FACTORS OTHER THAN DEFOCUS THAT INFLUENCE EMMETROPIZATION AND EYE GROWTH IN CHICKS

By

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Declaration

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository**, subject to the provisions of the Copyright Act 1968.

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Xiaoying Zhu

Statement of Collaboration

The work embodied in this thesis has been done in collaboration with other researchers. Here, I formally acknowledge the contribution of my collaborators:

Chapter 1: Introduction. Part of this chapter, Section 1.5, was adapted from a review titled *Temporal integration of visual signals in lens compensation (a review)*, published in *Experimental Eye Research* (2013). I wrote the manuscript. The late David Saffer gave me comments and proofread the manuscript.

Chapter 3: Evidence for a non-visual cue that guides recovery from abnormal eye sizes in the chick eye. I reviewed previous data that were collected over the years from Josh Wallman's laboratory at the City College of the City University of New York, and performed statistical analyses under the guidance of McFadden. I wrote the manuscript.

Chapter 4: The effect of eye size on monocular lens compensation in chicks. This chapter was completed with the assistance of McFadden S. A., Wallman J., Sidhu A., and Cernota N. R. I designed the experiments under the guidance of McFadden and Wallman. I collected the data, assisted by Sidhu A. and Cernota N. R. I analyzed the data under the guidance of McFadden and Wallman and wrote the manuscript.

Chapter 5: Chick eyes can shorten to compensate for myopic defocus. A manuscript based on the work presented in Chapter 5 has been published in *Investigative Ophthalmology & Visual Science* (2013), tilted *Eyes in various species can shorten to compensate for myopic defocus* by Zhu X., McBrien N. A., Smith E. L., Troilo D., and Wallman J. I designed the experiments and analyses under Wallman's guidance. I reviewed and analyzed the data on chicks that were collected over the years from Josh Wallman's laboratory at the City College of the City University of New York, and wrote the manuscript.

Chapter 6: Interaction between paired eyes: Symmetrical growth, yoking, and anti-yoking. I reviewed previous data that were collected over the years from Josh Wallman's laboratory at the City College of the City University of New York, collected new data, and performed statistical analyses under the guidance of McFadden. I wrote the manuscript.

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Abstract

Purpose: While it is well known that growing human and animal eyes respond to imposed defocus by changing their growth to compensate for and eliminate the defocus (referred to as the "defocus-factor" in this dissertation), non-visual factors may also be involved. For example, it is common knowledge that body parts are under an intrinsic homeostatic control to firstly obtain the "right" length or size during development and secondly maintain this size after development. Previous experiments have shown evidence supporting non-visual factors playing a role in eye growth, e.g., chick eyes can restore their normal shape during recovery from form deprivation even though the retina has been damaged by tunicamycin. Therefore, it is possible that an intrinsic, homeostatic, non-visual mechanism also exists to control eye growth and to prevent the eye from deviating from the age-appropriate eye length or size (referred to as the "size-factor" in this thesis). In addition, it has been discovered that there are interactions between the paired eyes in the same animal, another factor that might be involved in eye growth regulation. Specifically, previous studies have shown that the fellow eyes might change in either the same direction as the lens-wearing eyes (the "yoking" effect), or the opposite direction compared with the lens-wearing eyes (the "anti-yoking" effect), in terms of both refractive error and axial length. The aim of this thesis is to investigate the existence and role of factors other than local defocus that may influence eye growth control. This is undertaken using the well-known chick lens-compensation model as it provides the gold standard providing the largest effect sizes available within animal models.

Methods: The refractive error and axial dimensions of chick eyes were measured with a Hartinger refractometer and A-scan Ultrasound biometry, respectively. (1) The existence of a non-visual-factor was studied in Chapter 3: To investigate whether a non-visual factor exists in chick eyes to guide eye growth independent of the defocus-factor, recovery after wearing +7 D (n = 8) or –7 D (n = 11) lenses while the chicks were kept in darkness was compared to chicks that recovered in light after wearing +7 D (n = 8) and –7 D (n = 5) lenses. (2) After demonstrating the existence of a non-visual factor that can guide eye growth, the

effect of manipulating eye length or size on subsequent monocular lens-compensation was studied in Chapter 4: Chicks first wore a weak positive or negative lens (+7 D, n = 4; – 7 D, n = 25) over one eye for a few days then the lens power was stepped up to a strong positive or negative lens (+ 7 D to +15 D; –7 D to –15 D), respectively. The size- and defocus-factors would be working in opposite directions at the time when lens power was increased, so studying lens compensation after the step-up can reveal which of these factors predominates in guiding eye growth. Furthermore, recovery from prior lens treatment vs. lens compensation after the step-up in lens power was compared when experimental eyes in both groups experienced the same amount of defocus (chicks recovering from –7 D lens wear vs. chicks that wore +15 D lenses after compensating for +7 D lenses; and chicks recovering from +7 D lens wear vs. chicks that wore –15 D lenses after compensating for –7 D lenses. The major difference between the two groups was their asymmetric eye sizes, which could act to facilitate recovery and reduce further lens compensation after the step-up. (3) The previous Chapter found that local defocus dominated in the case of positive lens wear (myopic defocus caused the eye to further compensate for the strong positive lenses, against the size-factor), so analyses were performed in Chapter 5 to investigate the ability of chick eyes to shorten axially, against the size-factor, to compensate for myopic defocus. Previous data from chicks from the Wallman database that wore a positive lens over one eye (n = 219) was compared to that from a group of normal, untreated chicks (n = 48). (4) To study another non-visual factor, the inter-ocular interactions between the paired eyes in the same chick, axial length from both eyes from a large group of untreated chicks from the Wallman database (n = 2960) were obtained to study the correlation in axial length between paired eyes and changes with age (1-17 days) in Chapter 6. Another group of untreated chicks (n = 48) were measured on days 7 and 10 to study the axial length growth in paired eyes. In addition, another group of chicks (n = 169) wore spectacle lenses of various powers (+/– 5, 7, 10, and 15 D) over one eye for various durations (1 to 7 days) and were measured before and after the treatment. The change in axial length in the fellow eyes was compared to that estimated from eyes of age-matched untreated animals. (5) Taking into account the discoveries related to the effects if asymmetric eye sizes and interactions between the two eyes (yoking) in previous chapters, the effect of eye size versus defocus was re-examined

under binocular conditions in Chapter 7: Chicks first wore a weak positive or negative lens (+/–5 D, n = 6 and 14 for positive- and negative lens-wearing eyes, respectively) over one eye for a few days then the lens power was stepped up to a strong positive or negative lens (+/–10 D) on the same eye, respectively. At the same time, the fellow eyes started to wear a weak positive or negative lens, so both eyes would experience defocus of the same sign and magnitude after the step-up. Chapter 7 addressed whether the size-factor can still prevent the eyes from further elongating to compensate for the strong negative lenses if the defocus signal was similar in both eyes.

Results: (1) Chapter 3: Compared with chick eyes that recovered from prior lens treatment in the light (i.e. with visual input), chick eyes recovered more slowly in darkness. However, all chick eyes partially recovered from prior positive or negative lens treatment despite being kept in the darkness for 3 days, suggesting that a factor independent of visual input does exist and that it alone can guide eye growth. For convenience, we refer to this as a "size-factor". (2) Chapter 4: Chick eyes fully compensated for +15 D lenses after they had compensated for +7 D lenses, despite having reduced axial length at the time of lens step-up, suggesting that myopic defocus dominated the eyes growth response, despite inter-ocular differences in eye size. In contrast, while chick eyes could fully compensate for –15 D lenses if they wore them from the beginning, chick eyes did not fully compensate for –15 D lenses after having compensated for –7 D lenses, suggesting that some intrinsic factor interfered with the ability of the eye to respond to hyperopic defocus. Similar findings were discovered with weaker-powered lenses. It was also discovered that chick eyes that wore +15 D lenses after the step-up reduced their rate of ocular elongation more than those recovering from prior –7 D lens wear, confirming the dominance of the defocus-factor in positive lens treatment. On the other hand, eyes recovering from prior +7 D lens wear developed a greater myopic shift compared with –15 D lens-wearing eyes after stepping up from –7 D lenses, confirming the involvement of a non-defocus related factor in the eyes response to negative lens treatment. Similar findings were discovered with lower-powered negative lenses. (3) Chapter 5: Chick eyes wearing positive lenses reduced their rate of ocular elongation by two-thirds, including 38.5% of eyes in which the axial length became shorter than before (mean change in axial length over the course of the experiment, experimental vs. fellow eyes, 40

vs. 171 μm). The axial shortening was caused mostly by the reduction in vitreous chamber depth. (4) Chapter 6: Paired eyes in untreated chicks were well correlated in their axial lengths 24 hours after hatching (mean axial length 8.55 and 8.53 mm for the right and left eyes, respectively; $r^2 = 0.77$, $p < 0.0001$) and thereafter, demonstrating symmetrical length or size and symmetrical growth. While monocular lens treatment caused significant compensation in the treated eyes, there was still a significant correlation in axial length in paired eyes after 3 to 7 days of treatment. Furthermore, yoking and anti-yoking, as defined by significant differences compared to growth predicted from untreated animals, were observed in approximately half of the experiments. In general, monocular lens treatment tended to reduce eye growth in the fellow eyes after shorter lens wearing durations (1-2 days, anti-yoking for positive lens treatment and yoking for negative lens treatment) and to increase eye growth after longer lens wearing durations (longer than 4 days, yoking for positive lens treatment and anti-yoking for negative lens treatment), and had minimal effect on the fellow eyes if the treatment duration was around 3-4 days. (5) Chapter 7: When chicks experienced defocus of the same sign over both eyes, chick eyes fully compensated for the strong positive lenses and especially, the strong negative lenses after the step-up, suggesting that the defocus-factor dominated in binocular lens compensation and that there is yoking between paired eyes.

Conclusions: Other than the defocus-factor that plays a crucial role in regulating eye growth, there are other intrinsic, non-visual, homeostatic mechanisms that are also involved in eye growth regulation: One of the non-visual mechanisms, which we refer to as a "size-factor", can guide the eyes to grow towards the direction to regain the normal, age-appropriate eye size, in the absence of visual cues. Additionally, some unknown intrinsic mechanism, possibly non-visual, refrains the eye from becoming longer than normal in the case of monocular hyperopic defocus. However, defocus still has a huge impact in eye growth regulation, as shown by the results that chick eyes fully compensated for the strong positive lenses after the step-up (at the step-up, the size-factor could act to reduce further compensation for the strong positive lenses since the lens-wearing eyes were already shorter than normal after compensating for the weak positive lenses) and that chick eyes can shorten axially to facilitate compensation for the myopic defocus, both against that predicted by any

intrinsic size-factor. Another non-visual mechanism, the inter-ocular interactions between paired eyes (symmetrical growth, yoking and anti-yoking), also influences eye growth: Growth in paired eyes was well correlated despite monocular lens treatment. Yoking and anti-yoking seemed to be lens-wearing duration dependent. Importantly, experiments which use the fellow eye as a control under conditions which may induce yoking and anti-yoking can still be used but are conservative and may underestimate the actual effect sizes by up to 27% if the lens treatment duration is around 3-4 days. Shorter and longer treatment durations, on the other hand, seem to have a larger effect on the fellow eyes, and caution should be taken when interpreting results of longer term monocular treatments. Finally, it might be prudent to have a group of untreated animals as a control. These non-defocus factors have significant implications for human myopia control, and may partially explain why the current mainstream optical treatments for myopia control attempting to project myopic defocus to reduce axial elongation have only proven to be moderately effective at best. Therefore, it is worthwhile further investigating the molecular pathways underlying the possible non-visual mechanisms and developing potential pharmaceutical treatments that enhance this intrinsic growth limiting system. It might be possible to maximize the effect of myopia treatment if the optical and pharmaceutical treatments can be combined.

1. Introduction

1.1 Myopia and its impact, globally and ocularly

In the first stage of seeing, the optics of the eye facilitate the projection of its visual input onto its retinal photoreceptors. However, in order to see distant images clearly, an eye's physical length needs to precisely match its optical focal length, so that the images fall precisely onto the outer segments of the photoreceptors. Such an eye is said to be emmetropic and the refractive error is zero. When a mismatch occurs, the eye has a refractive error and the image is not focused on the photoreceptors (creating defocus). Refractive errors can fall into one of the two categories (Fig. 1.1): (1) Near-sightedness, or myopia, a disorder in which an eye's physical length exceeds its focal length, with the images focused in front of the retina; or (2) far-sightedness, or hyperopia, a disorder in which an eye's focal length exceeds its physical length, with the images focused behind the retina.

Myopia is often considered to be a benign condition, since it can be corrected with spectacle and contact lenses and with refractive surgery. However, it is currently a major public health concern for the following reasons:

(1) The prevalence of myopia has been rapidly growing across the world in the last several decades and is particularly high in East Asian populations, reaching epidemic levels[1-6], the so called "myopia boom"[7]. For example, the prevalence of myopia in the US in children between 12 and 17 years of age increased from 12% (between 1971 and 1972) to 31.2% (between 1999 and 2004)[8]; and in Taiwan, the prevalence of myopia in 7-year-old children increased from 5.8% in 1983 to 21% in 2000[9]. Furthermore, in urban areas of developed countries in east and southeast Asia, such as Singapore and China, 80-90% of children completing high school are myopic[9]. Williams *et al.* have shown that the prevalence of myopia in Europe also increased, although at a slower rate compared with Asia, with meta-analysis of 15 population-based, cross-sectional studies from European Eye Epidemiology Consortium (62 thousand participants): Age-standardized myopia prevalence increased from 17.8% to 23.5% in people born between 1910 and 1939 compared to those born between 1940 and 1979[10]. Interestingly, Hermann Cohen showed that the rate of myopia was already high among school children (average prevalence 59% at the time of high school examination) in central Europe in the 19th century (reviewed by Schaeffel (2016)[11]). Since

there is no precise knowledge of the global prevalence of myopia, by performing a systemic review and meta-analysis of 145 studies with 2.1 million participants, Holden *et al.* estimated that the global prevalence of myopia increased from 22.9% in 2000 (1,406 million people) to 28.3 in 2010 (1,905 million people)[6]. It is projected that, by 2050, 49.8% of the world population (4,758 million people) will develop myopia. Even though studies that review previous data have potential limitations, such as heterogeneity between studies (contributing studies inherently differed in study design)[10], and paucity of prevalence data in many countries and age groups, across representative geographic areas[6], the overall evidence supporting the steady and significant increase in the prevalence of myopia globally over the past a few decades is unequivocal and overwhelming.

