of −4D or −8D, combined with alternate occlusion, elongated more rapidly than that of the fellow eye (negative lens eye–positive lens eye, 0.21 ± 0.03 mm (S.E.M.), $P<0.01$ and 0.25 ± 0.06 mm, $P<0.05$, respectively) and became relatively more myopic (2.8 ± 0.26D, $P<0.01$ and 2.4 ± 0.61D, $P<0.05$ respectively). Eyes that wore −4D lenses monocularly elongated more rapidly and became myopic than fellow eyes. Eyes that wore +4D or +8D lenses were less strongly affected: animals that wore +8D lenses monocularly (without alternate occlusion) developed a slight relative hyperopia (0.99 ± 0.21D, $P<0.01$), with the more hyperopic eyes also slightly shorter (0.09 ± 0.05 mm) than their fellow eyes, but eyes wearing +4D lenses were not significantly different from their fellow eyes. Animals that wore −8D lenses monocularly (without alternate occlusion) developed a slight relative hyperopia after three weeks of lens-wear (0.85 ± 0.26D, $P<0.05$). These were the only eyes that responded in a non-compensatory direction to the optical challenge of spectacle wear, and we interpret this effect as one due to visual deprivation. After the removal of lenses, the degree of anisometropia slowly diminished in those groups of animals in which it had been induced, but in the three groups in which the largest effects had been produced by lens-wear the overall mean anisometropia (0.68 ± 0.24D, $P<0.01$) and vitreous chamber depth (VCD) discrepancy (0.09 ± 0.03 mm, $P<0.01$) were still significant at the end of the experiments, when the animals were 273 days old. The reduction of anisometropia in these groups was associated with an increase in the rate of elongation of the vitreous chamber in the eyes that had previously grown normally i.e. the less myopic eyes grew more rapidly than their fellow eyes: in the seven weeks following lens-wear these eyes became more myopic and longer than normal eyes (refraction $P<0.001$; VCD $P<0.001$). Control experiments showed that occlusion of one eye for 50% of the day had no effect on eye growth and refraction, and therefore that alternate occlusion itself had no effect.

Keywords: Eye growth; Myopia; Primate; Refractive development; Spectacles

1. Introduction

Regulation of postnatal eye growth and refractive development is of considerable interest in its own right as a biological problem, and also for its potential for throwing light on the causes of myopia, a sub-category of which is associated with pathological changes to the eye and a risk of blindness.

The eye is generally not emmetropic at birth, but hyperopic, with the degree of hyperopia varying substantially between individuals. These neonatal refractive errors are usually reduced in the first few weeks or months after birth. This trend towards emmetropia has been described in humans (Mohindra & Held, 1981; Ehrlich, Atkinson, Braddick, Bobier & Durden, 1995;

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Saunders, Woodhouse & Westall, 1995); chicks (Wallman, Adams & Trachtman, 1981); tree shrews (Norton & McBrien, 1992); macaques (Raviola & Wiesel, 1990), and in marmosets (Troilo & Judge, 1993); see Troilo (1992) for a review. The mechanism of this emmetropisation is unknown.

1.1. Deprivation myopia

If the retinal image is degraded early in life (by congenital cataract, lid suture, or the fitting of translucent goggles) the deprived eye elongates relative to its fellow and becomes myopic (Wallman, 1993). It has been shown that deprivation myopia can arise through a local effect in the eye, in that it still occurs when the optic nerve is cut (Raviola & Wiesel, 1985–rhesus monkey; Troilo, Gottlieb & Wallman, 1987, Wildsoet & Pettigrew, 1988–chick) or retinal ganglion cells are silenced by intra-ocular tetrodotoxin injection (Norton, Essinger & McBrien, 1994–tree shrew; McBrien, Moghaddam, Cottriall, Leech & Cornell, 1995, Wildsoet & Wallman 1995–chick).

Furthermore, if occluders that cover only part of the visual field are worn, myopia develops only in the corresponding part of the retina (Wallman, Gottlieb, Rajaram & Fugate-Wentzek, 1987; Hodos & Kuenzel, 1984–chick; Norton & Siegwart, 1991–tree shrew).

If deprivation myopia is caused by removal of a visual cue essential for emmetropisation, then one might hope to see evidence that the eye can recover from the effects of brief periods of deprivation when visual feedback is restored. There is evidence for this both in chicks (Wallman & Adams, 1987; Schaeffel & Howland, 1991; Troilo & Wallman, 1991) and tree shrews (Norton, 1990). Recovery from deprivation myopia seems not to have been observed in macaques or marmosets (Troilo & Judge, 1993).

1.2. Restricted environments, and lens-rearing

Direct ways to test whether ocular growth is regulated by optical demand are either to rear animals in restricted environments, or wearing spectacles. It has long been known that macaques raised in restricted environments become myopic (Young, 1961). More recently, in an ingenious experiment, Miles & Wallman (1990) showed that chicks raised in enclosures with low ceilings became myopic in their upper visual field.

Spectacle-rearing experiments in chicks have shown that growth of the eye can be altered to compensate for the presence of either positive or negative lenses aligned with the optic axis (Schaeffel, Glasser & Howland, 1988; Schaeffel & Howland, 1991; Wildsoet & Wallman, 1995; Irving, Callender & Sivak, 1991, 1992).

Chick eyes compensate fully to a wide range of lens powers, even when accommodation is prevented (Schaeffel, Troilo, Wallman & Howland, 1990), though the ability to respond to negative lenses is greatly impaired if the optic nerve is cut (Wildsoet & Wallman, 1995). If lenses are worn intermittently, positive lenses still induce some hyperopia, but negative lenses fail to induce myopia if there is a small period (e.g. 3 h/day) of normal vision (Schmid & Wildsoet, 1996). Another recent finding is that there is a degree of yoking between the two eyes of the chick: the growth of the eye contralateral to one wearing a spectacle lens is affected, though the responses are an order of magnitude smaller than those in the eye wearing the spectacle (Wildsoet & Wallman, 1995).

There are preliminary reports that the eyes of guinea pigs (McFadden & Wallman, 1995) also respond to altered optical demand, and that tree shrew ocular growth is altered by spectacles (Siegwart & Norton, 1993; McBrien, Cottriall & Crisp, 1996). Moreover, similar responses to those of chicks to intermittent wearing of negative lenses have been briefly reported in the tree shrew (Shaikh, Siegwart & Norton, 1997).

In pioneering studies on macaques, Hung, Crawford & Smith (1995) found that low power (+3 or −3D) monocular spectacle lenses fitted to infant animals generally induced compensatory changes in refraction, whereas higher power lenses produced small and inconsistent effects. Puzzling results with earlier experiments of this kind (e.g. Crewther, Nathan, Kiely, Brennan & Crewther, 1988; Chung, 1993; Smith, Hung & Harwerth, 1994) were attributed to the use of contact lenses, which seem to have a tendency to induce hyperopia regardless of the sign of their power (Hung & Smith, 1996), or to the use of lenses of too large a power. Another recent finding is that macaque eyes that have been made amblyopic have a strong tendency to be hyperopic (Kiorpes & Wallman, 1995). While it is known that a high degree of hyperopia early in life is a risk factor for the development of squint and amblyopia (Ingram, Walker, Wilson, Arnold & Dally, 1986; Atkinson, 1993; Atkinson, Braddick, Robier, Anker, Ehrlich, King, Watson & Moore, 1996), the opposite linkage has not previously been clearly demonstrated, though there is suggestive clinical evidence (e.g. Lepard 1975; Ingram, Gill & Goldacre, 1994)

1.3. The aims of our experiments

It is extremely important to know whether the results obtained by Hung, Crawford & Smith (1995) in macaques are representative of primates in general. The cost of working with macaques is such that experiments cannot be carried out on a large number of animals: Hung et al. raised eight animals with spectacle lenses. Marmosets are attractive as an alternative because they are small, grow rapidly, and breed readily in captivity, generally producing twins or triplets. Our first aim was therefore to determine whether marmoset eye growth early in life is affected by lens wear and, in particular, whether eye growth compensates to some degree for lenses of opposite power—i.e. whether negative lenses accelerate growth and positive lenses decelerate growth. Our second aim was to examine whether the use an animal makes of an eye affects its growth. We did this by using an alternate occlusion paradigm in which each eye was occluded for 50% of the daylight period so that the animal had some incentive to use each eye. We aimed to make as systematic and comprehensive an investigation as possible, using a range of lens powers and raising a number of animals (generally five) in each paradigm so that we could make intra- and inter-group statistical comparisons. As a rule we measured every animal at the same predetermined age. At each age we measured refraction in both horizontal and vertical meridia, corneal curvature, anterior chamber depth, lens thickness and vitreous chamber depth. Each animal was followed for nine months, by which time eye growth was very slow.

Preliminary reports of some of these findings have been presented (Judge & Graham, 1995; Graham & Judge, 1996).

2. Methods

Experiments were carried out in accordance with the requirements of the UK Animals (Scientific Procedures) Act 1986, on animals bred for the purpose, which lived in extended family groups. General conditions under which the marmosets were housed are described in Graham and Judge (1999).

2.1. *Choice of period of lens-wear*

We wished to study the effects of lens-wear in the period of emmetropisation when the eye grows most rapidly, in the hope that large effects of altered optical demand might be seen. Also, we wished to remove lenses well before the end of the period of eye growth so that there would be an opportunity for compensation of any induced effects. It would clearly be inappropriate to use a period of life when animals were not making use of vision. In the third and fourth weeks of life young marmosets still spend almost all of their time being carried around by older family members but they look around at the cage and its contents. After 4 weeks of age the young begin to make occasional forays around the cage on their own (it would perhaps be fairer to say that the older animals force this upon the youngsters): the young explore their cages and take an interest in what goes on outside the cage as well, looking at animals in other cages and at the activities of humans within the room. By 8 weeks of age the youngsters are weaned and left to themselves, spending much of their time playing together and observing animals in their own and other cages.