(2) Myopia is a major cause of visual impairment if not corrected[3]. A global systematic search and review of 53 surveys from 39 countries carried out by the World Health Organization (WHO) Prevention of Blindness and Deafness Programme showed that the principal cause of visual impairment is uncorrected refractive errors (43%)[12], and the WHO recognizes that myopia, if not fully corrected, is a major cause of visual impairment[3]. The global productivity lost due to uncorrected visual impairment is estimated to be $121.4 billion international dollars, the equivalent of $91.3 billion USD[13]. Visual impairment from uncorrected refractive errors can have negative impacts on patients' lives, e.g., lost educational and vocational opportunities[14], lost economic gain for individuals, families and societies, and impaired quality of life[12]. Myopia may also pose a financial burden to the patients. The direct cost of myopia includes the expenses for comprehensive eye exams, for spectacle and/or contact lenses (and solutions), and for refractive surgeries[14]. Thus, myopia is a huge public health issue that causes a tremendous economic burden[15-17].

(3) High myopia (spherical equivalent ≤ –5 or –6 D, or axial length longer than 26 to 27 mm for humans[18, 19]) increases the risk of vision-threatening myopic ocular pathologies that are not prevented by optical correction. It has been shown in many studies that patients with high myopia are at greater risk of developing a variable spectrum of characteristic fundus lesions, i.e., pathologic myopia (a more detailed review on complications of pathologic myopia can be seen in Cho *et al.* (2016)[19]). Specifically, typical ocular presentations of pathologic myopia include cataracts, lacquer cracks (breaks in Bruch's

membrane), choroiretinal atrophy, myopic choroidal neovascularization, myopic maculopathy, retinal detachment, and glaucoma, all of which are vision threatening[3, 19]. In fact, myopia has been considered as the leading cause of blindness in many developed countries, especially in Asia and the Middle East[20-23]. While the exact mechanisms responsible for these changes are unknown, it has been suggested that they are associated with retinal and choroidal thinning[24] and reduced blood circulation[25] caused by excessive axial elongation of the eye seen in high myopia[26]. A more detailed review on histological changes seen in pathologic myopia can be seen in Jonas and Xu (2014)[27].

Therefore, it is extremely important to investigate mechanisms involved in myopia development, treatment and prevention.

1.2 Etiology of myopia

The etiology of myopia was believed to be mostly genetic with only minor environmental influences about 50 years ago[3]. In light of results from animal studies over the last 4 decades, it has become clear that environmental factors also play a major role in school-age myopia[28]. The current view on human myopia is that major genetic contributions to myopia exist but that school-age myopia is multifactorial, involving major environmental factors[3]. Although high myopia or pathological myopia can be present from an early age and/or of congenital origin, a progression between myopia and high myopia is also common.

1.2.1 Genetic risk factors for myopia

It has been long noted that the concordance of refractive error is greater in monozygotic than dizygotic twins[29-32]. It was therefore claimed that "heredity is the basic determinant of ocular refraction"[33]. In addition, some studies discovered that children with myopic parents are more likely to become myopia[34-37]. However, this result can also be explained by environmental risks (see Section 1.2.2 below). Never the less, certain gene loci and variants have been identified to be associated with myopia[38, 39]. Refer to Table 1.1 for more reviews on this topic.

1.2.2 Environmental risk factors for myopia

There is also overwhelming evidence supporting the existence of environmental risk factors for myopia. First, the rapid increase in myopia prevalence all over the world (see Section 1.1), especially in young people with a higher level of education[20, 40, 41] and in young adults who do intensive professional studies[42, 43], argues against a genetic origin and suggests that near work might be myogenic. Near work is associated with greater accommodative lag[44], which may cause images to focus behind the retina and subsequent axial elongation[28]. For example, it has been shown that the prevalence of myopia in Orthodox boys who spent 16 hours daily studying religious text is much higher compared with that in Orthodox girls whose study load is similar to that in Western countries (81.3% vs. 36.2%)[45]. Young *et al.* showed a drastic increase in myopia prevalence in recently acculturated Eskimos within a single generation (from 1.5% in parents to 51.4% in children), also suggesting that participation in education plays a role in myopia development. In studies that show that children with myopic parents are at a greater risk to develop myopia[34-37], while genetics is certainly a possibility, it is also possible that these children may spend more time reading (following their parents' example). Therefore, the visual environment experienced may play a role in their myopia development.

Second, increased levels of accommodation during prolonged near work may also be myogenic, but epidemiological studies on this topic have produced conflicting results[3]: While Saw *et al.* showed that reading more than two books per week made it more likely for Singapore children to develop high myopia than those who read less[46], the Sydney Myopia Study found that the association between the time on near work and myopia progression was weak[47]. The US Orinda Longitudinal Study of Myopia showed weak but significant effects of increased hours of near work, and the authors argued that the evidence did not support a significant effect of near work[48].

Third, recent epidemiological surveys[49, 50] and animal studies[51] have shown that increased time outdoors and exposure to bright light in animal studies, can protect against myopia development. The underlying mechanisms for the bright light effect have been speculated to be[52, 53]: (1) Increased depth of focus seen with pupillary constriction under bright light, and (2) elevated release of dopamine from dopaminergic neurons simulated by

light[54] since dopamine has been shown to inhibit myopia development[55-60]. Perhaps the lack of spending time outdoors contributes of myopia development in school-aged children. See Table 1.1 for more reviews on this topic.

Table 1.1. Summary of recent reviews (from 2005 to 2016) on various aspects of myopia

Category	Review content	Source
Epidemiology	This review summaries epidemiology, causes, risk factors (both environmental and genetic) for myopia. It also discusses pathogenesis for and ocular presentations in pathological myopia and interventions to control myopia.	Morgan, Ohno-Matsui and Saw, 2012[3]
	This review summaries data on prevalence, incidence, progression, associations, risk factors, and impact from recent epidemiological studies on myopia.	Foster and Jiang, 2014[4]
	Myopia prevalence in Asia was estimated via meta-analysis of 50 population-based studies from 16 Asian countries or regions	Pan *et al.*, 2015[5]
	Myopia prevalence in Europe was estimated via meta-analysis of 15 population-based, cross-sectional studies from the European Eye Epidemiology Consortium.	Williams *et al.*, 2015[10]
	Global prevalence of myopia was estimated by systemic review and meta-analysis from data from 145 studies with 2.1 million participants.	Holden *et al.*, 2016[6]
Complications of pathologic myopia	Histological changes of high axial myopia.	Jonas and Xu, 2016[27]
	Prevalence, classification, pathophysiology, complication, progression and visual prognosis of pathologic myopia.	Cho *et al.*, 2016[19]
Genetic risk factors in myopia	This review covers various techniques used to study the molecular biology of myopia.	Schaeffel *et al.*, 2003[61]
	This review summarizes various ocular and systemic conditions associated with myopia, and possible genes associated with myopic discovered by linkage studies, candidate gene screening, large-scale genetic analysis and QTL mapping.	Jacobi *et al.*, 2005[38]
	This review compares the allelic frequencies of gene variants associated with myopia in different ethnic groups with an emphasis on the Asia-Pacific region.	Rong, Chen, and Pang, 2016[39]
Environmental risk factors in myopia	This review summarizes the etiology, causes, ocular presentations (for pathological myopia), and treatment for myopia.	Morgan and Rose, 2005[62]
	This review summarizes recent findings on the effects of various light levels on refractive development in animal models and associated mechanisms.	Norton and Siegwart, 2013[52]
	This review summarizes recent findings on the protective effects of outdoor activities on myopia development and associated mechanisms.	French *et al.*, 2013[53]

While there are many theories about the etiology of myopia, the exact causes are still unclear. Since it is virtually impossible to empirically test these theories on humans, it is important to investigate the etiology of myopia using animal models.

1.3 Animal research in myopia

Decades of myopia studies conducted on various animal models have produced a tremendous amount of results showing that the growth of the eye, like the growth of other organs in our body, is under homeostatic control, and that the homeostatic control mechanism depends, at least in part, on visual signals that exert strong control over the axial length of the eye[28].

1.3.1 Lens compensation, recovery, and form deprivation

When wearing a spectacle lens which causes defocus, the eye can negate the defocus using either a rapid focusing mechanism (accommodation, see Section 1.4.1 below) or a slower focusing mechanism (emmetropization). During emmetropization, the eye adjusts its rate of ocular elongation to align the location of the retinal imaging surface with the focal length of the eye. The process by which the eye reduces or eliminates externally imposed defocus (as induced with a spectacle or contact lens) to still achieve emmetropia is called lens compensation. Specifically, when wearing a positive lens so that the image is focused in front of the retina (so called "myopic defocus", since the eye is now functionally myopic, Fig. 1.1A), the eye reduces its rate of ocular elongation, effectively moving the retina forward to meet the focal plane (Fig. 1.1B). Given enough time, the eye will restore emmetropia with the positive lens in place, and will therefore appear hyperopic (with the defocus behind the retina) without the lens. If the positive lens is removed, the eye will increase its rate of ocular elongation, effectively pushing the retina backwards to meet the focal plane and regain emmetropia, a process called "recovery" from lens wear.

The opposite happens when wearing a negative lens that focuses images behind the retina ("hyperopic defocus", Fig. 1.1A): The eye will increase its rate of ocular elongation to compensate for the negative lens (Fig. 1.1B). The eye will appear myopic (with the defocus in front of the retina) if the negative lens is now removed. The eye will "recover" from the prior negative lens treatment by decreasing the rate of ocular elongation.

In addition, depriving the eye of any visual input of high spatial frequencies by lid-suture or fitting the eye with an occluder or a diffuser causes myopia arising from excessive ocular elongation, a phenomenon known as "form deprivation" myopia. After the normal visual input is restored, the eye will also recover from form deprivation myopia by reducing its rate of ocular elongation to regain emmetropia.

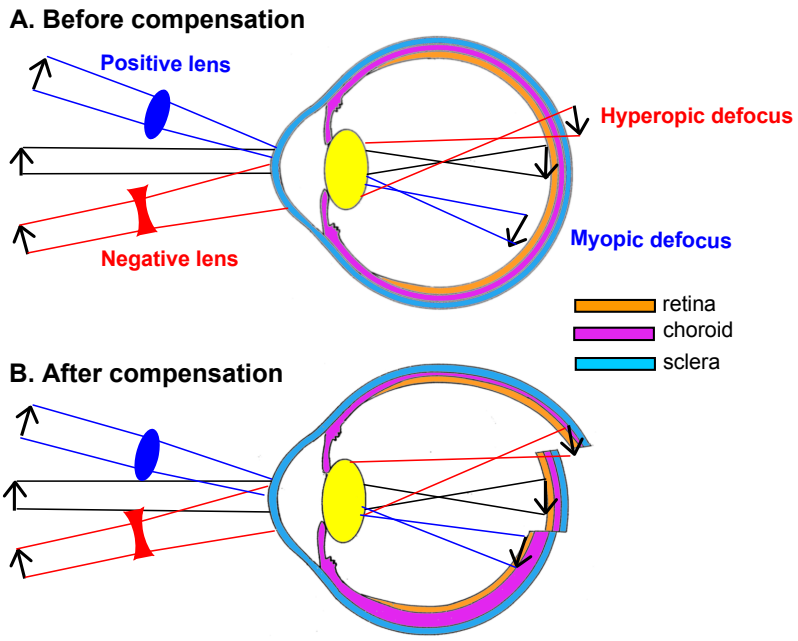

Figure 1.1. Schematics of ocular compensation for defocus of opposite signs. (A) shows an emmetropic eye with a schematic representation of the myopic and hyperopic defocus produced by wearing a positive and negative spectacle lens, respectively. (B) shows ocular compensation: The eye reduces axial length and increases choroidal thickness to compensate for the positive lens, and increases axial length and reduces choroidal thickness to compensate for the negative lens. In either case, the eye becomes emmetropic again with the spectacle lens in place, since the image is now again focused on the retina. Adapted from a review by Wallman and Winawer[28].

1.3.2 Animal models in myopia research

It was first discovered in monkeys in 1977 that lid-suture after birth caused excessive ocular elongation and a large myopic shift[63]. Similar findings were also discovered in tree shrews around the same time[64]. A year later, Wallman *et al.* discovered that restricting chicks' vision to the frontal visual field by fitting the eyes with translucent, hemispherical occluders caused extreme myopia (up to 24 D) with increased axial length[65]. Form deprivation myopia has been discovered in various animal models, such as, rhesus monkeys[63, 66], tree shrews[64], chicks[67-69]. It also occurs in human if the eye is deprived of form vision because of pathological conditions, such as ptosis (droopy upper eye lid)[70-72] and congenital cataract[72, 73]. The early findings from animal studies inspired researchers to further investigate how eye growth is regulated by post-natal visual input.

Among the species used in myopia research, chicks are commonly used because of the following advantages[74]: (1) Relatively large eyes (8 – 14 mm); (2) rapid early growth of approximately 70 μm / day[75] to 100 μm / day[76]; (3) high visual acuity (7 – 9 cycles / deg)[77]; (4) of all species studied, chick show the largest compensatory capacity (–10 to +20 D) within a relatively short period of time[78]; and (5) chicks are relatively inexpensive and easy to maintain. Young chicks have two distinguishing traits facilitating compensation: Their eyes (which grow at a relatively steady rate when measured until at least 42 days old[79]) change their rate of growth within a day or two to compensate for both myopic and hyperopic defocus, and their choroids show strikingly large and rapid changes in thickness to compensate for both myopic and hyperopic defocus[80]. Choroidal thickening can account for 50 – 60% or 7.2 D of a +15.5 D change in refractive error[81]. The choroid in chick eyes may also have an accommodative function (see Section 1.4.1 for details). The two compensatory components (axial length and choroidal thickness) have different temporal dynamics in chicks: Choroidal compensation happens more rapidly (within a few hours), whereas axial compensation takes a day or two to occur[82]. The strong relationship between these two components, means that the chick model offers very early access to putative growth signals.

Indeed, while compensatory changes in choroidal thickness have been found in tree shrews[83], marmosets[84], rhesus macaques[85], guinea pigs[86, 87], and even in humans[88, 89], when superimposed with defocus, the magnitude of change in choroidal thickness in chicks is by

far the largest and significantly changes the refractive error of the eye. The magnitude of change in choroidal thickness in other species is small and does not significantly change the refractive error. Therefore, the robust and somewhat unique choroidal compensatory capacity in chicks could also be viewed as a disadvantage of the chick model. Other differences between chick and human eyes include the lack of a fovea and differences in scleral composition compared to mammalian models: The chick sclera has an inner cartilaginous layer and an outer fibrous layer, while the mammalian sclera only has a fibrous layer.

Regardless of the limitations of the chick model, numerous studies have been performed using the chick model, and fundamental discoveries made in the chick model over the last few decades have had enormous impact on the current understanding in the mechanism of emmetropization and myopia control, and underpin our current understanding of putative myopia prevention and treatment strategies, i.e., using various optical treatments (Progressive Additional Lenses—PALs, multifocal soft contact lenses, and orthokeratology) to impose peripheral myopic defocus.

Chicks will be used in this thesis since they provide a gold standard in myopia research with exaggerated effect sizes, a rapid rate of lens compensation, and there is a large body of literature using this model.

1.4 Possible cues that guide ocular growth

One of the main challenges for the emmetropization system is to discern the sign of the defocus, aiming to minimize blur[28]. Both lens compensation and recovery from lens compensation or form deprivation myopia strongly support the notion that the chick eye detects both the magnitude of defocus and the sign of defocus and uses this information to guide its growth. Furthermore, since a brief episode of myopic and hyperopic defocus can induce rapid choroidal compensation that does not initially change the eye's refractive error, it has been suggested that chick eyes can distinguish between myopic and hyperopic defocus from the beginning of lens treatment, instead of using a trial-and-error mechanism[82].

However, it is still unclear exactly which aspect(s) of the visual information, or what cue(s) the eye uses for each of these tasks. Many visual cues have been proposed: the associated evidence for each is summarized below with an emphasis mostly on what has been found in studies using the chick model, although findings in other species are included. It should be born in mind that the way the avian eye compensates for defocus may differ to that used by other species, but due to the phylogenetically conserved nature of eye growth control, some overlap between species may be expected.

1.4.1 Accommodation

The average level of accommodation over time could be used as a cue to guide emmetropization: A higher level of accommodation would indicate the eye being hyperopic with a higher accommodative demand, and a lower level of accommodation would indicate the eye being myopic with a lower accommodative demand. However, the findings that chicks can still compensate for lenses after lenticular accommodation had been blocked either pharmacologically[90] or surgically[91, 92] argue against this possibility, at least in the chick. Furthermore, when chicks wore mixed spherical and cylindrical lenses that projected blur with Jackson crossed cylinders that could not be eliminated by accommodation, chick eyes still compensated for the spherical power of the lenses, as if they wore the spherical lenses alone, supporting the notion the chick eye does not use traditional lenticular accommodation as a cue to guide eye growth[93]. Furthermore, the fact that eyes can compensate for superimposed defocus locally[94, 95], but lenticular accommodation cannot, agrees with the same notion. However, other than lenticular accommodation, avian eyes have other mechanisms for accommodation, the second of which may be locally modified.

Specifically, consistent increases in corneal curvature have been observed during accommodation produced either by electrical stimulation of the Edinger-Westphal nucleus or by topical instillation of 0.4% nicotine sulfate to the cornea[96]. Corneal accommodation is achieved by contraction of the ciliary muscle, which flattens the peripheral cornea and steepens the central cornea[97]. It is estimated that corneal accommodation can contribute to 40-50% of ocular accommodation (about 6 to 9 D)[96, 98, 99]. Second, it has also been suggested that chick eyes change the thickness of their choroid to accommodate, albeit more slowly[80].

This may not just be an avian phenomena, since significant choroidal thinning with 6 D of accommodative command was recently discovered in humans[100].

Therefore, the role of accommodation in human myopia is still unclear and the strong correlations between myopia and apparent near work in humans, make this still a contentious issue. However, in chicks, the eye growth control system appears to be able to act in the absence of accurate lenticular accommodation.

1.4.2 The magnitude of blur or sharp vision

Since young animals are hyperopic and spend most of their time looking at near objects, it is possible that myopic eyes caused by wearing positive lenses, experience reduced blur or sharp vision while looking at near objects and increased blur while looking at distant objects. It is, therefore, plausible that the eye uses this information to guide its growth: The eye will grow normally if it experiences some sharp vision associated with positive lens wear and increase growth if experiencing blur associated with negative lens wear[101]. This hypothesis was supported by studies in which monkeys and tree shrews failed to compensate for strong positive lenses and positive lens compensation could only be achieved by using a series of weak positive lenses worn sequentially[102]. However, chick eyes that wore lenses composed of negative and positive cylindrical elements with orthogonal axis (i.e., Jackson Crossed Cylinders) that increase the amount of blur but with zero net spherical power, actually became slightly hyperopic, not myopic[93]. Later studies have shown that, at least in chicks, the eye can distinguish between myopic and hyperopic blur and compensate for defocus in the right direction without sharp vision. When restricted in a small drum such that vision was restricted to distances that were out of range, chick eyes compensated for myopic and hyperopic defocus in the right direction without experiencing any sharp vision, either cyclopleged to block accommodation[103] or with accommodation intact[104]. In addition, when chick eyes were deprived of any sharp vision but superimposed with myopic or hyperopic defocus by wearing positive or negative lenses attached with image-degrading diffuses, they still compensated for the lenses in the right direction[104]. Therefore, in the absence of sharp vision, chick eyes are well able to compensate for both the magnitude and sign of defocus.

1.4.3 Spatial frequency and image contrast

Since frosted eye occluders and diffusers used in form deprivation and defocusing lenses change spatial frequency and image contrast input, it is possible that eyes use these image features to guide eye growth. Wearing frosted occluders or diffusers that restrict the spatial frequency content and reduce image contrast increases eye growth and induces myopia in chicks[65, 69, 105], tree shrews[83], macaques[106], guinea pigs[86], mouse[107], and marmoset[108], and the more frosted the occluders are, the more myopia develops, indicating that the system can quantify these features over time[106, 109]. Similarly, after guinea pigs wore Bangerter foils of various strengths which differed in their cut-off special frequencies, it was reported that the extent of induced myopia and ocular growth were related to the amount of image degradation[110].

Previous studies have also shown that certain contrast and spatial frequencies may help facilitate lens compensation. Diether and Wildsoet reported that chick eyes compensated better for myopic defocus when it is combined with a spatially rich target vs. a regular target and that compensation for myopic defocus is reduced when the image contrast is reduced from 100% to 32% for the spatially rich target[111]. It has also been discovered that in chicks, middle to high spatial frequencies are needed for choroidal expansion seen with positive lens treatment[104], and for +7 D lens compensation[111]. Furthermore, Schmid *et al.* reported that target contrasts 4.2% and lower produced relative myopia of similar amount to that observed in response to a 0% contrast target, while target contrasts 47.5% and higher did not significantly alter the eye's refractive error in chicks, and concluded that image contrast provides important visual information for the eye growth control system[112]. On the other hand, chick eyes still developed compensatory hyperopia after wearing positive lenses regardless of reduced spatial frequency content and image contrast, suggesting that these are not the only cues needed for emmetropization[103]. Schmid and Wildsoet showed that image contrast (ranging from 9 – 78%) were only slightly less effective than the normal cage environment in preventing form-deprivation myopia and that spatial frequency had a differential effect in preventing form-deprivation myopia depending on the frequency, and concluded that emmetropization is relatively less insensitive to image contrast but sensitive to spatial frequency[113]. Many other studies have been carried out with

chicks manipulating spatial frequency and/or contrast and generally show that ocular growth is sensitive to such cues, but their significance is limited since the majority of studies did not precisely analyze the image features that the animals experience, so overall it is not clear exactly which visual aspects are necessary for emmetropization. However, there are several notable exceptions. For example, Diether *et al.* meticulously calculated the modulation transfer for various lens powers and predicted the amount of contrast adaptation (a spatial frequency-selective increase of suprathreshold contrast sensitivity after exposure to low-contrast patterns) and concluded that shifts in contrast adaptation may represent a signal related to refractive error development[114].

1.4.4 Image size

Since positive lenses magnify images whereas negative lenses minify images, it is plausible that image size is used as a cue to guide emmetropization. Previous results found in chicks, however, argue against this possibility. It has been shown that chick eyes correctly compensated for –11 D lenses that had magnifying effects (+1.9% and +6.9%, instead of –2.9%)[115]. In addition, wearing afocal iseikonic lenses that produced 10% of image magnification (a little less than the degree of magnification produced by +10 D lenses) did not cause hyperopia despite image magnification[116]. Therefore, it is unlikely that the avian eye uses image size alone to guide emmetropization.

1.4.5 Chromatic aberration

If the eye is focused on a black-and-white edge, green light would be focused on the retina, with blue light focused in front of the retina and red light behind the retina, called longitudinal chromatic aberration. If the eye is too long (myopic), red light would be more in focus than blue and green light, whereas blue light would be more in focus if the eye is too short (hyperopic). It is very likely that the eye uses chromatic aberration (i.e., the asymmetric image when the eye is too long vs. too short) as a directional cue to regulate eye growth. An early study showed that rearing fish in red light caused significantly enlarged eye size (nasotemporal diameters)[117]. Similarly, it has been discovered that chicks raised in

red light become 1.25 D more myopic than normal chicks raised in white light[118]. It has also been found that chick eyes that were exposed to chromatic simulation of hyperopic defocus (sinusoidal gratings with greater contrast in the blue component for the image than in the red) for 3 days without any lens treatment showed elevated scleral glycosaminoglycan synthesis (an indicator of eye growth) and thinned choroids compared with fellow eyes that were exposed to chromatic simulation of myopic defocus (sinusoidal gratings with greater contrast in the red component for the image than in the blue)[119]. On the other hand, raising animals in monochromatic light does not prevent lens compensation, indicating chromatic cues might not be necessary for lens compensation when other cues are available[92, 120, 121]. In addition, it has been shown that lens compensation was compromised when chicks were raised under dim Ultraviolet light[121, 122], suggesting that the emmetropization system may require Ultraviolet input. A more detailed review on the role of chromatic aberration can be seen in Rucker (2013)[123].

1.4.6 Higher-order aberration

For a perfect optical system in monochromatic light, all the rays emitted from one light source on one side converge to a single image point on the other side of this system. Higher-order aberrations (HOAs) are small optical irregularities or imperfections of the eye, uncorrectable by spherical and cylindrical lenses[124]. HOAs contribute to the blur the eye experiences, and may change the point spread functions (PSFs, the response of an imaging system to a point source)[125]. Therefore, it is possible that the chick eyes may be able to detect the HOAs by discerning the shape of the PSFs. It has been shown that HOAs increase soon after chick eyes wore -15 D lenses[126]. Spherical aberration, one type of HOA, occurs when light rays going through the edge are refracted more compared with light rays going through the center of the system. It creates an asymmetry with respect to the sign of defocus, i.e., when the eye is too long vs. too short, in both chicks[127]and humans[128], and this asymmetry can potentially be used to guide eye growth. It has been shown that human subjects can learn to identify the sign of defocus of images of point sources of light in a forced-choice psychophysical task[129]. However, contradictory results have been found when researchers have measured spherical aberration in emmetropes and myopes. Several studies found higher

levels of aberration in myopes compared with emmetropes[130, 131], whereas other studies did not[132-134]. Therefore, the role of spherical aberration in emmetropization is still unknown. A more detailed review on higher-order aberration can be seen in Charman (2005)[135].

1.4.7 Conclusions

From the preceding brief summary, it is clear that multiple visual cues may be used by the eye to discern the direction and amount of eye growth required for both emmetropization or for lens compensation. It is possible that the array of negative results for any one single cue is because the eye uses multiple mechanisms to adjust its eye growth, without relying on any one single method. Such a fail-safe system is common in biological control.

1.5 Integration of myopic and hyperopic defocus

In real life, every region of the retina experiences a dynamic mixture of myopic and hyperopic defocus, changing constantly depending on one's fixation point, accommodative state, and the surrounding environment. Because the pattern of defocus in the retina changes rapidly over space and time, but the compensatory growth mechanism is relatively slow, the eye must integrate visual information over space and time to infer whether it needs to increase or reduce its length, or to maintain its current size.

It is thought that the eye growth system is more sensitive to myopic defocus than to hyperopic defocus. It has been shown that a short period of "normal vision" (viewing without any lens or occluder on the eye) each day cancels myopia from wearing negative lenses or occluders during the rest of the day in chicks[136, 137]; by contrast, it takes a much longer period of normal vision to cancel out hyperopia from wearing positive lenses during the rest of the day in chicks[137], tree shrews[138] and monkeys[139-141]. Furthermore, when positive and negative lenses are worn alternately, the eye is more responsive to myopic defocus and develops hyperopia, rather than averaging out the defocus of the opposite signs in chicks[142-144] and tree shrews[145]. On the other hand, myopic defocus was found to be less protective than

normal vision in rhesus monkeys, possibly because the degree of imposed myopic defocus was too large [141].

The long-lasting effect of myopic defocus is possibly because axial inhibition caused by positive lens-wear lasts longer than axial elongation caused by negative lenses[146]: It was hypothesized that there was an intrinsic emmetropization signal that controls the axial response to positive or negative lens wear, and it has been shown that the signal controlling axial inhibition (in the case of positive lens wear) lasts much longer (fall time 24.4 hours) than the signal controlling axial elongation (in the case of negative lens wear, fall time 0.4 hour)[146].

Myopic defocus also seems more potent than hyperopic defocus in guiding eye growth when the two are presented simultaneously. It has been shown that presenting chick eyes with simultaneously competing myopic and hyperopic defocus, using either mixed astigmatic (toric) lenses with opposite lens powers on the two perpendicular meridians[93], lens-cone devices with two target planes[111], multi-zone contact lenses with alternating powers[147], or dual-power lenses that had different combinations of lens powers[148], caused hyperopia. In addition, projecting myopic defocus onto the peripheral retina (while allowing the central retina to receive clear images) has been shown to slow myopia progression in chicks[149].