2.2. *Design of experiments and data analysis*

Animals wore lenses from 4 to 8 weeks of age and the lenses worn by each group are shown in Table 1. With the exception of the group of nine normal animals, described in detail in Graham and Judge (1999), there were five animals in each group.

Because the growth of the two eyes of each animal is normally very well-matched, whereas there is considerable inter-individual variation between animals, a within-animals design has greater statistical power: smaller differences between growth of the two eyes of the same animals are statistically significant than differences between eyes of different animals. We organised the experiments so that as a rule different optical demands were presented to the two eyes of each animal and therefore the within-animal comparison was always of interest.

In one series of experiments animals wore lenses of a range of powers (− 8, − 4, + 4, + 8D) over one eye and no lens over the other (groups M − 8, M − 4G, M − 4, M + 4G and M + 8 in Table 1).

As far as possible we used twins, and assigned one animal to an experiment with a negative lens of a given power and the other to an experiment with a positive lens of the same absolute power. There were two groups of − 4D animals to control for the possibility that two different methods of attaching the lenses might produce different results (see below). Most of these monocular lens-wear experiments were carried out before the binocular lens-wear experiments described below.

In a second series of experiments, binocular lenses of equal and opposite power were worn over the two eyes. We prevented the animals from focusing all of the time

Table 1
Lens-wearing of marmoset groups, and composition of the two pseudonormal groups emerging out of analysis

Group	Lens power (D)		N	N in pseudo-normal	
	Right	Left		Group A	Group B
M − 8	− 8	None	5	5	5
M − 4	− 4	None	5	0	0
M − 4G	− 4	None	5	5	0
M + 4G	None	+ 4	5	0	5
M + 8	None	+ 8	5	5	5
AO ± 8	− 8	+ 8	5	5	0
AO ± 4	− 4	+ 4	5	0	0
AO4 ± 0	Plano	+ 4	5	5	5
OCC	None	OCC	5	5	0
Normal	None	None	9	9	9

Lenses were worn from 4 to 8 weeks of age. G: taped goggles; all other animals except normals wore spectacles attached to skull pedestals; M: monocular lens-wearers; AO: binocular wear combined with alternate occlusion.

with one eye, and encouraged the animal to make equal use of the two eyes, by alternately occluding each eye with translucent tape. Initially 8D lenses of opposite sign were used in front of the two eyes (AO ± 8 in Table 1), on the assumption that the anisometropia introduced (16D) was large enough that differences between the two eyes would also be large. Following these experiments, animals wore 4D lenses of opposite sign (AO ± 4 in Table 1), to compare the effects of introducing 16D or 8D of optical anisometropia.

A third group of animals wore + 4D lenses in front of one eye combined with plano lenses in front of the contralateral eye and alternate occlusion, in order to investigate whether alternate occlusion might reveal a small effect of + 4D lenses (AO4 + 0 in Table 1).

Control experiments using occlusion of one eye consistently for different fractions of the day in five different individual animals were carried out to examine whether alternate occlusion itself might have affected eye growth (OCC in Table 1).

Finally, we compared the growth of the eyes of the lens-wearing animals with that of the normal animals described in the accompanying paper (Graham & Judge, 1999) (Normal in Table 1.)

Following the usual principles of biological experimentation, we concentrated our attention on the mean effects in groups of animals wearing the same lenses. We used SPSS for Windows to carry out Analysis of Variance (ANOVA). Because the number, *n*, of animals in each group was necessarily small (usually five) it was not normally possible to subdivide groups for more detailed analysis. However, it was possible to look at variation between individuals in another way. We estimated the 95% confidence intervals associated with measurement of vitreous chamber depth (VCD) or refraction. In the case of VCD, as described in Graham and Judge (1999), we made a very large number of measurements in the same animals over the course of a few days, and so were able to determine directly the standard deviation of measurement error as less than 0.04 mm. Assuming measurements in the two eyes are independent random variables, the standard deviation of the measurement error on the difference between VCD in the two eyes is therefore $\sqrt{2}*0.04 = 0.06$ mm, and the 95% confidence intervals on measurement about ± 0.1 mm. This allows us to say that differences between VCD in the two eyes of more than 0.1 mm in an individual animal are significant at the 5% level. The 95% confidence intervals on measurement of anisometropia were estimated by examining the distribution of the differences between the two measurements of the refraction of the right eye of consecutively measured animals in a randomly selected epoch in the middle of the experiments. These measurements were made on 25 animals, measured at a wide variety of different ages. This gives an estimate of ± 0.75D for the 95% confidence interval on refraction measurement. By using these estimates we were able to identify one condition (wearing − 4D lenses monocularly) in which the analysis based on mean effects may well have been misleading. This issue will be considered in Section 4.

2.3. Alternate occlusion

Alternate occlusion was accomplished as follows. Half way through the daylight period a piece of translucent tape (3M Magic Tape 810) was removed from the lens in front of one eye, and another piece of translucent tape was applied to the opposite lens. This tape eliminated all but the lowest spatial frequencies: Snellen acuity of a human subject with 6/6 vision fell to 0.06/60 or so when Magic Tape 810 was applied to the inside of his spectacles. By alternating the occlusion at the midpoint of each day, each eye viewed through an unoccluded lens for equal proportions of the day, and for equal times through an unoccluded lens in the morning and in the afternoon over the 4 week period of lens-wear—thus building into the experimental design some protection against the possibility of circadian modulation in susceptibility to lens-wear.

At the end of the 4 week period of lens-wear, the lenses were taken off and not replaced.

2.4. Measurements

As described in Graham and Judge (1999), animals were cyclopleged with cyclopentolate, and anaesthetised for optometric measurements with a combination of 0.9% (w/v) alphaxolone and 0.3% (w/v) alphadolone acetate (Saffan, Pittman Moore, UK, 0.09 ml/100 g i.m.), with further doses of 0.05 ml/100 g i.m. often being necessary after 40 and 60 min. Measurements were taken on the day of onset of lens-wearing; after 2, 3 and 4 weeks of lens-wear (i.e. at ages of 28, 42, 49 and 56 days); 2 weeks after lens removal (i.e. at 10 weeks of age), and at 15, 24 and 39 weeks (105, 168 and 273 days) of age. These points of follow-up measurement were chosen to lie at approximate equal intervals on a logarithmic scale, because it has previously been shown that VCD increases logarithmically with age in the marmoset over this period of life (Troilo & Judge, 1993). With one exception (Fig. 9) data are presented without correction for artifact of retinoscopy (Glickstein & Millodot, 1970), but with correction for the working distance of the retinoscopist. Refraction was measured twice in both the horizontal and vertical meridia, and the mean spherical refraction calculated. Four axial ultrasonograms of each eye were taken, and the means calculated for each component of each eye (Graham & Judge, 1999).

Goggles were sewn to elastic adhesive bandage (Elastoplast; Smith and Nephew) which was shaped so as to fit to the forehead, nose, temple and cheek of the animal. Gentle pressure was applied to stick the tape to the shaven head. This enabled consistent positioning of the lens in front of the eye. If the tape lifted from any area around the eye it was glued in place using veterinary tissue adhesive (Vetbond; 3M). Animals generally did not interfere with the tape or the goggles. On a few occasions the goggles were initially positioned too close to the lateral canthus and the eyelashes of the animals rubbed on the back of the goggle. If this occurred the animals would pull at the goggle and tape, in which case the goggle was removed and re-positioned.

2.5. Implant surgery

On the day prior to surgery the head of the animal was shaved. Immediately prior to fitting the implant, prophylactic antibiotic (Tribrissen, 3 mg/100 g sub-cutaneous) and atropine sulphate (0.005 mg/100 g sub-cutaneous) were administered and anaesthesia was induced with Saffan (0.09 ml/100 g i.m., with further doses of 0.05 ml/ 100 g i.m. often being necessary after 40 and 60 min to maintain surgical anaesthesia). The head of the animal was cleaned with an antiseptic wash (Betadine; Napp Laboratories, UK). The skin was dabbed dry and the animal was intubated (Jackson no. 3 cat catheter, external diameter 1 mm; Rocket, UK) and draped in a prone position on a piece of Vetbed cushioning (Pets Life, UK). A heating pad (Safe and Warm; Creative Concepts, UK) was placed under the Vetbed to keep the animal warm. The breathing rate of the animal was monitored at all times during surgery. A respiratory stimulant (Doxapram hydrochloride; Dopram-V, Willows Francis Veterinary, UK) was available that was administered sub-lingually on the rare occasions it was necessary.

An arc-shaped incision was made across the top of the head, running posteriorly from points slightly anterior to the ears. This created a flap of skin which was then reflected forward over the forehead. This was kept moist with a gauze soaked in sterile saline throughout the remainder of the surgery. The periosteum was removed by light scraping with a scalpel. Because the frontal skull sutures were better closed than the coronal or sagittal sutures, the implant was placed across the frontal sutures. Using a small hand-held drill two holes were made in each of the frontal skull plates. The holes were approximately 5 mm lateral to the frontal suture, with the first pair approximately 6 mm anterior to bregma and the second pair placed a further 5 mm anteriorly. Miniature stainless steel screws with 1.5 mm diameter shafts were inserted into the holes. The bone was then swabbed dry and acrylic cement (Kem-dent; Associated Dental, UK) applied in thin layers to form a smooth mound just burying the screws, a small amount of vaseline having been applied to the slot of each screw to facilitate removal of the implant later in life. The head of a 2 mm diameter stainless steel bolt was embedded in the acrylic with the bolt held in a vertical position while the acrylic dried. The reflected flap of skin was then pulled back over the top of the bolt and an incision made for the bolt to pass through. This incision was large enough for a stainless steel washer to be fitted over the threads of the bolt without crushing the skin at the edge of the hole. Topical antibiotic ointment (Chloromycetin; Parke-Davis, UK) was applied under the skin which was then re-aligned and sutured using absorbable suture (5/0, 1.0 metric, coated Vicryl; Ethicon, UK). Running sub-cuticular sutures were generally used, plus a small number of single-interrupted check (lock) sutures. The animal was placed on Vetbed, in an incubator with the air temperature set to 30°C, until it had fully recovered from the effects of the anaesthetic. The animal was then returned to the home cage where it was observed for approximately 30 min to make sure that it showed no signs of distress and that it was accepted by its parents and siblings.