In the current thesis, only single-powered positive and negative spectacle lenses will be studied, and each will be studied separately. However, the limitations of this approach and the implications for how the results might apply to real world viewing, and myopia treatment, in particular, will be discussed in the General Discussion.

1.6 Myopia control

Inspired by results from animal studies, most treatment approaches try to manipulate the visual impact of defocus on the retina. The current main stream of myopia control is: (1) Optical treatment for myopia control by projecting myopic defocus to the peripheral retina,

using undercorrection, bifocal or multifocal spectacles (including Progressive Addition Lenses, PALs), soft bifocal contact lenses, and Orthokeratology, and (2) pharmaceutical treatment by administering topical Atropine eye drops of various concentrations. A recent review shows that pharmaceutical treatment (with muscarinic antagonist such as Atropine and Pirenzapine) is the most effective, and certain specially designed contact lenses, including orthokeratology and peripheral defocus modifying contact lenses, had moderate effects, whereas specially designed spectacle lenses showed smaller effects[150]. In addition, the therapeutic effect tended to disappear after a couple of years, and myopia tends to rebound after the treatment had been ceased[151, 152]. The following section reviews some findings related to such limits on optical treatments for myopia control and possible implications for other possible non-visual factors that may affect ocular growth during development.

1.6.1 Undercorrection

Two undercorrection clinical trials were conducted in school-aged children and neither proved to be effective. One study found that undercorrecting children by approximately –0.75 D significantly increased the rate of myopia progression compared with control group that was fully corrected[153], and a later study found no difference in myopia progression between the treatment group that was undercorrected by approximately –0.50 D and the control group[154].

If myopic defocus can reduce axial elongation and induce a hyperopic shift, why did not undercorrection, that would create myopic defocus, show the expected effect? One of the possibilities might be that while the central retina experienced myopic defocus caused by undercorrection, a large extent of the peripheral retina still experienced hyperopic defocus[155, 156]. But it still does not explain why undercorrection would cause the eyes to become more myopic that those in the control group in Chung *et al.*'s study[153]. These studies suggest that there might be non-defocus related mechanisms involved in eye growth regulation.

1.6.2 Bifocal and multifocal spectacle lenses

Both bifocal and multifocal spectacle lens designs serve the same purpose of superimposing the eyes with myopic defocus, especially for those with increased accommodative lag. While most of the clinical trials show significant slowing with myopia progressions with multifocal spectacles[157-164], the effect is of little clinical significance (i.e., data from the largest clinical trial reports –0.25 D less myopia progression compared with the control group[159]), even in children with high accommodative lag and near esophoria, who should benefit from these optical correction the most. In addition, the therapeutic effect seems to disappear a couple of years after the initiation of the correction. The lack of satisfactory outcome, despite the theory behind it, also begs the question mentioned above. Taken together these studies suggest that other than the visual input, it is possible that there are other factor(s) that bifocal and multifocal spectacle lens treatments do not address that are also involved in eye growth regulation.

1.6.3 Soft bifocal contact lenses

Compared with multifocal spectacles, the soft bifocal contact lens-wearing eyes (center corrected for distance and the positive add power in the periphery of the contact lens) showed more encouraging results: On average, soft bifocal contact lenses reduced myopia progression by approximately 46%[165-168]. Anstice and Phillips conducted a clinical trial in which the fellow eye was used as a control, i.e., one eye wore a soft bifocal contact lens while the fellow eye wore a single vision soft contact lens[165]. After 10 months of clinical trial, the lenses were swapped and worn for another 10 months. They discovered that after the lenses were swapped, the rate of myopia progression in the eyes that previously wore soft bifocal contact lenses increased to the baseline progression rate of the single vision contact lens-wearing eyes. As a result, both eyes had similar amounts of myopia at the end of the trial. Therefore, it is possible that the eye is pre-determined to grow at a certain rate or to reach a certain length, and will catch up with the pre-determined growth rate once the treatment is stopped.

1.6.4 Orthokeratology

Orthokeratology is a special corneal reshaping technique, using a rigid contact lens that is worn overnight. It has been shown to correct myopia through flattening central cornea and steepening the midperipheral cornea to reduce peripheral hyperopia and project peripheral myopia, but only with moderate results. One review looked at 8 studies and found that orthokeratology reduced axial elongation by an average of 43%[152]. Another review performed network meta-analysis on 16 interventions and showed that, compared to placebo or single vision spectacles, orthokeratology on average reduced axial length by 0.15 mm per year (95% Confidence Interval, –0.22 to –0.08 mm), corresponding to myopia reduction of only 0.25 to 0.50 D per year[150]. In addition, one case report showed that myopia developed at a faster rate after the patient discontinued orthokeratology[169]. Therefore, these results may also imply that the eye is programmed to grow at a certain rate or to reach a certain length, and will catch up with the pre-determined growth rate once treatment ceases.

This thesis studies the relevance of non-defocus based mechanism(s) in eye growth modulation, using the chick model. It is hypothesized that there may be non-visual mechanism(s) involved in eye growth modulation, and that their function is to prevent the two eyes from deviating from each other in size, or from some predetermined age-matched normal size. If true, and if this applies to human myopia development, it may have significant implications for both the limits of optical treatments, and suggest alternative factors that might need to

1.7 Non-visual mechanisms regulating eye growth

1.7.1 Circadian rhythm

It is well known that many ocular processes have diurnal rhythms that serve to optimize retinal function (a detailed review on circadian rhythm can been seen in Nickla (2013)[170]. Among many parameters and biochemical processes studied, the rhythms in axial length and choroidal thickness are the most studied and relevant to emmetropization.

In normal chick eyes, axial length shows a diurnal rhythm, with the peak around 6 am and trough around 6 pm, with a daily variation of approximately 25 μm[75]. Choroidal thickness also has a diurnal rhythm that is in antiphase to the diurnal rhythm in axial length. It has been speculated that these diurnal rhythms are controlled by a non-visual, endogenous oscillator, since these diurnal rhythms still exist in constant darkness[171]. Eyes in young adult rabbits also show endogenous, consistent 24-hr changes in axial length and lens thickness[172]. In addition, it has been shown that marmoset eyes have a diurnal rhythm in both axial length and choroidal thickness[173]. Specifically, the choroid thickened during the night and thinned during the day. The rhythm in axial length was age-dependent: Axial length in juveniles (with faster-growing eyes) increased during the day and decreased at night; the pattern was reversed in adolescents (with slower-growing eyes). Axial length, and choroidal thickness in humans also show diurnal rhythms: Axial length in humans also has a diurnal rhythm, with the maximal length occurring at midday[174]. The diurnal rhythm in choroidal thickness appears to be in antiphase to axial length[175].

The normal diurnal rhythm is interrupted with various types of visual manipulations. Depriving chick eyes of visual input of high special frequency (called "form deprivation"), for example, caused the eye to grow rapidly during both day and night[176]. On the other hand, wearing positive lenses caused the eyes to slow its rate of elongation, and there was phase delay in the axial length rhythm and a phase advance in the choroidal rhythm, and the two phases were moved in phase with another[75].

Many studies have shown that interrupting the normal diurnal rhythm can also affect lens compensation[170]. It has been recently shown that interrupting the night with a two-hour long of light from 12 to 2 am for 7 days actually increased axial elongation, thinned choroidal thickness, and caused a myopic shift in chick eyes that wore positive lenses[177].

1.7.2 Eye size

It is common knowledge in the world of Biology that all body parts are under intrinsic homeostatic controls to firstly obtain the "right" size during development and secondly maintain this "right" size after the body parts reach adult size. In addition, the intrinsic homeostatic control of organ and limb size during development helps ensure left-right

symmetry for organs and limbs that exist on both sides of the body. As a result, for example, the two arms of an individual grow separately and yet ultimately reach approximately the same size, which will be maintained throughout adulthood. These goals are achieved by multiple pathways coordinating growth in multiple dimensions and cell growth, inhibition and apoptosis (a more detailed review on achieving bilateral symmetry can be seen in Allard and Tabin (2009)[178]). An intrinsic mechanism also acts to restore the original or "normal" size of an organ or tissue after damage. For example, a rat's liver restores its original size after the liver is partially removed[179]. Transforming Growth Factor-beta1, a known inhibitor for hepatocyte proliferation in culture[180], is speculated to terminate liver regeneration after it has reached the appropriate size[181]. Furthermore, other examples indicate that organ size is actively controlled, e.g., gonads in tropical birds vary based on the length of the day[182] and internal organs of a python fluctuate in size based on its digestive state[183]. Therefore, it is possible that a similar intrinsic homeostatic mechanism also exists in the eye to control its growth to ultimately maintain its "right" size, referred to as the "size-factor" in this thesis, as first postulated by van Alphen[184]. Recent studies have discovered evidence supporting the Hippo pathway is involved in the differentiation of the retina in Drosophila[185, 186] and in the lens in mouse[187].

Several studies have shown evidence for non-visual control of eye growth in chicks. First, dark-reared chicks continue to change their ocular dimensions after being returned to normal lighting even after emmetropization has been re-attained[188]. Second, Schaeffel and Howland[120] discovered that chick eyes recovered from form deprivation myopia (eyes already longer than normal) even when the refractive error was optically corrected with negative lenses of the appropriate power, concluding that there is a "non-visual mechanism highly sensitive to abnormal eye shape". Most dramatically, eyes made very asymmetric in shape and myopic in only the nasal retina by wearing diffusers that only covered the nasal visual field recovered normal symmetry even after the retina has been lesioned by tunicamycin, showing that the non-visual factors could return the eye back to its normal shape [189]. Furthermore, previous findings have shown that while form deprivation induced variable amount of myopia in chicks, they returned to their individual set-point refractions

during recovery, suggesting that there appears to be an endogenous, possibly genetic, definition of the set-point of emmetropization in each animal[190].

It is worth noting that eye size can be affected by anatomical factors such as the orbit size: Previous data have shown that even though myopic eyes in humans tend to expand in all directions, they elongate more axially than vertically and horizontally, possibly due to the anatomic constraint of the bony orbital walls (the orbital walls are much closer to the sides of eyes than behind the eye)[156, 191]. Similar results have also been found in primates[192].

It is important to note that the term of the "size-factor" is only conveniently used to represent some non-defocus related factor(s) involved in eye growth and that the nature of these factors are unknown. On the same note, the term the "defocus-factor" is used to refer the signal(s) driving emmetropization the lens-wearing paradigm. In reality, the exact signal driving emmetropization may not be defocus *per se*, but could be the consequence of defocus, e.g., accommodation, even though previous experiments argue against this possibility (see Section 1.4.1 above for details).

While some studies have suggested the involvement of non-visual mechanisms that are sensitive to abnormal eye shape or size in eye growth, the effect of eye size on eye growth has never been directly studied. If a size-factor exists to help maintain the "right" eye size, it should prevent the eye from growing too long or short despite the eye experiencing hyperopic or myopic defocus and therefore reduce lens compensation. Evidence for the existence of a size-factor is presented in Chapter 3, and its effect when in competition with defocus, is presented in Chapters 4 and 7.

1.7.3 Interactions between paired eyes

Chick eyes have independent innervation[193], blood supply[194], and accommodation[195]. The two bony orbits are separated by an interorbital septum (an ossified partition)[196]. An artery ophthalmica interna that travels medial of the optic nerve provides blood supply for each eye[194]. The optic nerves project in a highly ordered manner onto their primary visual

target areas with complete decussation at the chiasm[193]. The visual space of the external world is represented as an array of receptive fields on a map in the visual target areas.

Allometric growth of the eyes during normal development[197] results in approximately symmetrical growth rates in the two eyes. Symmetrical growth in paired eyes has also been shown in normal young rhesus monkeys[198] and chicks that were treated either monocularly[146] or binocularly[190]. Research on emmetropization (with either form deprivation or lens treatment) and eye growth regulation in animal models and human subjects often involves treating one eye (with diffusers or lenses, or with some pharmaceutical agents, the experimental eye) and comparing change in refractive error and growth in that eye with data from the untreated fellow eye. This experimental approach has the advantage of controlling for genetic variability and increasing statistical power thus reducing the number of animals needed to reach statistical significance. In this design, however, the experimental procedure is presumed to affect mostly the experimental eye and the contralateral fellow eyes are assumed to be unaffected. However, it has been shown on many occasions that the untreated fellow eye may also show changes in refractive error, axial growth rates, and certain molecular pathways that differ from that in untreated age-matched animals: This change in the fellow eyes could be either in the same direction as the experimental eyes, i.e., yoked growth, or in the opposite direction, i.e., anti-yoked growth[199].

1.7.3.1 Yoked growth

It has been noted that when the refractive error and growth rate in the experimental eye were changed by wearing a positive or negative lens, the fellow eye also tended to change in the same direction as seen in the experimental eye. Sometimes the change in the untreated fellow eyes might be different from what is observed in age-matched normals. This deviation from binocular symmetric growth is referred to as yoked growth. Similar findings have also been discovered in certain proteins and genes. See Table 1.2 below for a summary of literature that shows yoking between the treated and fellow eyes.