2.6. Fitting of lenses

Lenses were always fitted with the animal fully awake as this gave the best guide for centration of the lenses in front of the eyes. Two grooved washers, separated by a standard stainless steel washer, were placed over the head bolt and held in place with a 2 mm stainless steel nut. This allowed one to clamp the two stainless steel wires which acted as ‘sidearms’ for the spectacles. The wires were bent into shape with the ends of the wires running around each side of the central post. The bend on the wires was adjusted by trial and error until the rings were positioned in front of the eyes, but not touching the skin at any point around the eyes. Acrylic cement was applied to the lateral and lowermost edges of the rings to form a baffle so that the animals could not see out round the spectacles. The nose and eyebrows made it impossible for animals to see out elsewhere.

2.7. Lens-wearing

Animals were checked 4 or 5 times a day when wearing lenses. The fronts of lenses were cleaned early each morning and at the mid-point of the light cycle every day. As necessary, the goggles, monocles or spectacles were removed in order that the backs of the lenses could be cleaned. No fogging (by condensation) of lenses was seen, but the lenses did collect particles of food and litter materials, thus necessitating the regular cleaning regime.

When fitted, lenses were approximately 5 mm in front of the cornea if the animal had an implant, or 6 mm in front of the cornea if the animal wore a goggle. This resulted in small differences between the nominal and effective power of the lenses (approximately ±0.10D for 4D lenses and ±0.30D for 8D lenses).

2.8. Removal of implants

Implant viability was checked daily. Approximately 3 weeks after the end of spectacle-wearing animals were anaesthetised with Saffan and implants were removed. Preparation for this surgery was the same as that for fitting the pedestals. After inducing anaesthesia, intubation (Jackson no. 4, external diameter 1.3 mm) and draping, midline incisions were made both anterior and posterior to the head bolt. The skin was freed-up from the surface of the implant and retracted in order to allow good visibility of the entire implant. An aluminium handle about 15 cm long was attached to the central head post of the implant and used to hold the implant so that it could be removed with very little torque being applied to the skull. Acrylic was carefully removed from around the screw heads using a small hand-held drill fitted with an annular cutter. Any remaining acrylic on the screw heads was carefully removed with rongeurs and the four screws were unscrewed from the skull. The implant was then lifted free of the skull. The edges of the wound adjacent to the bolt position were trimmed or scraped to provide a fresh wound edge, chloromycetin was applied under the skin, and the skin was sutured using Vicryl suture, as for the fitting of the implants.

3. Results

Prior to the onset of lens-wear there were no significant differences between the two eyes in either the mean refraction or mean ocular dimensions in each group of animals.

3.1. Overview of effects

Fig. 1 gives an overview of the results of the two main series of experiments, with the results of the

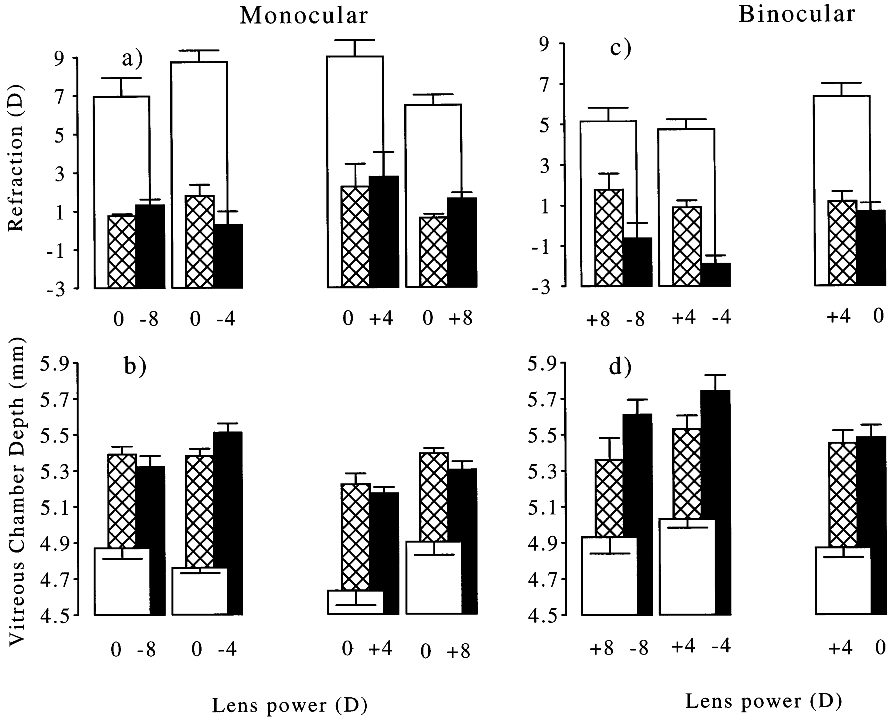

Fig. 1. This figure gives an overview of the results of the two main series of experiments. On the left are the results of the monocular lens-wear experiments (a and b), and on the right the results of the binocular lens-wear experiments with alternate occlusion (c and d). Each column shows data for one group of animals: refraction in the upper graphs (a and c) and VCD in the lower graphs (b and d). The power of lens worn over each eye is given below the histogram bars. The white bars show values at the onset of lens-wear (not significantly different between the two eyes of animals within each group). The cross-hatched and black bars show values at the end of 4 weeks of lens-wear, for each eye of the same animals. For the monocular experiments there were no lenses over the zero power eye, in the binocular experiments a plano lens was worn over the zero power eye. The most obvious result is that in binocular viewing with alternate occlusion the eyes wearing negative lenses grow more and become more myopic/less hyperopic than eyes wearing positive lenses (c and d). The effects in monocular viewing are less clear-cut, partly because of somewhat greater variation between groups in initial value of refraction and VCD. Error bars are ±1 S.E.M. $n = 5$ except in the 0, −4D monocular group (second column from left) where $n = 10$. Refraction data are corrected for working distance but not for artifact of retinoscopy. The base level of the bars (−3D or 4.5 mm) is arbitrary.

monocular lens-wear experiments on the left and of the binocular lens-wear experiments with alternate occlusion on the right. Each column shows results from a group of animals wearing lenses of the powers indicated below the bars. The white bars show initial values at the onset of lens-wear (not significantly different between the two eyes, within each group). The cross-hatched and black bars show values at the end of 4 weeks of lens-wear, for the same animals. The base levels of the bars (− 3D and 4.5 mm) are arbitrarily chosen. The most obvious result is that in binocular viewing with alternate occlusion the eyes wearing negative lenses grew more and became more myopic/less hyperopic than eyes wearing positive lenses (c and d). The effects in monocular viewing are less clear-cut, partly because of somewhat greater variation between groups in initial value of refraction and VCD.

Fig. 2 examines the same data in another way. For each group, we plot the mean difference in VCD between one eye and its fellow on the x-axis, against the difference in refraction between one eye and its fellow on the y-axis. This removes the substantial inter-animal variation in initial values. By plotting both variables on the same graph we are also able to show the co-variation, across all conditions, of the effect on the two variables.

The y-axis of Fig. 2 is the degree of anisometropia that developed by the age of 8 weeks (when lenses were removed) and the x-axis is the difference between the VCD of the two eyes at 8 weeks of age. Symbols show the mean and standard error of the mean for each group of animals. Across all groups, there is a covariation between anisometropia and difference in VCD ($R^2 = 0\ 79$, slope $= 9.25\mathrm{D\ mm}^{-1}$, $P < 0.0001$).

Fig. 2 shows that the largest effects are produced by binocular lens-wear combined with alternate occlusion, intermediate effects by the monocular wear of − 4D lenses, smaller effects with + 8D monocular lenses, and still smaller effects with other lenses. Effects are in a compensatory direction except for those with − 8D lenses.

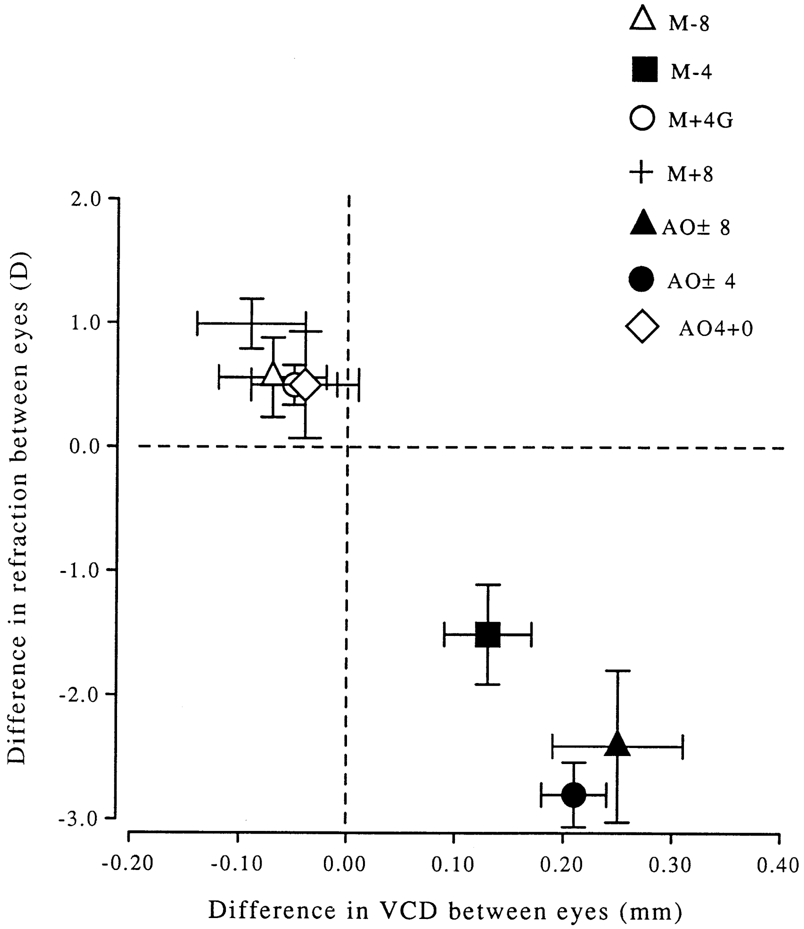

Fig. 2. Overview of results. Difference in VCD (x-axis) versus difference in refraction (y-axis) between eyes after 4 weeks of lens-wear, in groups of animals wearing lenses. Means ± 1 S.E.M. The largest differences (filled symbols) arise because most eyes wearing negative lenses elongate and become relatively myopic compared with their fellow eyes: note that the − 8D group (hollow triangles) are an exception. Five animals in each group.

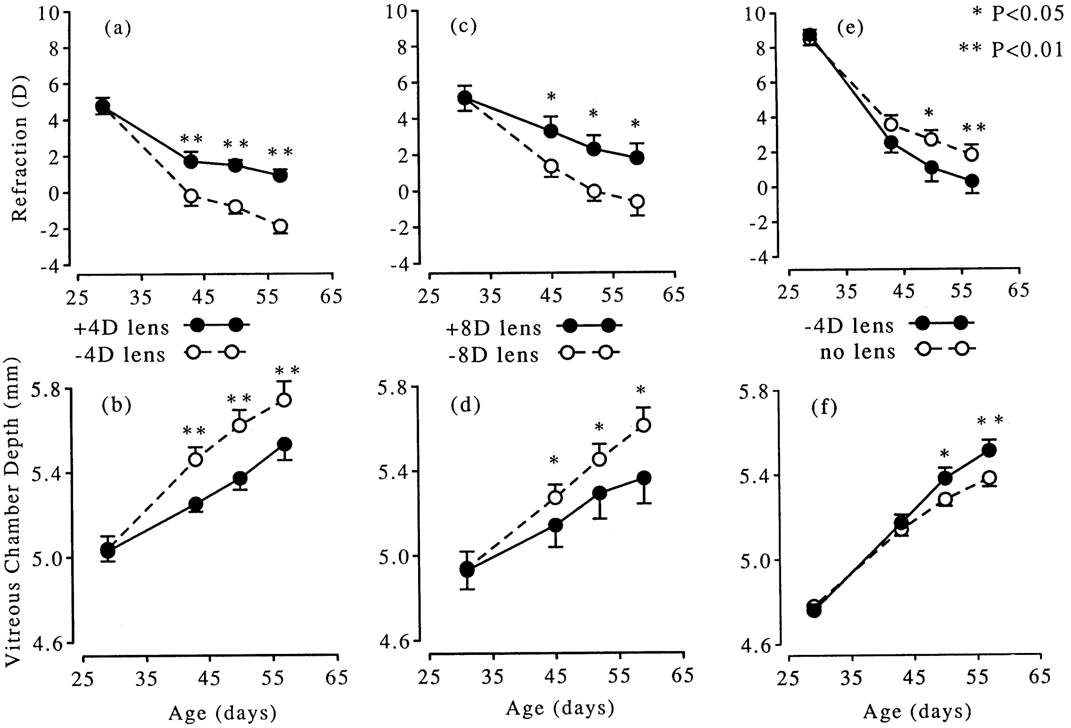

Fig. 3. In alternately occluded animals, eyes viewing through −4D lenses (hollow symbols, dashed lines) became relatively myopic (a) and their vitreous chambers became longer (b) than fellow eyes viewing through +4D lenses (filled symbols, solid lines) [means (±1 S.E.M.) of five animals], and eyes viewing through −8D lenses (hollow symbols, dashed lines) became relatively myopic (c) and their vitreous chambers became longer (d) than fellow eyes viewing through +8D lenses (filled symbols, solid lines) [means (±1 S.E.M.) of five animals]. In unoccluded animals wearing −4D lenses monocularly, those eyes (filled symbols, solid lines) became relatively myopic (e) and their vitreous chamber depths became greater (f) than those of fellow eyes wearing no lens (hollow symbols, dashed lines). Means (±1 S.E.M.) of ten animals. Significance values from paired t-tests.

3.2. Part I: within-group comparisons of effects of lens-wear

3.2.1. Conditions under which eyes viewing through negative lenses elongate faster and become myopic relative to fellow eyes

3.2.1.1. Binocular lens-wear with alternate occlusion. Raising marmosets wearing lenses of opposite sign combined with alternating occlusion was sufficient to induce significant anisometropia and differential growth between the two eyes after just 2 weeks of lens-wear. In groups AO ± 4 and AO ± 8, eyes viewing through negative lenses became significantly more myopic (Fig. 3a and Fig. 3c, matched pairs t-test, $P < 0.01$ and $P < 0.05$ respectively) and had deeper vitreous chambers (Fig. 3b and Fig. 3d, matched pairs t-test, $P < 0.01$ and $P < 0.05$ respectively) than eyes viewing through positive lenses. These differences were maintained without further significant increase over the subsequent weeks of lens-wear. There were no significant differences between the two eyes with respect to the other ocular components throughout the period of lens-wear (one way ANOVA, AO ± 4: lens thickness $P = 0.59$, anterior segment depth $P = 0.37$, corneal radius of curvature $P = 0.16$; AO ± 8: lens thickness $P = 0.51$, anterior segment depth $P = 0.56$, corneal radius of curvature $P = 0.33$). Despite the difference in the degree of optical anisometropia between the two groups (8D in AO ± 4 and 16D in AO ± 8) there was no significant difference in the degree of induced anisometropia (2.8D versus 2.4D, unpaired t-test, $P = 0.58$) or induced VCD differences (0.21 mm versus 0.25 mm, unpaired t-test, $P = 0.62$).

As we have already said, differences in refraction and VCD are significant ($P < 0.05$) after 4 weeks of lens-wear. As an aside we note that the differences in refraction and VCD were robust in the sense that even if one animal with unusually large effects (difference in refraction 4.75D and difference in VCD 0.46 mm after 4 weeks) was removed from the AO ± 4 group (reducing the number (n) from five to four) the effects were still significant after 4 weeks of lens-wear (matched pairs t-tests with $n = 4$, refraction $P < 0.01$, VCD $P < 0.01$).

3.2.1.2. Monocular −4D lenses. In the ten animals viewing through −4D monocular lenses, mean refraction of the eye wearing the lens became significantly more myopic and mean VCD was significantly greater than in contralateral eyes after three weeks of lens-wear

(Fig. 3e and Fig. 3f; matched pairs t-tests, refraction $P < 0.01$, VCD $P < 0.05$). After 4 weeks of lens wear the mean difference between the two eyes of the ten animals was 1.51 ± 0.40D in refraction ($P < 0.01$) and 0.13 ± 0.04 mm in VCD ($P < 0.01$). During the period of lens-wear there were no significant differences between the two eyes in the other ocular components (one-way ANOVA, lens thickness $P = 0.41$, anterior segment depth $P = 0.96$).

There was no significant difference between the degree of myopia (t-test on data at the end of lens-wear, $P = 0.99$) and differential vitreous chamber growth (t-test, $P = 0.88$) induced by the two methods of lens-attachment. The average (over all ten animals) amount of myopia and the difference in VCD between the two eyes was significantly less (t-tests: refraction $P < 0.05$; VCD $P < 0.05$) than in the groups which had worn binocular lenses.

Group means may conceal as well as reveal: examination of the behaviour of individual animals within the -4D groups shows that some animals had very small effects which were within the 95% confidence intervals of the measurement error associated with the techniques of retinoscopy and ultrasonography in our hands. If the four such animals are removed from the -4D group then the mean VCD and refraction of the remaining six animals after 4 weeks of lens-wear (2.32 ± 0.35D and 0.21 ± 0.03 mm) are not significantly different from those produced in animals wearing binocular (± 4D or ± 8D) lenses (one-way ANOVA, refraction $P = 0.57$; VCD $P = 0.58$). The importance of this point will be considered in Section 4.

3.2.2. Conditions which produce small degrees of hyperopia

3.2.2.1. Monocular +8D lenses. In animals wearing monocular +8D lenses, experimental eyes became significantly more hyperopic than controls during the period of lens-wear. The difference between the two eyes was statistically significant after just 2 weeks of lens-wear (Fig. 4a, matched pairs t-test, $P < 0.01$). This difference was maintained over the subsequent weeks of lens-wear. The VCD differences between the two eyes were significant after 2 and 3 weeks of lens-wear (Fig. 4b, matched pairs t-test, $P < 0.05$) but not after 4 weeks of lens-wear, with the experimental eyes having shallower vitreous chambers than control eyes.

There were no significant differences in the other ocular components between the two eyes (one-way ANOVA; lens thickness $P = 0.80$, anterior segment depth $P = 0.26$, corneal radius of curvature $P = 0.62$).