Table 1.2. Summary of studies reporting effects of monocular lens wear on the untreated eye and ocular interactions

Species	Monocular Treatment	Findings	Source
Chick	Form deprivation	Binocular form deprivation caused more myopia than monocular form deprivation.	Sivak, 1989[200]
Chick	Intravitreal injection	Monocular Tetrodotoxin injection caused a hyperopic shift in both the injected eyes and the fellow control eyes.	McBrien *et al.*1995[201]
Rhesus Macaques	Spectacle Lens	For positive lens treated monkeys: The monkeys used the positive lens wearing eye as the fixating eye (to minimize accommodative effort), and the fellow eye as the non-fixating eye. The opposite occurred for negative lens-wearing monkeys. Therefore, compared with the fixating eye, the non-fixating eye experienced hyperopic defocus behind the retina. It was discovered that while the non-fixating eyes were always less hyperopic or myopic than the fixating eyes, the refractive-error changes for both the fixating and non-fixating eyes were significantly correlated with the fixating eyes' initial effective refractive status.	Hung, Crawford, and Smith, 1995[202]
Chick	Spectacle Lens	The refractive error in the fellow eyes shifted in the same direction as the lens-wearing eyes, both in intact eyes and in eyes with optic nerve section. This happened during both lens treatment and recovery.	Wildsoet and Wallman, 1995[81]
Rhesus Macaque	Contact lens	The refractive error in the fellow eyes shifted in the same direction as the lens-wearing eyes	Bradley *et al,*, 1999[203]
Rhesus Macaque	Monocular form deprivation	The refractive error in the non-treated fellow eyes became at least 1 D less hyperopic during the treatment period (71-80 weeks), the same direction as seen in the form-deprived eyes. This myopic shift is greater than what is observed in normal monkeys of the same age (on average 0.17 D per year).	Smith *et al.,* 1999[204]
Chick	Form deprivation and spectacle lens	Recovery from form deprivation and positive lens wear increased the fraction of ZENK-expressing glucagon amacrine cells in both treated and fellow eyes.	Fischer, 1999[205]
Rhesus Macaque	Form deprivation	Some untreated eyes became less hyperopic or less myopic than the age-matched normal controls. They also exhibited recovery toward more ametropia during recovery	Smith and Hung, 2000[106]
Chick	Form deprivation	Depriving one eye of form vision with occluders not only caused these eyes to completely lose their growth rhythms, it also caused the, follow eyes to change their growth patterns and grow more at night than during the day.	Ohngemach *et al.*, 2001[206]
Tree shrew	Form deprivation	FD changed mRNA levels for proteins involved in extracellular matrix synthesis and degradation (α1 collagen, decorin, matrix metalloproteinase 2 and 3, and Tissue Inhibitors of Metalloproteinases 1 in both form deprived and fellow eyes.	Siegwart and Norton, IOVS, 2002[207]
Rhesus Macaques	Form deprivation	One fellow eye became more myopic with longer vitreous chamber depth compared with the normal.	Smith *et al.*, 2002[139]
Chick	Spectacle Lens	Monocular +12 and –12 D lens treatment caused similar changes in the percentage of ZENK-positive glucagon cells in paired eyes.	Bitzer and Schaeffel, 2002[208]

Table 1.2. Cont.

Species	Monocular Treatment	Findings	Source
Chick	Spectacle Lens	Monocular +7 and –7 D lens treatment caused similar changes in choroidal gene expression of the nuclear transcription factor ZENK, retinaldehyde dehydrogenase 2, and Transforming Growth Factor β in the treated and untreated fellow eyes.	Simon *et al.*, 2004[209]
Chick	Spectacle Lens (+/–7 D)	Twenty-four hours of lens treatment upregulated retinal selenoprotein P gene expression, in both the treated and fellow eyes, with the changes more prominent in the fellow eyes compared with the lens-wearing eyes.	Ohngemach *et al.*, 2004[210]
Tree shrew	Spectacle Lens	Compared with –5 D lens wearing eyes, the fellow eyes showed similar changes in mRNA expression for certain matrix metalloproteinases and tissue inhibitors of metalloproteinases in the fibrous sclera, during both lens treatment and recovery.	Siegwart and Norton, 2005[211]
Chick	Spectacle lens	Monocular –7 D lens treated caused elevated levels of MMP-2 mRNA in both treated and fellow eyes in the cartilaginous sclera.	Schippert *et al.*, 2006[212]
Guinea Pig	Form deprivation	Form deprivation in the treated eyes caused the vitreous chamber of the fellow, untreated eye to elongate.	Howlett and McFadden, 2006[86]
Rhesus Macaques	Form deprivation	The untreated fellow eyes exhibited relative myopic errors that fell outside the normal range during the treatment period	Smith *et al.*, 2007[213]
Chick	Bilateral spectacle lens wear and monocular intravitreal injection of atropine	Atropine inhibited myopia development and reduced axial elongation not only in the atropine-injected eyes, but also in the saline-injected fellow eyes.	Diether *et al.*, 2007[214]
Tree shrew	Spectacle Lens	Monocular –5 D lens wear caused the fellow eyes to develop a small but consistent myopic shift, compared with eyes in untreated animals.	Norton *et al.*, 2010[215]
Tree shrew	Spectacle Lens	One day of monocular –5 D lens wear caused down-regulation of certain signaling molecules, matricellular proteins, metalloproteinases, tissue inhibitors of metalloproteinases and cell adhesion proteins, in both the lens-wearing eyes and the untreated fellow eyes.	Gao *et al.*, 2011[216]
Tree shrew	Spectacle Lens	The fellow eyes of –5 D lens-wearing eyes became slightly but significantly more myopic with longer eyes compared with age-matched normals. There were a few scleral proteins whose expression in the fellow eyes was also changed in the same direction as the treated eyes and significantly different from those found in age-matched normals.	Frost and Norton, 2012[217]
Tree shrew	Spectacle Lens	Monocular –5 D lens wear and recovery caused a rapid 2- to 3-fold increase in the elastic modulus of scleral collagen fibrils.	Grytz and Siegwart, 2015[218]

1.7.3.2 Anti-yoked growth

In contrast to yoking, asymmetrical growth between the eyes has also been reported, whereby the rate of growth in the control eye of a monocular treated animal grows in the opposite direction (growth rate increases or decreases) from that induced in the experimental eye, and shows a significantly different growth rate compared to the eyes of age-matched untreated control animals, referred to as anti-yoking. Similar to yoking, anti-yoking has also been found in certain biochemical pathways. See Table 1.3 below for a summary of literature that suggest anti-yoking between the treated and untreated fellow eyes.

Table 1.3. Summary of literature reporting opposite changes between the two eyes

Species	Monocular Treatment	Findings	Source
Tree shrew	Form deprivation	While 30 days of form deprivation caused myopia with elongated vitreous chamber depth, the fellow eyes developed a significant hyperopic shift with significantly shorter vitreous chamber depth compared with age-matched normals.	McBrien and Norton, 1992[219]
Chick	Spectacle lens	The fellow eyes of –10 D lens-wearing eyes became more hyperopic with shorter vitreous chamber depth than those of +10 D lens-wearing eyes.	Schmid and Wildsoet, 1996[137]
Tree shrew	Form deprivation	While form deprivation increased the level of latent gelatinase in the posterior sclera in the form deprived eyes, the level of latent gelatinase in the fellow eyes became lower than that in normal eyes.	Guggenheim and McBrien, 1996[220]
Tree shrew	Spectacle lens	After 4 days of monocular –5 D lens wear, while the creep rate and the rate of axial elongation in the treated eyes were increased, those in the control eyes became lower than age-matched normals.	Siegwart and Norton, 1999[221]
Rhesus Macaques	Form deprivation	Some untreated eyes showed hyperopic errors that were larger than those found in the age-matched normal controls.	Smith and Hung, 2000[106]
Rhesus Macaques	Form deprivation	The fellow, non-deprived eyes were more hyperopic with shorter vitreous chamber depth compared with the eyes in age-matched normal monkeys. The anti-yoking effect on the fellow eyes seemed to depend on the duration of form deprivation on the treated eyes: Longer daily periods of form deprivation generally caused larger ametropia in the non-treated fellow eyes.	Smith *et al.*, 2002[139]
Guinea pig	Form deprivation	During the first 2 weeks of form deprivation, the fellow eyes of the lid-sutured eyes developed a small and transient hyperopic shift with shorter vitreous chamber compared with the eyes from normal animals.	Lu *et al.*, 2006[222]
Rhesus Macaques	Form deprivation	The untreated fellow eye exhibited no sign of emmetropization and was >2 SD more hyperopic than the age-matched control	Smith *et al.*, 2007[213]
Chick	Spectacle lens	While kept under blue or red light, monocular positive lens-wear caused the fellow eyes to elongate more than the fellow eyes of negative lens-wearing eyes.	Rucker and Wallman, 2008[223]
Chick	Form deprivation	After recovery from monocular form deprivation, scleral glycosaminoglycan synthesis in the fellow eyes became twice as high compared with that found in the fellow eyes after monocular form deprivation.	Rucker *et al.*, 2009 [199]
Rhesus Macaques	Form deprivation	The untreated fellow eyes developed atypical central refractive errors or abnormal patterns of peripheral refractions.	Smith *et al.*, 2009[224]
Marmoset	Soft contact lens	The fellow eyes of positive lens-wearing eyes became significantly longer and more myopic than those of the negative lens-wearing eyes.	Troilo, Totonelly, and Harb, 2009[225]
Chick	Spectacle lens	Choroids in the fellow eyes of negative lens-wearing eyes were thicker than those of positive lens-wearing eyes.	Zhu and Wallman, 2009[146]

Despite the large number of studies that have reported phenomena consistent with either yoking and anti-yoking, these effects have not been systematically studied. An analysis was undertaken to study the interactions between paired eyes in chicks in Chapter 6. It is hypothesized that eye growth in paired eyes is well correlated (symmetrical growth) and that there is a yoking effect in addition to symmetrical growth.

1.8 Aims and hypotheses

The overall aim of this thesis is to investigate the effect of non-visual and/or size-factors in eye growth control and their interaction with those initiated by defocus. Single-powered positive and negative spectacle lenses will be used for this thesis, and the interaction between defocus and eye length or size will be studied for positive and negative lenses separately.

It is hypothesized that the non-visual factors could be related to a system that registers or is predisposed to match the growth of the two eyes. This general aim was pursued with the following specific aims in five Experimental Chapters (3-7) as described below.

1.8.1 Chapter 3: Evidence for a non-visual cue that guides recovery from abnormal eye sizes in the chick

The existence of the hypothesized intrinsic size-factor in chick eyes was investigated in this Chapter, by studying recovery from prior positive and negative lens treatment while the chicks were kept in darkness (to eliminate visual factors).

It was hypothesized that if a size-factor exists in chick eyes and is involved in regulating eye growth, it would be able to guide the eye to recover from prior lens treatment and regain normal eye length without any visual input.

1.8.2 Chapter 4: The effect of eye size on monocular lens compensation in chicks

After establishing the existence of a size-factor in chick eyes in Chapter 3, its effect in lens compensation was further studied in Chapter 4 in the following two ways:

1.8.2.1 Constant vs. Stepped Lens Powers

To investigate if chick eyes could compensate for a stepped change in defocus when the size- and defocus-factors required opposite directions for correct compensation, the time course of lens compensation for a weak lens then stepping up to a stronger lens of the same sign but of a larger magnitude was compared with a control group that wore the stronger lens from the beginning of the experiment. **It was hypothesized that if the ocular growth pathway is sensitive to abnormal eye size, then this "size-factor" could prevent the eye from deviating from its pre-determined normal length and thus reduce compensation for the stronger lens.**

1.8.2.2 Stepped Lens Powers vs. Recovery

The ocular growth response was compared between the two groups when they both experienced the same amount of defocus but the size-factor worked in the opposite directions to that provided by the defocus cues: Spectacle lenses from one group were removed so the eyes could recover from prior lens treatment (both the defocus- and size-factors working in the same direction), whereas lens powers in the second group were stepped up (the defocus- and size-factors working in the opposite directions). **It was hypothesized that the recovery group would show a more complete compensatory ocular response than the step-up group since both the defocus- and size-cues were working in the same direction, thus complementing each other.**

1.8.3 Chapter 5: Chick eyes can shorten to compensate for myopic defocus

After discovering that the defocus-factor dominated the size-factor in the case of positive lens compensation in Chapter 4, and since tissues are continuously remodeled under

a homeostatic control, analyses were performed of the change in axial length during positive lens wear, across 11 studies from the data base from Josh Wallman's laboratory at the City College of the City University of New York: Change in both axial length and vitreous chamber after wearing positive lenses for 3 days was compared to those in normal, untreated chicks during the same duration.

Specifically, it was hypothesized that chick eyes would be able to shorten axially to compensate for myopic defocus caused by positive lenses when compared to chick eyes from untreated animals.

1.8.4 Chapter 6: Interaction between paired eyes: Symmetrical growth, yoking, and anti-yoking

Since multiple studies have reported instances of either yoking and anti-yoking effects, but there are no systematic investigations, in Chapter 6 several analyses were performed to compare the changes in axial length in the fellow eyes of monocular lens-wearing chicks to that in eyes from age-matched, untreated animals. **It was generally hypothesized that there may be interactions between the two eyes that affects eye growth, i.e., yoking and anti-yoking.**

In particular, it was hypothesized wearing a positive lens over one eye would reduce the rate of axial elongation in the fellow eyes, compared to eyes from age-matched, untreated animals. It was also hypothesized that wearing a negative lens over one eye would increase the rate of axial elongation in the fellow eyes, compared to eyes from untreated animals. Lastly, it was hypothesized that the magnitude of this inter-ocular interaction would be positively affected by the duration of lens treatment.

1.8.5 Chapter 7: The effect of eye size on binocular lens compensation in chicks

In the last experimental chapter, the effect of the size-factor in binocular lens treatment was studied. It is possible that, while the size-factor may dominate the defocus-factor in the case of monocular negative lens wear as demonstrated in Chapter 4, the yoking-

effect demonstrated in Chapter 6 might override these effects. This hypothesis was tested in the following two ways:

1.8.5.1 Stepped vs. Constant Lens Powers

Chicks were raised first with a weak positive or negative lens over one eye, then the weak lens was stepped up to a stronger lens of the same sign and the fellow eye started to wear a weaker lens of the same sign. It was reasoned that such binocular treatment would facilitate compensation for the strong negative lens.

Specifically, it was hypothesized that superimposing defocus of the same sign and magnitude in both eyes would maximize negative lens compensation.

1.8.5.2 Lens Wear vs. Recovery

Chicks were raised first with a weak positive or negative lens over one eye, then the weak lens was removed for recovery, and the fellow eye started to wear a weak lens of the opposite sign, so both eyes were superimposed with the same defocus after the time of the lens change. **It was hypothesized that superimposing defocus of the same sign and magnitude on both eyes, would facilitate faster recovery compared with superimposing defocus on only one eye during recovery.**

Table 1.4. Summary of hypotheses for Chapters 3 to 7

Chapter	Hypotheses
3. Evidence for a non-visual cue that guides recovery from abnormal eye sizes in the chick	If a size-factor exists in chick eyes and is involved in regulating eye growth, it would be able to guide the eye to recover from prior lens treatment and regain normal eye length without any visual input.
4. The effect of eye size on monocular lens compensation in chicks	1. If the ocular growth pathway is sensitive to abnormal eye size, then this "size-factor" could prevent the eye from deviating from its pre-determined normal length and thus reduce compensation for the stronger lens. 2. The recovery group would show a more complete compensatory ocular response than the step-up group since both the defocus- and size-cues were working in the same direction, thus complementing each other.
5. Chick eyes can shorten to compensate for myopic defocus	Chick eyes would be able to shorten axially to compensate for myopic defocus caused by positive lenses when compared to chick eyes from untreated animals.
6. Interaction between paired eyes: Symmetrical growth, yoking, and anti-yoking	1. There may be interactions between the two eyes that affects eye growth, such as yoking and anti-yoking, whereby: a). Wearing a positive lens over one eye would reduce the rate of axial elongation in the fellow eyes, compared to eyes from age-matched, untreated animals. b). Wearing a negative lens over one eye would increase the rate of axial elongation in the fellow eyes, compared to eyes from untreated animals. 2. The magnitude of these inter-ocular interactions would be positively affected by the duration of lens treatment.
7. The effect of eye size on binocular lens compensation in chicks	1. Superimposing defocus of the same sign and magnitude in both eyes would maximize negative lens compensation. 2. Superimposing defocus of the same sign and magnitude on both eyes, would facilitate faster recovery compared with superimposing defocus on only one eye during recovery.

2. General Methods

2.1 Animals

White leghorn chicks were used in all experiments. Most chicks were hatched from eggs obtained from Cornell University (Cornell K-strain; Ithaca, NY) unless otherwise indicated. Once hatched, all chicks were housed in heated brooders (91 x 76 cm), with a 14:10 hour light:dark cycle under daylight balanced fluorescent illumination (approximately 300 lux, lights off from 10 pm to 8 am) prior to the start of experiments, with food and water *ad libitum*[146]. Chicks were kept in heated, sound-attenuated chambers (76 x 61 cm) during experiments, with a 14:10 hour light:dark cycle under daylight balanced fluorescent illumination, unless otherwise specified.

Unless otherwise specified, chicks were one week old when experiments started, and refractive errors (myopia and hyperopia) were induced using glass spectacle lenses (see Section 2.2 below), worn in front of one eye (the experimental eye, "X"), and the contralateral eyes were left untreated (the fellow eye, "N"). Care and use of all animals adhered to the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. The protocols used were approved by the Institutional Animal Care and Use Committee at the City College of the City University of New York and by the Animal Ethics Committee at the University of Newcastle (Protocol #575).

Data from Chapters 3 and 5 was based on retrospective analyses of previous date from the Wallman Database at the City College of the City University of New York, whereas data from Chapters 4, and 7 was based on prospective analyses of data collected for the purpose of this thesis. For Chapter 6, data from groups 30 to 46 was from retrospective analyses, whereas data from groups 47 to 63 was from prospective analyses.

2.2 Spectacle lenses used

Glass lenses (not conspicuously curved) of various powers with a diameter of 12 mm were used (see Methods for each chapter for details). Each lens was glued between a rigid plastic ring and a Velcro ring. The lens was then attached to a mating Velcro ring glued to

the feathers around the chicks' eyes. This allowed the lenses to be regularly replaced with clean lenses as necessary. Lenses were cleaned at least twice a day to keep them clean[146].

The vertex distance was estimated to be 4 mm. Using the equation of Fc=F/(1-xF), with Fc being the power corrected for vertex distance, F being the original lens power, x being the change in vertex distance in meters, the corrected powers for –5, –7, –10, –15, +5, +7, +10, +15 D lenses are –4.90, –6.81, –9.62, –14.15, +5.10, +7.20, +10.42, and +15.96 D, similar to the original lens powers. The vertex distance would make the negative lenses slightly weaker than the original powers by an average of 3.5%, and make the positive lenses slightly stronger than the original powers by an average of 3.9%.

2.3 Measurements of refractive error and ocular dimensions

Measures were made in both the treated and fellow untreated eyes. The Spherical Equivalent Refractive Error in diopter (D), calculated by adding half of the cylindrical power to the spherical power, was measured during anesthesia with a Hartinger refractometer (Jena Coincidence Refractometer, Carl Zeiss, Jena, Germany, Fig. 2.1) modified for small pupils, as previously described[105]. Chicks were anaesthetized with 1.5% of isoflurane in oxygen[226] without cycloplegic agents, prior to the refraction measurement. We discovered that isoflurane produces a moderate degree of mydriasis and presumably of cycloplegia. The alignment of the refractometer was facilitated by acquiring central corneal reflections of a ring of light attached to the scope facing the eye. Six to 8 readings were taken at both 0 and 90 degrees for each measurement to ensure accuracy. This method yields repeated refraction measurements and low interoperator variability (average SD within ± 0.3 D)[227].

Figure 2.1. A photograph of the Hartinger refractometer use.

Internal ocular dimensions on-axis were measured during anesthesia using A-scan ultrasonography (Fig. 2.2). A-scan was conducted with a 30 MHz transducer (Panametrics Model 176599) and sampled at 100 MHz with a Sonix 8100 A/D board[75]. The internal ocular dimensions consisted of the anterior chamber depth (defined as the distance between the posterior surface of the cornea and the anterior surface of the lens), lens thickness, vitreous chamber depth (defined as the distance between the posterior surface of the lens and the anterior surface of the retina), retinal thickness, choroidal thickness, and scleral thickness. Axial length was defined as the sum of anterior chamber depth, lens thickness, vitreous chamber depth, and the thicknesses of the retina, choroid, and sclera. Note that changes in the choroidal thickness only affected the vitreous chamber depth and did not affect axial length.

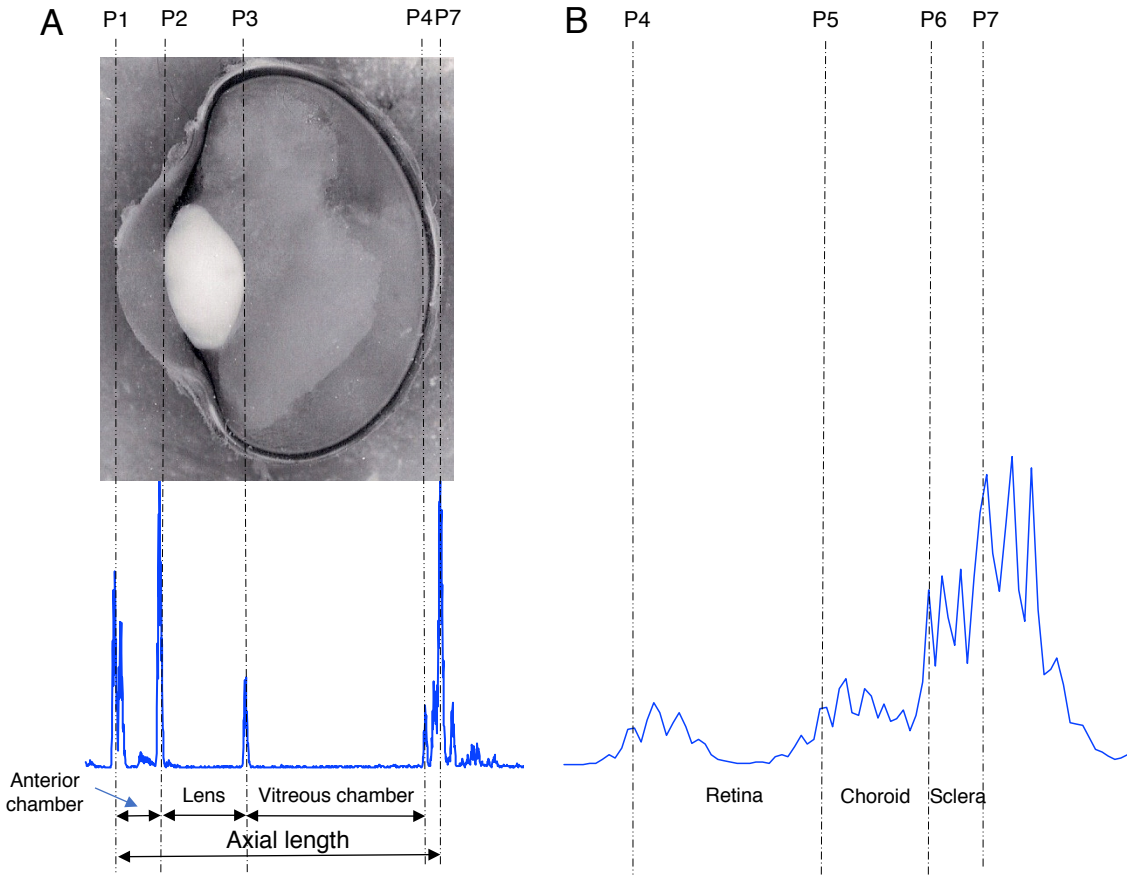

Figure 2.2. Axial ocular dimensions measured using A-scan high-frequency ultrasound biometry. (A) Peaks 1 to 4 are landmarks indicating the anterior surface of the cornea, anterior surface of the lens, posterior surface of the lens, the interface between the vitreous chamber and the retina. (B) Peaks 4 to 7 (in an enlarged view) are landmarks indicating the interface between the vitreous chamber, the anterior surface of the choroid, and anterior and posterior surfaces of the sclera.

Alignment of the transducer with the eye's optic axis was maximized by observing the corneal reflections of a small ring of light that was mounted around the transducer along the axis of the ultrasound beam. In addition, the transducer was mounted on a small pantograph arm which allowed the transducer to rotate normal to an imaginary sphere centered about a fixed point along the axis of the ultrasound beam. Proper alignment of the transducer was shown by the presence of the clear echoes from the anterior cornea, both lens surfaces, and the retina, the choroid, and the sclera (Fig. 2.2)[105]. The velocities at which ultrasound travels at various ocular compartments were determined by measuring the difference in time it takes for ultrasound to travel in saline in a beaker with and without

a lens[105]. The velocities in lenses, aqueous/vitreous humors were determined to be 1.6078 mm/μsec (SD 0.0064 mm/μsec) and 1.534 m/sec[105]. The repeatability of the measurement was estimated to be approximately ± 20 μm for all ocular components[226].

2.4 General data analyses

Parametric distribution for results critical to interpretation was undertaken by Normality Testing (Shapiro-Wilk) and Equal Variance Testing using SigmaPlot (V 12.5, Systat Software, Inc., CA, USA). Power analyses were not included for each comparison, but they were in general undertaken for the most important outcomes by G*Power (V 3.1.9.3). Power analyses show that the sample sizes were higher than necessary for the effect size with high *p* values.

For within-group comparisons: (1) For each variable (refractive error and each ocular dimension), the mean change over either the course of the experiment or a certain interval in the treated eye (ΔX) was compared with the mean change in the untreated fellow eye (ΔN) in the same animal using 2-tailed, paired *Student*'s t-tests, or with that in normal eyes (from age-matched untreated animals) using 2-tailed, unpaired *Student*'s t-tests. The difference between the changes in each eye were calculated for each animal and averaged for each group. (2) Separately for each variable, the mean values in the experimental and fellow eyes were compared at different time points (for example, before and after a lens power change) using Two-Way Mixed Measures Analysis of Variance (ANOVA). (3) Mean inter-ocular differences (X – N) at different time points were compared using One-Way Repeated Measures ANOVA. For both (2) and (3), post hoc comparisons used the Holm-Sidak adjustment method.

For between-group comparisons: (1) Inter-ocular difference (X – N) at various time points were compared using Two-Way Mixed Measures ANOVA with the Holm-Sidak adjustment for multiple group comparisons. (2) The relative changes (change in the experimental eyes over the course of the experiment minus the change in the untreated eyes, ΔX-ΔN) from two groups were compared using 2-tailed, unpaired *Student*'s t-tests.

The above analyses were conducted using SigmaPlot (V 12.5, Systat Software, Inc., CA, USA). In addition, DataDesk (V 7.0.2, Data Description Inc., NY, USA) was used to

calculate the *p* values for the Coefficient for linear regression, and to conduct a Two-Way ANOVA to see if the adjusted change in axial length in the fellow eyes was significantly different across all lens-wearing durations and lens powers. Data given in experimental chapters 3, 4, 6, and 7 is the mean and the standard error of the mean (SEM), while in Chapter 5, frequency distributions are shown, and the data given is the mean and the standard deviation (SD).

3. Evidence for a Non-Visual Cue That Guides Recovery from Abnormal Eye Sizes in the Chick Eye

3.1 Forward

This series of experiments tests for the existence of a proposed intrinsic size-factor regulating eye growth by studying the ability of eyes with altered growth, to recover in darkness (i.e. without a defocus-factor). This chapter includes data from previous experiments conducted at Josh Wallman's laboratory at the City College of the City University of New York.

The following hypothesis is proposed:

A non-visual factor (referred to as a "size-factor") exists in chick eyes and can initiate and guide the direction of eye growth. Specifically, eyes that are already too long (myopic) or too short (hyperopic) can regain their normal length while kept in the darkness for only 2 or 3 days. This is different from previous studies in which treated animals were kept in constant darkness or much longer durations. "Normal" is defined as the growth in the untreated fellow eye under the same light conditions.

Some of these results have been presented in an abstract form (Zhu X, Wallman J, and McFadden SA, *Invest. Ophthalmol. Vis. Sci.* 2016, E-Abstract 3791).

3.2 Abstract

Purpose: The size of all body-parts, including the eye, is regulated by intrinsic homeostatic developmental mechanisms (here referred as the "size-factor"). Eyes have an additional, visual, homeostatic mechanism, permitting compensation for superimposed defocus (here referred to as the "defocus-factor"). These mechanisms can work in either the same or opposite directions. The existence of the size-factor in chick eyes was investigated in this chapter by studying if chick eyes can recover from prior positive and negative lens treatment in darkness without the defocus-factor.

Methods: All chicks wore either a +7 or –7 D lens over one eye for a few days, and the fellow eye was left untreated. Then the lenses were removed, and recovery in darkness (n = 8 and 11 for +7 D and –7 D lens treated animals, respectively) was compared to that under normal light (n = 8 and 5 for +7 D and –7 D lens treated animals, respectively). Refractive error and ocular dimensions were measured before and after lens treatment, and repeatedly at various intervals during lens treatment and recovery with a Hartinger refractometer and A-scan biometry, respectively.

Results: While chick eyes completely recovered from prior lens treatment under normal light after 2 days, they also partially recovered from prior hyperopia (by 60%) and myopia (by 69%), respectively, after being kept in darkness for 3 days, supporting the existence of a non-visual factor that guides the direction of eye growth.