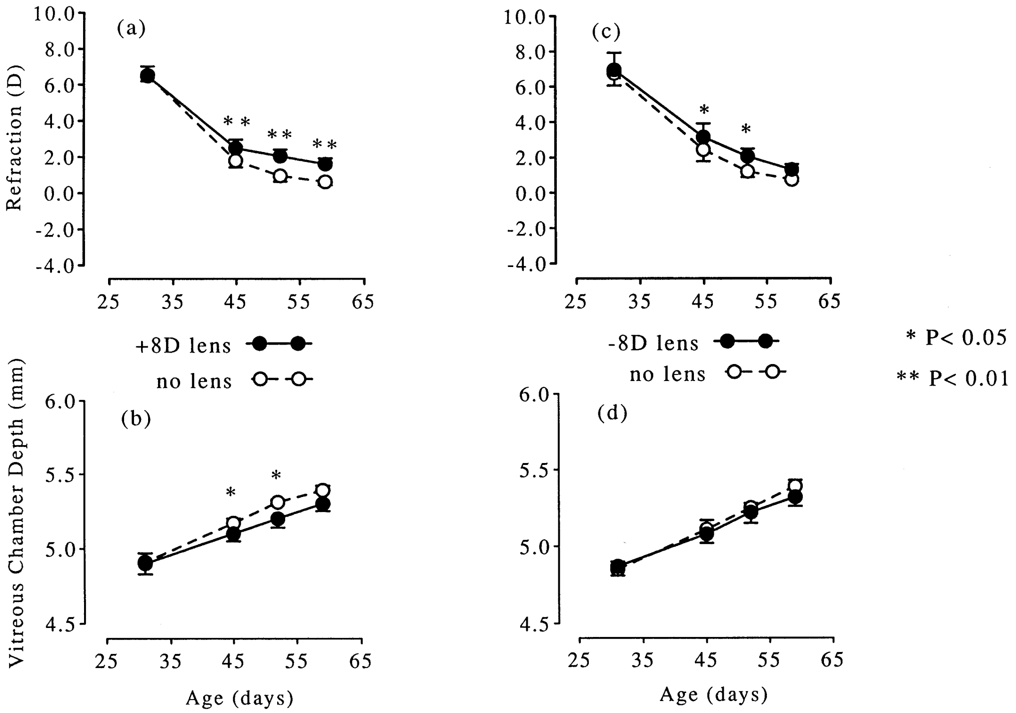

Fig. 4. Eyes wearing +8D lenses (filled symbols, solid lines) became relatively hyperopic (a) and their vitreous chamber depths usually were smaller (b) than those of fellow eyes wearing no lens (hollow symbols, dashed lines). There was no occlusion in these animals. Means (±1 S.E.M.) of five animals. Eyes wearing −8D lenses (filled symbols, solid lines) tended to have a slight relative hyperopia (c) compared with fellow eyes wearing no lens (hollow symbols, dashed lines). There was no occlusion in these animals. Means (±1 S.E.M.) of five animals. Significance values from paired t-tests.

3.2.2.2. Monocular −8D lenses.

Eyes viewing through −8D lenses also became significantly more hyperopic than their fellows during the period of lens wear (Fig. 4c), but did not develop significantly shorter vitreous chambers. The difference in refraction between the two eyes was statistically significant after 2 weeks of lens-wear (matched pairs t-test, $P < 0.05$), but decreased and was no longer significant after 4 weeks of lens-wear. There were no significant differences in VCD between the two eyes over this 4 week period (Fig. 4d, one-way ANOVA, $P = 0.57$) though the mean VCD of the experimental eyes was shallower than that of the control eyes.

There were no significant differences in the other ocular components between the two eyes at any time during lens-wear (one way ANOVA: lens thickness $P = 0.16$, anterior segment depth $P = 0.66$, corneal radius of curvature $P = 0.09$).

3.2.3. Conditions which produced no anisometropia

3.2.3.1. Monocular +4D lenses and +4D/plano lenses with alternate occlusion.

Animals wearing a +4D lens monocularly with unobstructed viewing through either eye did not develop significant refractive or ocular differences between the two eyes during the period of lens-wear (one-way ANOVA, refraction $P = 0.38$, VCD $P = 0.92$, lens thickness $P = 0.68$, corneal radius of curvature $P = 0.78$). Moreover, wearing a +4D lens in front of one eye and a plano lens in front of the other eye with alternate occlusion also did not produce anisometropia and differential vitreous chamber growth in the two eyes. At no time during the period of lens-wear were there any significant differences between two eyes with respect to refraction (one-way ANOVA, $P = 0.12$), VCD (one-way ANOVA, $P = 0.80$) or the other ocular components (one-way ANOVA, lens thickness $P = 0.63$ or anterior chamber depth $P = 1.00$).

3.3. Part II: inter-group comparisons in effects of lens-wear

We wished to make inter-group comparisons, both between the above groups, and with the normal animals described in the accompanying paper (Graham & Judge, 1999), to answer such questions as whether there was any yoking of growth between the two eyes when one eye but not the other wore a lens, and whether the growth of both eyes was abnormal in the experiments where opposite power lenses were worn over the two eyes.

We have made these inter-group comparisons only for refraction and VCD as these were the only parameters that differed significantly within groups, and moreover the P-values of the non-significant differences within groups in other parameters were high, making it unlikely that merging groups would reveal significant differences in the other parameters.

3.3.1. Significant differences between initial values in some groups

Experiments were carried out group-by-group rather than in a fully randomised design, and there were some differences between the initial mean values between groups. We made plots of the variation between groups in initial VCD and refraction, and carried out an analysis of variance, in which we considered parentage as a possible factor affecting the unequal initial values. (Because marmosets breed as stable pairs it was not necessary or possible to consider mother and father separately.) The major initial differences between the groups were the unusually small VCDs and high hyperopia in the +4D lens group, and the unusually large VCDs and low hyperopia in the binocular ±4D lens group. There was a more minor effect in the shorter than average VCDs and higher than average hyperopia in the −4D ‘implant’ lens group. Excluding the first two groups, but not the third, we found that there were still some significant differences in initial values even when parentage was accounted for (either using a unique or sequential sum squares two factor ANOVA). By excluding the third group as well, we were able to arrive at a substantial subset of the data (seven of the ten groups, including 38 of the 54 animals) in which there were no significant differences between initial refraction or VCD in either eye. We refer to this subset as ‘Pseudo-normal group A’ (see Table 1).

3.3.2. No evidence of yoking

One eye of each animal in six of these seven groups wore no lens or a plano lens, and in one (the binocular ±8D group) the growth of the eye wearing the positive lens was much more nearly normal than the other, so one test of yoking of eye growth is whether these eyes differ in any way. If there were yoking, they should differ because some fellow eyes wore +8D lenses, some +4D lenses, some no lens or a plano lens and others a −4D or −8D lens. There were no significant differences in refraction or VCD between these eyes at the end of lens (or occluder) wear at 8 weeks of age (two-factor ANOVA by eye and group, refraction $P = 0.14$; VCD $P = 0.36$; $n = 38$).

3.3.3. Initial VCD is weakly or not at all related to increase in VCD whereas initial refraction is correlated with change in refraction

We also examined, in this group of 38 eyes (Pseudo-normal group A) whether the change in VCD and refraction between the age of 4 weeks, when lenses were first worn, and when the lenses were removed at the age of 8 weeks was correlated with the initial VCD and refraction (Fig. 5). VCD data showed a significant

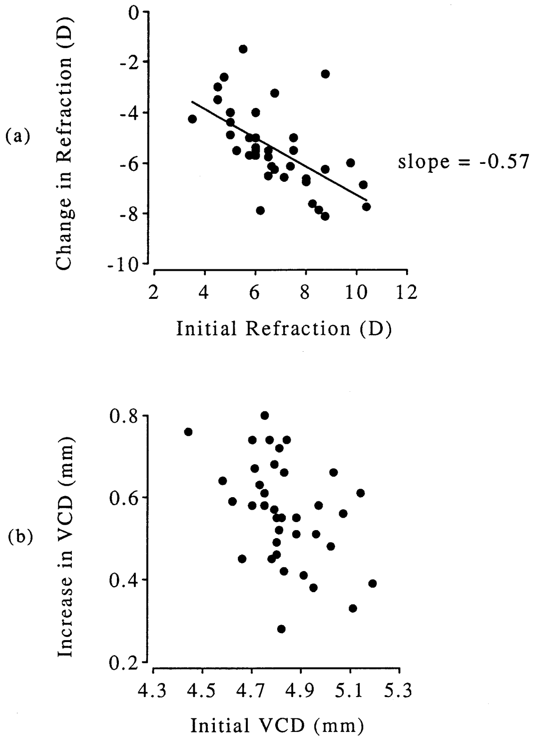

Fig. 5. Initial refraction and change in refraction over a 4 week period of lens wear were significantly negatively correlated (a), but initial vitreous chamber depth and increase in vitreous chamber depth over the 4 week period of lens-wear were weakly correlated (b). 'Normal' eyes only. See text for details.

negative correlation, but this became non-significant if the two most extreme VCDs were removed, so we are reluctant to place much emphasis on this finding. The change in refraction however was significantly and robustly correlated with the initial refractive error (Fig. 5a, $R^2 = 0.34$, slope $= -0.57$, $P < 0.001$).

3.3.4. *In binocular lens-wear only the eye wearing the negative lens grows abnormally*

The same data, presented above, which led to the conclusion that there is no evidence of yoking of eye growth, shows that only the eye wearing the negative lens grows abnormally. In particular, because the eye wearing the $+8$D lens in the AO $\pm$ 8D lens group is not significantly different from all the eyes wearing no lens or plano lenses; the eye wearing the $+8$D lens in the AO $\pm$ 8D group grows normally, and it is only the eye wearing the -8D lens that grows abnormally.

The case of the AO $\pm$ 4D group is more difficult to analyse, because the eyes of this group initially had unusually large VCDs and unusually low hyperopia. For that reason they were excluded from Pseudo-normal group A. We used the above regressions between initial VCD and refraction in the pseudo-normal animals to predict the expected VCD and refraction in the AO $\pm$ 4D animals, and then tested whether the measured values in the eyes wearing the $+4$D lenses differed from those predicted. There was no significant difference between the observed and predicted values (matched pairs t-tests, VCD $P = 0.92$; refraction $P = 0.14$). It is therefore also true that in these animals it is only the eyes viewing through the -4D lenses which grow abnormally.