Conclusions: A non-visual homeostatic factor seems to exist in chick eyes, that can guide the eyes to grow towards the direction to regain the same length as the fellow untreated eye. It is likely that this non-visual homeostatic factor is involved in emmetropization.

3.3 Introduction

Numerous animal studies over the last four decades have established that post-natal growth is actively controlled by visual signals. Indeed, to perceive clear images of far objects on the retina (i.e. without accommodation), the physical length of the eye must match its optical length, and such an eye is described as emmetropic. Most eyes are not emmetropic at the time of birth, and the eyes emmetropize by actively modulating eye growth guided by the visual signals that the eye experiences during post-natal development. Animal studies have shown that eyes emmetropize by changing their axial length in response to the defocus experienced by the eye during development[28]. Specifically, when a young growing eye wears a negative lens that focuses distant images behind the retina (hyperopic defocus), the eye increases its rate of ocular elongation effectively compensating for the imposed hyperopia. On the other hand, eyes that wear a positive lens that displaces the image plane anteriorly (myopic or relative myopic defocus), the eye reduces its rate of ocular elongation. This has been demonstrated in chicks[78, 195], monkeys[202], marmosets[228], tree shrews[229], guinea pigs[230], and in some species of mice[231]. In addition, chick eyes also change the thickness of their choroid, which effectively changes the location of the retinal plane to meet the focal plane and allows the rapid establishment of emmetropia with the lens in place[80].

Animals which have compensated for lenses, also recover from their abnormal growth rates when the lens is removed. For example, once the eye has compensated for positive lens wear by slowing its rate of axial elongation, over time the eye becomes relatively short compared to a non-lens-wearing eye, so that subsequent removal of the positive lens will cause the eye to be relatively hyperopic [80, 105]. The eye then increases its rate of ocular elongation (and decreases its choroidal thickness) to compensate for this hyperopia or to recover from prior positive lens wear and eventually regain emmetropia. The opposite process happens when an eye recovers from myopia induced with form deprivation or from wearing a negative lens. When the diffuser or negative lens is removed, the eye readily inhibits its ocular elongation. These processes of reversing prior lens compensation are called “recovery”.

Recovery from myopia has been demonstrated in all species studied to date, including tree shrews[64], rhesus monkeys[63, 202, 232], chicks[78, 80, 195], marmosets[233, 234], guinea pigs[235], and mice[107, 236].

This chapter investigates whether vision is necessary for this recovery process. Several studies have suggested that non-visual factors may be involved in the control of eye growth. First, prolonged dark-reared chicks (for 4 weeks) continue to change their ocular dimensions after being returned to normal lighting even after emmetropization has been re-attained[188]. Secondly, Schaeffel and Howland[120] discovered that chick eyes recovered from form deprivation myopia (eyes already longer than normal) even when the refractive error was optically corrected with negative lenses of the appropriate power, concluding that there is a "non-visual mechanism highly sensitive to abnormal eye shape". Furthermore, McFadden *et al.* have shown that guinea pigs can recover from form deprivation myopia after 3 days of darkness[237]. Most dramatically, it was discovered that chicks eyes made very asymmetric in shape and myopic in only the nasal retina by wearing diffusers that only covered the nasal visual field recovered normal symmetry even after the retina has been lesioned by tunicamycin, showing that the non-visual factors could return the eye to its normal shape[189]. In addition, it has been recently speculated that a variety of non-visual mechanisms might potentially explain the prolate shape changes associated with axial myopia development [238].

In the current study, recovery from prior positive or negative lens treatment was investigated while chicks were kept in darkness for 2 to 3 days. If a non-visual factor can guide recovery, eyes made shorter (hyperopic) or longer (myopic) than normal should be able to recover in the darkness without any visual stimuli.

It was discovered that chick eyes partially recovered from prior lens treatment while kept in darkness, suggesting that some non-visual homeostatic factor(s) seems to exist in chick eyes and can guide the eyes to grow towards the direction to regain the normal, age-appropriate eye size or length

3.4 Methods

3.4.1 Animals

White Leghorn chicks (n = 32) were obtained and housed as described in the General methods (Section 2.1). Lighting was the same as that described in the General methods (Section 2.1).

3.4.2 Experimental procedures

In four separate groups, 32 chicks wore either a +7 D (groups 1 and 2) or a –7 D (groups 3 and 4) spectacle lens in front of one eye (the experimental eye) for at least 4 days (sufficient to induce robust compensation), and the other eye was left as the untreated control (the fellow eye). Glass lenses of –7 and +7 D were used, as previously described[146]. Lenses were removed at various ages, and animals either continued to be held in their normal 14:10 hour light:dark cycle for 2 days, or were put into complete darkness for 3 days. During dark rearing, chicks' crops were checked daily to make sure that they were eating normally, and no issues were discovered. The chicks were kept in light-proof chambers, and food and water containers were removed from the cage and lenses detached from the Velcro rings in total darkness. Containers were replenished and lenses were cleaned in light with the cage door closed, then moved back into the cage and lenses re-attached to the Velcro rings in darkness.

Refractions and biometry measurements were taken before and after lens treatment, and again after a recovery period, all under normal lighting conditions. Details of treatment details, length of lens-wear and ages, measurement times and samples size for each experimental group is shown in Table 3.1.

Table 3.1. Summary of treatment details, the predicted effects of the size- and defocus-factors, and sample size (n)

Group #	Lens type	Details (age in days)	Size- vs. defocus- factor direction during recovery*	n
1	Plus	+7 D lens wear for 7 days (7-14), then recovery in **normal light** for 2 days (14-16)	S: ↑ growth; D: ↑ growth	8
2		+7 D lens wear for 4 days (7-11), then recovery in **darkness** for 3 days (11-14)	S: ↑ growth; D: absent	8
3	Minus	–7 D lens wear for 5 days (1-6), then recovery in **normal light** for 2 days (6-8)	S: ↓ growth; D: ↓ growth	5
4		–7 D lens wear for 4 days (7-11), then recovery in **darkness** for 3 days (11-14)	S: ↓ growth; D: absent	11

* S: Size-factor; D: Defocus-factor

Shadowed and un-shadowed rows are groups used for comparison, respectively

3.4.3 Measurements

Measurements are detailed in the General Methods, Section 2.3. In brief, refractive error and ocular dimensions were measured while the chicks were anesthetized with 1.5% of isoflurane using a modified Hartinger refractometer and A-scan ultrasonography respectively.

3.4.4 Analyses

Analyses are described in the General methods, Section 2.4. Briefly, data are shown as mean ± SEM for inter-ocular differences (X – N) in Table 3.2. The detailed values for each eye for these parameters on various days are shown in Table A1.1 in Appendix 1.

Two-Way Mixed Measures ANOVA (SigmaPlot V12.5) were used to compare mean values in the experimental eyes (X) and fellow eyes (N) on various days (X vs. N at different time points), and *p* values from post-hoc comparisons for X vs. N at each time point are shown in Table 3.2. Two-tailed, unpaired *Student's* t-tests were used to compare the change in ocular dimensions and refractive error in experimental eyes and their fellow eyes between various intervals (ΔX vs. ΔN).

Table 3.2. Summary of inter-ocular difference (X – N, Mean ± SEM) for ocular dimensions and refractive error

Group	Age	Anterior chamber depth (mm)	*p*	Lens thickness (mm)	*p*	Vitreous chamber depth (mm)	*p*	Choroidal thickness (mm)	*p*	Axial length (mm)	*p*	Refractive error (D)	*p*
1	7	-0.02 ± 0.01	0.072	0.00 ± 0.01	0.782	0.00 ± 0.01	0.855	0.03 ± 0.03	0.086	0.03 ± 0.03	0.393	-0.46 ± 0.49	0.393
	14	0.00 ± 0.01	0.731	-0.01 ± 0.01	0.445	-0.29 ± 0.03	**≤ 0.001**	0.09 ± 0.02	**≤ 0.001**	-0.19 ± 0.03	**≤ 0.001**	6.34 ± 0.71	**≤ 0.001**
	16	0.00 ± 0.01	0.829	-0.05 ± 0.01	**≤ 0.001**	-0.05 ± 0.02	**0.020**	0.01 ± 0.01	0.741	-0.09 ± 0.03	**0.003**	0.27 ± 0.49	0.614
2	7	-0.02 ± 0.01	0.386	0.01 ± 0.01	0.580	0.00 ± 0.01	0.920	0.02 ± 0.02	0.186	0.01 ± 0.02	0.760	0.89 ± 0.65	0.348
	11	-0.06 ± 0.02	**0.005**	-0.04 ± 0.01	**0.041**	-0.23 ± 0.02	**≤ 0.001**	0.12 ± 0.02	**≤ 0.001**	-0.20 ± 0.03	**≤ 0.001**	7.08 ± 0.80	**≤ 0.001**
	14	-0.07 ± 0.03	**0.004**	-0.02 ± 0.02	0.119	-0.08 ± 0.04	**≤ 0.001**	0.05 ± 0.00	**0.003**	-0.12 ± 0.04	**≤ 0.001**	3.90 ± 0.75	**≤ 0.001**
3	1	0.00 ± 0.01	0.940	0.00 ± 0.00	0.985	0.08 ± 0.04	**0.024**	-0.02 ± 0.02	0.337	0.06 ± 0.04	0.199	0.14 ± 0.41	0.718
	6	-0.05 ± 0.03	0.151	-0.05 ± 0.02	0.904	0.42 ± 0.02	**≤ 0.001**	-0.06 ± 0.01	**0.029**	0.25 ± 0.05	**≤ 0.001**	-4.62 ± 0.71	**≤ 0.001**
	8	-0.04 ± 0.04	0.058	-0.02 ± 0.02	**0.017**	0.16 ± 0.03	**≤ 0.001**	0.12 ± 0.02	**0.017**	0.21 ± 0.05	**≤ 0.001**	1.31 ± 0.33	**≤ 0.001**
4	7	0.01 ± 0.01	0.677	-0.01 ± 0.01	0.526	-0.02 ± 0.02	0.293	0.01 ± 0.02	0.814	-0.01 ± 0.02	0.858	-0.50 ± 0.32	0.393
	11	-0.01 ± 0.02	0.835	-0.02 ± 0.01	0.184	0.20 ± 0.02	**≤ 0.001**	-0.05 ± 0.03	**0.027**	0.11 ± 0.03	**≤ 0.001**	-4.69 ± 0.47	**≤ 0.001**
	14	-0.05 ± 0.04	**0.036**	-0.03 ± 0.02	0.069	0.14 ± 0.02	**≤ 0.001**	-0.01 ± 0.02	0.715	0.02 ± 0.04	0.523	-1.81 ± 0.56	**0.002**

p: The mean values in the experimental and fellow eyes were compared at different time points measured using Two-Way Mixed Measures Analysis of Variance (ANOVA), with the Holm-Sidak adjustment method. See Table A1.1 in Appendix 1 for the mean values in the experimental and fellow eyes on various days. *p* values of statistical significance are underlined and bold.

Refer to Table 3.1 above for group definitions.

3.5 Results

In summary, chicks developed hyperopia or myopia after wearing +7 D or –7 D lenses, respectively. Eyes rapidly recovered from this prior hyperopia or myopia after only 2 days in normal light. In comparison, chick eyes only partially recovered when kept in darkness for 3 days.

3.5.1 Recovery from prior positive lens wear

Wearing +7 D lenses over one eye for either 7 (group 1) or 4 days (group 2) caused robust compensation, and the lens-wearing eyes became significantly more hyperopic than their fellow eyes (mean inter-ocular difference at 14 days of age for group 1 and 11 days of age for group 2, both shown as day 0 in Fig. 3.1, X – N, +6.34 D for group 1, +7.08 D for group 2; $p < 0.001$ between the two eyes in each group, Fig. 3.1A, see Table 3.2 for details), with significantly reduced axial length (–0.19 mm for group 1, –0.20 mm for group 2; $p < 0.001$ in each group; Fig. 3.1B) and vitreous chamber depth (–0.29 mm for group 1, –0.23 mm for group 2; $p < 0.001$ in each group; Fig. 3.1C), and thickened choroids (+0.09 mm for group 1, +0.12 mm for group 2, $p < 0.001$ in each group; Fig. 3.1D). The inter-ocular

difference at the end of lens-wear (normalized age day 0 in Fig. 3.1) between groups 1 and 2 was not significantly different for refractive error, axial length, vitreous chamber depth, or choroidal thickness (p = 0.503, 0.842, 0.107, 0.138 respectively, Holm-Sidak comparisons after ANOVA).

After the positive lenses were removed at 14 days of age for group 1, as expected, chick eyes significantly recovered from prior hyperopia by 92% after staying in normal light for only 2 days (Relative difference in 2 days, ΔX – ΔN between 14 and 16 days of age, 2 days after lens removal, Mean ± SEM, –6.07 ± 0.64 D; X – N, the day of lens removal on day 14 vs. day 16, $p < 0.001$, Table 3.2 and Fig. 3.1A). Specifically, the axial reduction caused by prior +7 D lens wear recovered by 46% (ΔX – ΔN 2 days after lens removal, +0.10 ± 0.05 mm; X – N, the day of lens removal on day 14 vs. day 16, $p > 0.05$, Fig. 3.1B), vitreous chamber depth recovered by 83% (ΔX – ΔN, +0.24 ± 0.03 mm; X – N, day 14 vs. day 16, $p < 0.05$, Fig. 3.1C), and choroidal thickening recovered by 133% (ΔX – ΔN, –0.08 ± 0.02 mm; X – N, day 14 vs. day 16, $p > 0.05$, Fig. 3.1D).

In comparison, after the positive lenses were removed at 11 days of age for group 2, chick eyes recovered from prior hyperopia by 60% after staying in darkness for 3 days (ΔX – ΔN between 11 and 14 days of age, 3 days of recovery, –3.18 ± 0.59 D; X – N, the day of lens removal on day 11 vs. day 14, p = 0.066, Table 3.2 and Fig. 3.1A). Indeed, all treated eyes (8 out of 8) developed a myopic shift. The prior reduction in axial length and vitreous chamber depth changed to an increased elongation and recovered by 42% (for axial length: ΔX – ΔN, +0.08 ± 0.02 mm; X – N, day 11 vs. day 14, $p > 0.05$, Fig. 3.1B) and 65% (for vitreous chamber: ΔX – ΔN, +0.15 ± 0.04 mm; X – N, day 11 vs. day 14, $p < 0.05$, Fig. 3.1C), respectively. Choroidal thickening recovered by 70% (ΔX – ΔN, –0.07 ± 0.02 mm; X – N, day 11 vs. day 12, $p > 0.05$, Fig. 3.1D).