There are no significant differences in refraction between eyes wearing a $+4$D lens and fellow eyes wearing either no lens or a plano lens (see above), suggesting that wearing a $+4$D lens has no effect.

3.4. *Part III: main effects after lens removal*

Graphs of the data suggested that after lens removal the groups of animals fitted into two distinct subsets. Those which wore binocular $\pm$4D or $\pm$8D lenses, or monocular -4D lenses and developed significant anisometropia and VCD differences, ($n = 16$ in total) showed different patterns of growth, after lens removal, to the other lens-wearing groups, which were indistinguishable from normal animals. From 70 days of age (analysing data from 70, 105, 168 and 273 days of age) the refraction of the latter eyes was not significantly different from that of normal eyes (two-factor ANOVA by age and group, M + 8 group $P = 0.96$, M − 8 group $P = 0.20$, M + 4 group $P = 0.09$, AO4 + 0 group $P = 0.75$). We will refer to these animals, together with the normal animals ($n = 29$ overall), as 'Pseudo-normal group B', the 'B' indicating that this is a different group from the Pseudo-normal group A referred to earlier. The former, 'A' group of eyes were not significantly different from normal eyes during lens-wear whereas the latter 'B' group were not significantly different from normal eyes after lens-wear. The composition of Pseudo-normal group B is shown in Table 1.

Within Pseudo-normal group B, if the artifact of retinoscopy (Glickstein & Millodot, 1970) is allowed for, then not only was there no effect of (sub)group on refraction, but also there was no effect of increasing age on refraction (two-factor ANOVA, $P = 0.41$), i.e. in these eyes refraction has stabilised to its adult value (see Fig. 9).

3.4.1. *Reduction of induced anisometropia*

To investigate the nature of the decrease in the difference in refraction and VCD between eyes after the period of lens-wear, the data from the AO $\pm$ 4 AO $\pm$ 8 groups and those animals which wore monocular -4D lenses and developed significant anisometropia and VCD differences, ($n = 16$ in total) were compared with that from the control eyes of normal and pseudo-normal animals.

At the end of the period of lens-wear the refraction of the positive-lens-viewing eyes of the animals in the AO $\pm$ 4 and AO $\pm$ 8 groups, and of the non-lens-wearing eyes of the six −4D animals developing an anisometropia, was not signIficantly different from the eyes in pseudo-normal group B (Fig. 6, one-way ANOVA, AO ± 4 group $P = 0.57$; AO ± 8 group $P = 0.62$; M − 4 group $P = 0.28$). The same was true of the VCD (one-way ANOVA, AO ± 4 $P = 0.09$; AO ± 8 $P = 0.80$; M − 4 $P = 0.85$). In contrast to this, the differences between the negative-lens-viewing eyes and the pseudo-normal eyes were significant (one-way ANOVA, refraction: AO ± 4 $P < 0.001$; AO ± 8 $P < 0.05$; M − 4 $P < 0.05$; VCD: AO ± 4 $P < 0.001$; AO ± 8 $P < 0.05$; M − 4 $P < 0.01$). This difference between the negative lens-viewing eyes and pseudo-normal eyes was maintained as animals got older (i.e. a two-factor ANOVA, with eye and age as factors, showed no significant interaction between eye and age (refraction: AO ± 4 $P = 0.29$; AO ± 8 $P = 0.10$; M − 4D $P = 0.55$, VCD: AO ± 4 $P = 0.74$; AO ± 8 $P = 0.83$; M − 4D $P = 0.94$).

By 105 days of age the eyes which had worn + 4D, + 8D, or no lenses, developed significantly more myopic refractive errors and deeper vitreous chambers than normal eyes (one-way ANOVA, refraction: AO ± 4 $P < 0.01$; AO ± 8 $P < 0.01$; M − 4D $P < 0.05$; VCD: AO ± 4 $P < 0.001$; AO ± 8 $P < 0.05$; M − 4D $P < 0.05$). Within the groups, these eyes were not significantly different from their fellow eyes which had worn negative lenses, and remained so over time (two-factor ANOVA, refraction $P = 0.85$, VCD $P = 0.99$). If data from all three groups were consolidated however, and the mean difference between refraction (or VCD) in the two eyes calculated as a function of time, then although the differences created by lens-wear are greatly reduced afterwards they are never abolished, and remain statistical significant (Fig. 7).

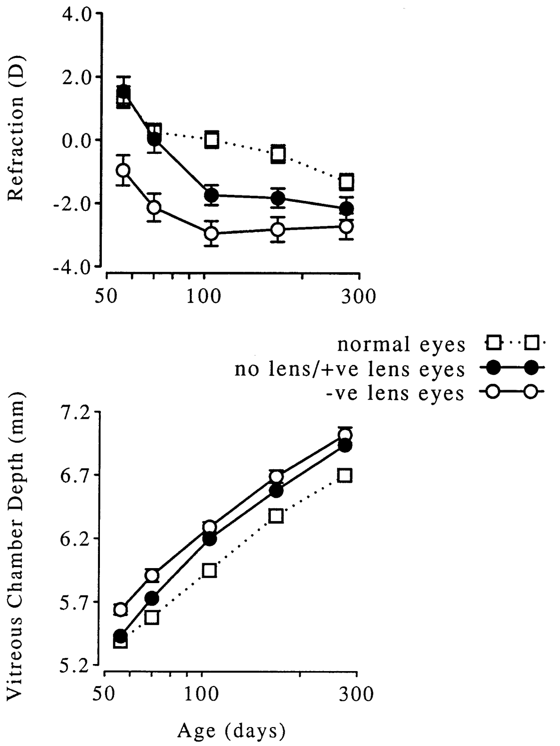

Fig. 6. Refractive error (a) and vitreous chamber depth (b), of eyes which developed induced myopia after wearing negative lenses. Previously normal eyes grew to have abnormally deep vitreous chambers as the anisometropia diminished. Hollow circles show means from 16 animals which viewed through negative lenses in groups AO ± 4, AO ± 8 and those animals which had a significant anisometropia from the M − 4G and M − 4 groups. Filled circles are means of 16 fellow eyes which viewed though either + 4D or + 8D lenses or without lenses. Hollow squares represent data for 29 normal and pseudo-normal eyes. Error bars represent ± 1 S.E.M. of each group.

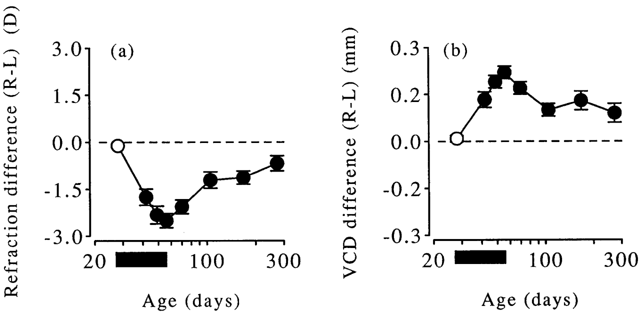

Fig. 7. Difference between eyes in refraction (a) and vitreous chamber depth (b) as a function of age in 16 animals which viewed through negative lenses in groups AO ± 4, A0 ± 8 and those animals which had a significant anisometropia from the M − 4G and M − 4 groups. Solid bars show the period of lens-wear. Error bars ± 1 S.E.M.

3.5. Part IV: within-groups comparisons—minor effects after lens removal

3.5.1. Monocular + 8D lenses

Induced anisometropia was eliminated in those animals which wore monocular + 8D lenses. The decrease in the difference in refraction between the two eyes was such that the eyes were not significantly different 2 weeks after lens removal, or thereafter (Fig. 8a, one-way ANOVA, $P = 0.27$). There were no significant changes in the VCD differences between the experimental and control eyes after the lenses were removed (Fig. 8b, one-way ANOVA, $P = 0.16$).

3.5.2. Monocular − 8D lenses

The difference between the two eyes in animals wearing − 8D lenses monocularly decreased after lens-removal and then actually reversed such that by 15 weeks of age experimental eyes were significantly more myopic than control eyes (Fig. 8c, *t*-test, $P < 0.05$). At 24 weeks of age the previously lens-wearing eyes were still significantly more myopic than the untreated, fellow eyes and their vitreous chambers were longer, with the difference between the two eyes approaching significance (Fig. 8d, *t*-test, $P = 0.053$). These differences do not persist and there was very little difference between the eyes at 39 weeks of age.

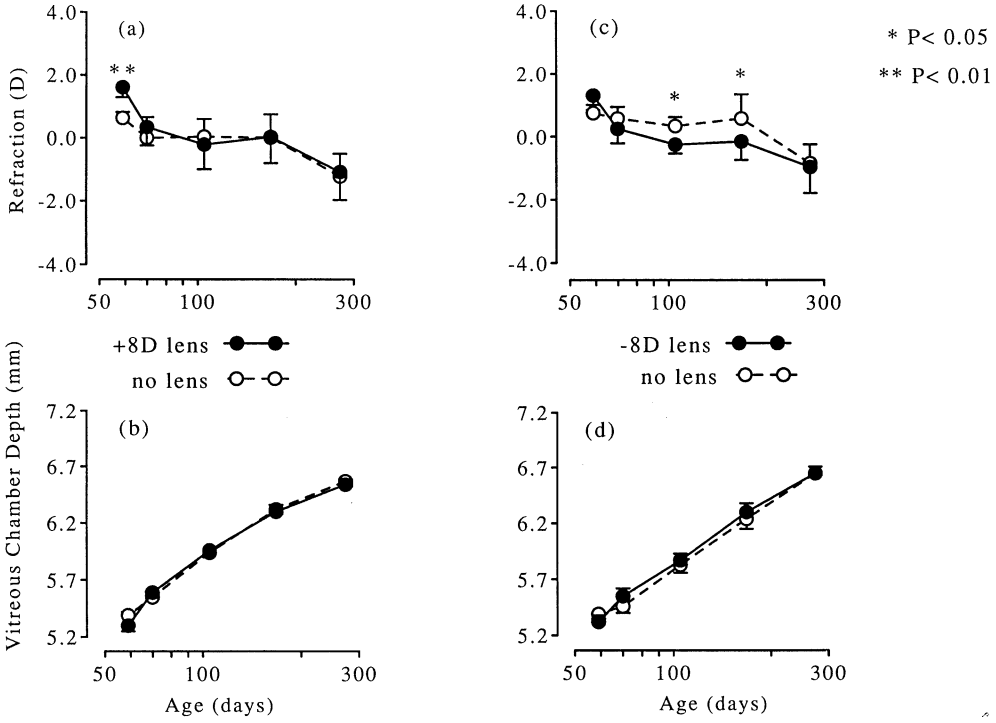

Fig. 8. After removal of a monocular +8D lens, anisometropia rapidly resolved (a). Vitreous chamber depth differences were not present (b). Means (±1 S.E.M.) of five animals. After lens removal, eyes which had previously viewed through −8D lenses (filled symbols, solid lines) became relatively myopic (c) compared with fellow eyes (hollow symbols, dashed lines) which had worn no lens. Means (±1 S.E.M.) of five animals. Significance values from paired t-tests.

3.5.3. *Monocular +4D lenses and +4D/plano lenses with alternate occlusion*

In the groups of animals wearing either monocular +4D lenses (M+4) or binocular +4D/0D lenses (AO4+0) there continued to be no significant difference between the two eyes after lenses were removed (one-way ANOVA; M+4, refraction $P=0.52$, VCD $P=0.52$; AO4+0, refraction $P=0.47$, VCD $P=0.78$).

3.6. *Part V: absolute refraction*

The data reported above are plotted in terms of the measured refraction without lenses or correction for the retinoscopic artifact. Fig. 9 shows the refraction in binocular (AO±4 and AO±8) groups and monocular −4D lens-wearing animals taking these factors into consideration. We call this 'absolute refraction'. See accompanying paper (Graham & Judge, 1999) for details.

Absolute (or 'true') refraction changed significantly during the period when lenses were worn, with all eyes becoming more myopic over time (one-way ANOVA on the four measurements while lenses worn: AO±4 group, $P<0.001$ both eyes; AO±8 group, $P<0.001$ (−8D eye) and $P<0.01$ (+8D eye); M−4 animals, $P<0.001$ both eyes). These changes occur only during the first 2 weeks of lens-wear. Absolute refraction did not change significantly during the second 2 weeks of lens-wear (one-way ANOVA, AO±4 group, $P=0.15$ (−4D eye) and $P=0.78$ (±4D eye); AO±8 group, $P=0.20$ (−8D eye) and $P=0.56$ (+8D eye); M−4 animals, $P=0.23$ (−4D eye) and $P=0.63$ (no-lens eye)).

Following the period of lens-wear, the refraction of eyes which had viewed through negative lenses did not change significantly over time (one-way ANOVA: AO±4 group, $P=0.47$; AO±8 group, $P=0.41$; M−4 animals, $P=0.84$). In the first 2 weeks after lens-removal, eyes which had viewed through positive lenses became significantly more myopic (one-way ANOVA: AO±4 group, $P<0.05$; AO±8 group, $P<0.05$) as they 'caught up' to the refraction of their fellow eyes. After 70 days of age there was no further change in refraction (one-way ANOVA: AO±4 group, $P=0.27$; AO±8 group, $P=0.43$). Eyes which viewed without lenses did not show significant changes in refraction after the lenses had been removed from their fellow eyes (one-way ANOVA: $P=0.16$).

3.7. *Control for possible effect of alternate occlusion on eye growth*

Although previous experiments in chicks have shown that it is necessary to occlude eyes for a very large fraction of each day to produce deprivation myopia (Nickla, Panos, Fugate-Wentzek, Gottlieb & Wallman,

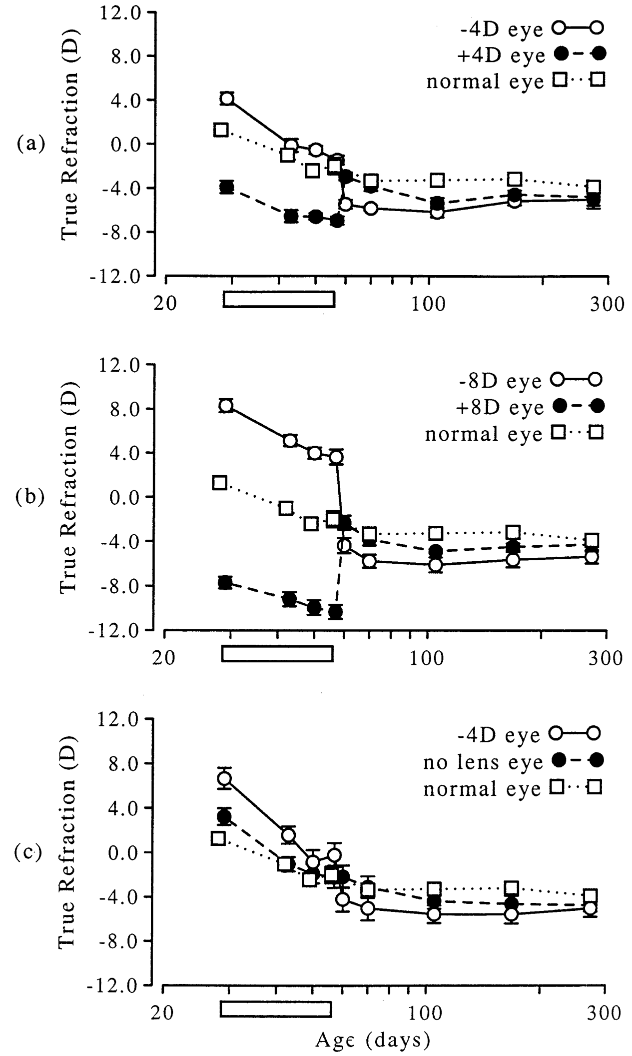

Fig. 9. Absolute refraction (i.e. refraction corrected for both the artifact of retinoscopy and for the lens worn) as a function of time, for (a) five animals wearing binocular ± 4D lenses, and the group of normal animals; (b) five animals wearing binocular ± 8D lenses and the group of nine normal animals, and (c) the six animals who developed anisometropia after wearing monocular − 4D lenses, and normal eyes. (Because not all the normal animals could be measured at every measurement epoch, the number of normal animals at each age (in days, in brackets) was: 6 (28), 7 (42), 7 (49), 7 (56) 9 (70), 9 (105), 9 (168), 9 (273)). Means ± 1 S.E.M. The hollow bars beneath the x-axes denote the periods during which animals wore lenses. Note that all eyes become more myopic over the first 2 weeks of lens wear, irrespective of the lenses worn, and that the same is true for normal animals and eyes which viewed without lenses.

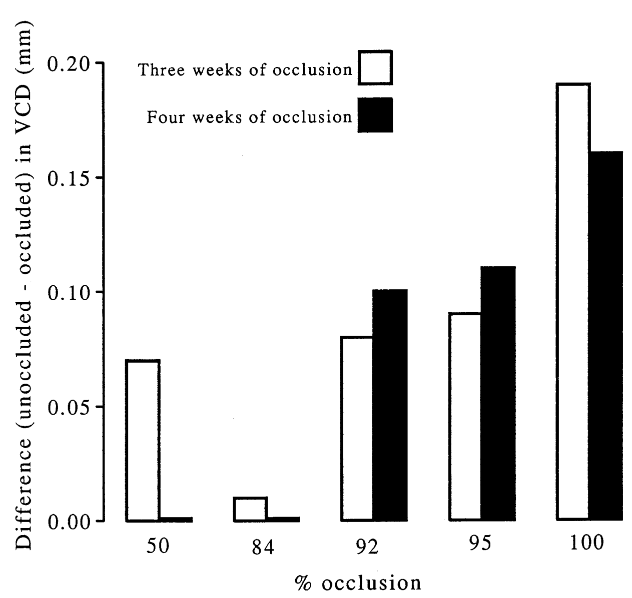

Fig. 10. Difference in VCD between unoccluded and occluded eyes of five individual animals occluded for 50, 84, 92, 95 and 100% of the daylight period. Open bars show differences after 3 weeks of occlusion; filled bars after 4 weeks of occlusion. Note that occluded eyes are always shallower than unoccluded eyes, i.e. the effect of occlusion is to slow vitreous chamber elongation. From separate studies of the repeatability of VCD measurements we have determined the standard deviation of measurement error to be 0.03–0.04 mm. Differences of more than 0.1 mm are therefore likely to be significant at the $P < 0.05$ level i.e occlusion for 92% and more of the day significantly increases VCD.

1989; Napper, Brennan, Barrington, Squires, Vessey & Vingrys, 1995, 1997), there are no such data on primates, and we were therefore anxious to examine the effects of occlusion for various fractions of the day on ocular development. Fig. 10 shows the difference in VCD between occluded and fellow eyes of five individual animals. Different animals were monocularly occluded for 100, 95, 92, 84 or 50% of the daylight period. It can be seen that only the 100, 95, and 92% occluded animals developed differences between the eyes. There are therefore no grounds for thinking that alternate occlusion itself would have affected ocular growth. It should be noted that in the 100, 95, and 92% occluded animals, the effect of deprivation was to slow vitreous chamber elongation and make the occluded eye more hyperopic than its normal fellow eye—in other words that the effect of deprivation, at least in the short-term, is to produce hyperopia rather than myopia, as has been reported previously (Troilo & Judge, 1993).

4. Discussion

One important constraint on our study was the considerable inter-individual variation in initial values of VCD and refraction, which led us to carry out an experiment in which the main stress was on within-animal differences in the growth of eyes wearing different lenses. It would of course have been better not to have been so constrained, and to have used a design in which one or both eyes of animals were fitted with lenses of the same power. We should perhaps ask the question of why inter-individual difference were considerable. We suppose that this was largely genetic (we were not working with deliberately in-bred strains of animals).

By contrast, presumably the strains of chicks used in ocular growth experiments are in-bred and so one might expect them to grow more consistently.

4.1. Responses to minus lenses stronger than to plus lenses

The evidence presented here for the marmoset shows that, over the period of life studied, ocular growth and refraction respond more strongly to the optical challenge presented by negative rather than positive lenses. Whereas the wearing of −4D lenses causes vitreous chambers to elongate more rapidly than normal and that eye to become relatively myopic, +4D lenses have no effect (Fig. 1 & 2). The wearing of −8D lenses causes vitreous chambers to elongate more rapidly than normal, and that eye to become relatively myopic. Although +8D lenses cause vitreous chambers to elongate less rapidly than normal, and become relatively hyperopic, the changes are smaller in magnitude than those caused by −8D lenses (Figs. 1–4).

In macaque monkeys there is evidence that eyes can respond to positive lenses (Hung, Crawford & Smith, 1995), because in four out of five animals wearing +3D or +6D spectacles, these eyes became more hyperopic than before lens-wear—a point that is brought out particularly clearly by Fig. 1 of Wallman & McFadden (1995) commentary on Hung, Crawford & Smith, (1995). We never saw such a hyperopic shift in our experiments.

In the chicken it is clear that responses to imposed defocus are bi-directional, i.e. refraction is shifted myopically by hyperopic defocus (viewing through negative power spectacle lenses) or hyperopically by myopic defocus (viewing through positive power spectacle lenses) (Schaeffel, Glasser & Howland, 1988; Irving, Callender & Sivak, 1992; Wildsoet & Wallman, 1995). The myopic shift is caused by an increase in the growth rate of the sclera and some thinning of the choroid; whereas the hyperopic shift is caused by an initial increase in the thickness of the choroid, followed by a slowing of scleral growth which eventually allows the choroid to revert to normal thickness (Wildsoet & Wallman, 1995). It is not clear that the thickness of the choroid of the primate eye can be altered significantly, but it seems extravagant to suppose that the lack of this specific mechanism would make it impossible for primates to respond in a compensatory manner to positive lenses.

It is possible that the asymmetrical effects we have observed from the wearing of negative and positive lenses relate to the functional demand for accommodation in the infant marmoset. If animals spend most of their time looking at nearby objects, then a moderate plus lens is perfectly functional. For example, Fig. 9b shows that an infant wearing a +8D lens has a true refraction of between −8D and −10D—which is appropriate if the animal is trying to look at objects no more than 10 cm or so away. In as much as the infant marmosets spend most of their time walking rather than jumping around the cage, their most distant fixation might rarely be beyond arms-reach and so within this far-point.

Another possibility is that the asymmetrical effect of negative and positive lenses reflects the fact that we have studied a period of life when the hyperopia of early infancy is being rapidly eliminated. If there is always the demand for eye (VCD) growth to eliminate hyperopia, then perhaps a bi-directional control of rate of eye growth is not necessary.

4.2. Does use of the eye affect eye growth?

Our reason for using the alternate-occlusion technique in the second series of experiments was to explore the possibility that the effect of lens-wear might depend on whether the animal focused with that eye or with the fellow eye. Alternate occlusion was a simple way of encouraging the animal to use (and particularly to focus images seen in) each eye, at least for part of the day. We found significant differences between the effects of lens wearing with and without alternate occlusion.

First, the mean effect of viewing through a −4D lens monocularly (Fig. 3e and Fig. 3f) was significantly less than that of viewing alternately through a −4D and +4D lens (Fig. 3a and Fig. 3b). Remembering that growth of the eye wearing the +4D lens was normal, this shows that the eye viewing through a −4D alternately-occluded lens grows more than that worn monocularly. The question is what this means. When we looked at the behaviour of individual animals in the −4D monocular groups, we found that we could divide these animals into two groups—four in which the effects were within the 95% confidence intervals of the measurement error of the techniques involved, and six where this was not so. (In the AO ± 4 group all the individual animals had effects that were significant in this sense.) When we compared the eyes of the six M − 4 animals which responded significantly, with the alternately occluded eyes, the difference was not significant. This makes us reluctant to emphasise the greater mean effect associated with alternate occlusion. It is quite possible, for example, that what is happening is that some monocular lens-wear animals fail to view through the −4D lenses and growth is unaffected, whereas the alternately-occluded animals are all forced to view through the −4D lens for at least part of the day, and therefore all have effects.

4.3. Effect of initial refraction

It was interesting (Fig. 5) that whereas initial refraction was robustly and negatively correlated with change in refraction over 4 weeks of infant life, there was at best a weak effect of initial VCD on change in VCD. There must, of course, be such a negative correlation between initial refraction and change in refraction if emmetropisation is to occur—initially more hyperopic eyes have further to go. What is interesting is the weakness of the equivalent correlation in VCD. This would seem to suggest that there may be some anterior segment changes involved in emmetropisation.

4.4. Higher-power negative spectacle lenses may cause deprivation effects

Troilo & Judge (1993) showed that periods of monocular deprivation (produced by lid-closure) as short as 3 weeks resulted in changes in the growth of the deprived eye of the marmoset. Initially the deprived eyes became shorter and more hyperopic than their fellows, but later they became more myopic and longer.

We interpret the initial hyperopic shift seen in the − 8D group as a deprivation effect, and this interpretation is supported by a drift towards myopia in the experimental eyes some time after lens removal (Fig. 8c). We think it is plausible that there is a deprivation effect when a − 8D lens is worn monocularly without alternate occlusion, on the assumption that marmosets, like macaques (Hung, Crawford & Smith, 1995), prefer to view through the eye where least accommodative effort is required. In this case, the retinal image in the − 8D lens eye will be greatly defocused, and it is possible that this simulates retinal conditions under which deprivation myopia occurs. In experiments in which a monocular + 8D lens was worn, we saw no sign of a deprivation effect in the fellow eye.

Bradley, Fernandes, Tigges & Boothe (1996) have reported that diffuser contact lenses worn from birth by macaques in one eye during daylight hours cause that eye to grow more slowly and become hyperopic, and draw the conclusion that less severe deprivation has the opposite effect on eye growth to severe deprivation. Because simply wearing an extended-wear contact lens produces hyperopia in the macaque (Crewther, Nathan, Kiely, Brennan & Crewther, 1988; Hung & Smith, 1996), the experiment of Bradley et al. is open to the interpretation that the effects reported are a result of lens-wear per se rather than of the diffusion. When diffusing spectacle lenses were worn by macaques, Smith & Hung (1995) have reported that the initial effect was a relative hyperopia, followed after 2 to 4 weeks by a myopic shift—an overall pattern of results similar to those in the marmoset.

4.5. Lack of evidence for inter-ocular yoking of eye growth

If chicks wear monocular spectacle lenses, compensatory changes occur in both eyes rather than in just the lens-wearing eyes (Wildsoet & Wallman, 1995), i.e. negative lenses produce an increase in the growth rate of both eyes, though the effects are much smaller (10% of the effect seen in lens-wearing eyes) in contralateral eyes and the two eyes, though both significantly different to normal eyes, are still significantly different from each other. This ‘yoking’ of the effects of lens-wear between the two eyes has also been found in guinea pigs (McFadden & Wallman, 1995) and in rhesus monkeys (Hung, Crawford & Smith, 1995), where both eyes of animals wearing weak (+ 3D) positive spectacle lenses become more hyperopic than they were prior to lens-wear whereas normal animals decrease their refractive errors towards emmetropia. We have not seen such effects in young marmosets wearing lenses, though it should be pointed out that a yoking effect no stronger than that in the chick would have been below the noise level in our experiments (10% of the group mean max effects of 2.5D change in refraction and 0.25 mm in VCD would only be 0.25D and 0.025mm).

4.6. Why are compensatory changes incomplete?

We do not know why the compensatory changes in eye growth and refraction exhibited in the marmoset are insufficient to fully compensate for the level of imposed defocus. In each of the experimental groups where compensatory changes are seen the degree of difference between the two eyes is similar, irrespective of the degree of imposed anisometropia between the eyes (4–16D). The majority of the changes occur in the first 2 weeks of lens-wear and are then maintained over the remainder of the lens-wear period. This suggests that incomplete compensation is not a result of the periods of lens-wear being too short for maximal changes in growth between the two eyes to occur. It cannot be the case that the eyes are only responsive over this time period or the changes seen after lens removal, with the two eyes becoming more similar again, would not be seen.

4.7. Cross-species comparisons

It is interesting to note the apparent differences, across species, in compensatory responses to lenses. Chick eyes compensate completely to 10D lenses (about a 6% change in ocular power). Macaques compensate to 3D lenses (about a 3% change in power). Our marmosets compensated partially, with a change in ocular power of 2 to 3D at most (less than 2% change in power). It is not clear why there should be such differences.

4.8. Response to anisometropia present after lens-removal

One of the most interesting observations we made was that in animals that had been made anisometropic by lens-wear, the anisometropia was largely corrected after lens-wear by the previously normal eye's vitreous chamber elongating more rapidly than that of its fellow eye, rather than growth slowing below normal in the more myopic eye with the deeper vitreous chamber. This finding raises several questions to which we do not yet know the answer. Is it not possible to slow elongation of the more myopic eye? Also, since both eyes end up with deeper vitreous chambers than normal, are there changes to the anterior segment of the eye that compensate to some degree for this? Might different results be obtained if alternate occlusion were continued in the period after the lenses have been removed?

4.9. Intermittency of lens-wear

If eye growth is affected by lens-wear, then one of the obvious questions is to what extent effects depend on continuous lens-wear and what the effect might be of intermittent lens-wear. One of the follow-up experiments we plan to do is to vary the proportion of the day which an animal views through a negative lens so as to explore this issue.

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