The relative change in refractive error in group 1 after two days of recovery in normal light was significantly greater than those in group 2 after three days of recovery in darkness (ΔX – ΔN 2 and 3 days after recovery for groups 1 and 2, respectively, group 1 vs. group 2, –6.07 vs. –3.18 D, $p < 0.01$). Interestingly, no statistical difference was found in axial length, vitreous chamber depth, or choroidal thickness for the relative recovery amounts between groups 1 and 2. No significant difference in relative difference was found for anterior

chamber depth or lens thickness between groups 1 and 2, either (Fig. A1.1 and Table A1.1 in Appendix 1). It is possible that the difference in refractive error was caused by a change in corneal curvature or the refractive index of the crystal lens, which were not measured in this thesis.

To summarize, chick eyes partially recovered from prior hyperopia after 3 days of darkness, and all the treated eyes reversed their direction of change in axial length, vitreous chamber depth, and choroidal thickness.

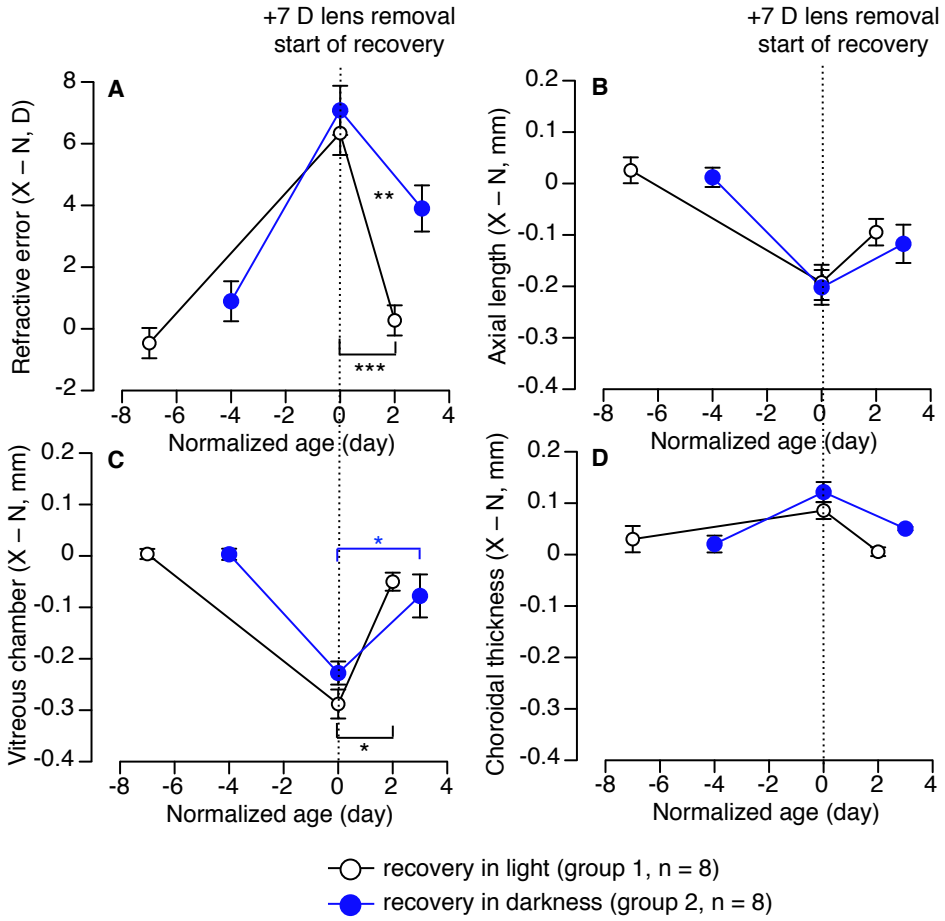

Figure 3.1. Comparison of recovery in the light and the dark after positive lens wear. Chicks wore +7 D lenses over one eye for 4 to 7 days (shown on the left side of the dashed line), then the lenses were removed and chicks were kept in either normal light (group 1, white circles) or darkness (group 2, blue circles, shown on the right side of the dashed line). Data is shown as the inter-ocular difference between the experimental and fellow eyes (X – N, Mean ± SEM) at various ages for (A) refractive error, (B) axial length, (C) vitreous chamber depth, and (D) choroidal thickness. Note that ages have been normalized so the day of lens removal (the start of recovery) is represented by zero on the X-axes, so days –7, 0, and 2 for group 1 in this figure correspond to 7, 14, and 16 days of age in Table 3.2, respectively; and days –4, 0, and 3 for group 2 in this figure correspond to 7, 11, and 14 days of age in Table 3.2, respectively. Asterisks with bars indicate statistical significant difference for inter-ocular difference (X – N) between various ages in each group (* $p <$ 0.05, *** $p < 0.001$, One-Way Repeated Measures ANOVA), and asterisks without bars indicate statistical significant difference for relative difference (ΔX – ΔN) between groups 1 and 2 during recovery (** $p < 0.01$, unpaired, 2-tailed *Student*'s t-test).

3.5.2 Recovery from prior negative lens wear

Wearing –7 D lenses over one eye for either 5 days (group 3) or 4 days (group 4) caused robust compensation, and the lens-wearing eyes became significantly more myopic than their fellow eyes (mean inter-ocular difference at 6 days of age for group 3 and 11 days of age for group 4, normalized age day 0 in Fig. 3.2; X – N, –4.62 D for group 3, –4.69 D for group 4; $p < 0.001$ between the two eyes in each group, Fig. 3.2A, see Table 3.2 for details), with significantly increased axial length (+0.25 mm for group 3, +0.11 mm for group 4; $p < 0.001$ in each group; Fig. 3.2B) and vitreous chamber depth (+0.42 mm for group 3, +0.20 mm for group 4; $p < 0.001$ in each group; Fig. 3.2C), and significantly thinned choroids (–0.06 mm for group 3, –0.05 mm for group 4; $p < 0.05$ for in each group; Fig. 3.2D). The inter-ocular difference at the end of lens wear (normalized age day 0 in Fig. 3.2) between groups 3 and 4 was significantly different for axial length ($p < 0.05$, Fig. 3.2B) and vitreous chamber depth ($p < 0.01$).

After the negative lenses were removed at 6 days of age for group 3, as expected, chick eyes significantly recovered from prior myopia by 125% after staying in normal light for only 2 days (Relative difference in 2 days, ΔX – ΔN, 2 days of recovery between 6 and 8 days of age, Mean ± SEM, +5.93 ± 0.80 D; X – N, the day of lens removal on day 6 vs. day 8, $p < 0.001$, Table 3.2 and Fig. 3.2A). Specifically, the axial elongation caused by prior –7 D lens wear significantly recovered by 20% (ΔX – ΔN, –0.04 ± 0.01 mm; X – N, day 6 vs. day 8, $p > 0.05$, Fig. 3.2B), vitreous chamber depth recovered by 68% (ΔX – ΔN, –0.23 ± 0.04 mm; X – N, day 6 vs. day 8, $p < 0.001$, Fig. 3.2C). The choroids thickened enormously to recover from prior myopia (ΔX – ΔN, +0.18 ± 0.02 mm; X – N, day 6 vs. day 8, $p < 0.001$, Fig. 3.2D), which explained the rapid reduction in vitreous chamber depth (Fig. 3.2C).

In comparison, after the negative lenses were removed at 11 days of age for group 4, chick eyes recovered from prior myopia by 69% after staying in darkness for 3 days (ΔX – ΔN 3 days after recovery between 11 and 14 days of age, +2.88 ± 0.49 D; X – N, on the day of lens removal on day 11 vs. day 14, $p < 0.01$, Table 3.2 and Fig. 3.2A). Specifically, all treated eyes (11 out of 11) developed a hyperopic shift. The prior increase in axial length and vitreous chamber depth changed to a reduction and recovered by 75% (for axial length, ΔX – ΔN, –0.09 ± 0.03 mm; X – N, day 11 vs. day 14, $p = 0.051$, Fig. 3.2B) and 27% (for

vitreous chamber, ΔX – ΔN, –0.06 ± 0.02 mm; X – N, day 11 vs. day 14, $p < 0.05$, Fig. 3.2C), respectively. Choroidal thinning recovered by 67% (ΔX – ΔN, +.04 ± 0.02 mm; X – N, day 11 vs. day 14, $p > 0.05$, Fig. 3.2D).

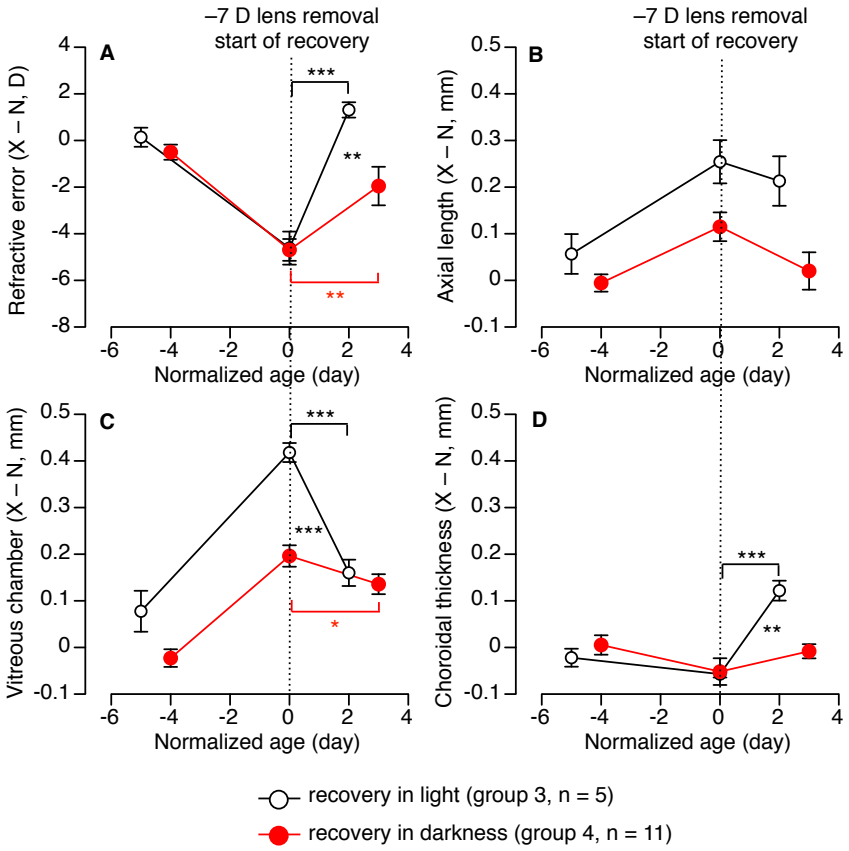

Figure 3.2. Comparison of recovery in the light and the dark after negative lens wear. Chicks wore –7 D lenses over one eye for 4 to 5 days (shown on the left side of the dashed line), then the lenses were removed and chicks were kept in either normal light (group 3, white circles) or darkness (group 4, red circles, shown on the right side of the dashed line). Data is shown as the inter-ocular difference between the experimental and fellow eyes (X – N, Mean ± SEM) at various ages for (A) refractive error**,** (B) axial length**,** (C) vitreous chamber depth, and (D) choroidal thickness. Note that ages have been normalized so the day of lens removal (the start of recovery) is represented by zero on the X-axes, so days –5, –2, 0, and 2 for group 3 in this figure correspond to 7, 14, and 16 days of age in Table 3.2, respectively; and days –4, 0, and 3 for group 4 in this figure correspond to 7, 11, and 14 days of age in Table 3.2, respectively. Asterisks with bars indicate statistical significant difference for inter-ocular difference (X – N) between various ages in each group (* $p < 0.05$, *** $p < 0.001$, One-Way Repeated Measures ANOVA), and asterisks without bars indicate statistical significant difference for relative difference (ΔX – ΔN) between groups 3 and 4 during recovery (** $p < 0.01$, *** $p < 0.001$, unpaired, 2-tailed *Student*'s t-test).

The relative change in refractive error in group 3 two days after recovery in normal light was significantly more than that in group 4 three days after recovery in darkness ($p < 0.01$, unpaired, 2-tailed *Student*'s t-test). The relative changes in both vitreous chamber depth and choroidal thickness in group 3 were significantly different from those in group 4 ($p < 0.001$ for vitreous chamber depth, $p < 0.01$ for choroidal thickness). No statistical difference was found in axial length between groups 3 and 4. See Fig. A1.2 in Appendix 1for changes in anterior chamber depth and lens thickness.

To summarize, after wearing positive or negative lenses for a few days, all the treated eyes recovered in the correct direction while in darkness.

3.6 Discussion

Briefly, the fact that chick eyes partially recovered from prior lens treatment while kept in darkness (in the absence of a visually mediated defocus-factor) supports the existence of some factor not related to vision that appears to "know" the expected length of the eye. This is referred to as a "size-factor" in our Discussion below.

3.6.1 The effect of dark rearing

It may be thought that some of the recovery observed here is simply a reflection of darkness and a lack of light cycle *per se*. Previous studies have shown that constant darkness (from days, weeks, to months) caused excessive axial elongation and a hyperopic shift in monkeys[239-241], kittens[242], and chicks[79, 171, 188, 243]. It should be noted that the effects of dark rearing may differ between species, since it has been shown that tree shrews (on day 27 of visual experience, approximately 7 weeks of age) developed significant myopia (–4.3 ± 0.5 D) with elongated vitreous chamber depth (0.09 ± 0.02 mm) and slight corneal flattening (average decrease 1.4 ± 0.3 D) after 10 days of constant darkness[244].

In chicks, the hyperopic shift with dark rearing is caused by corneal flattening[79, 188]. For example, after 4 weeks of dark rearing, chick eyes developed hyperopia (+8.24 D), significantly different from normal age-matched controls (+2.60 D), and the vitreous

chamber depth in these eyes were significantly longer than the age-matched normals (6.69 mm vs. 5.86 mm)[188]. The corneal curvature was significantly increased after both 2 and 6 weeks of dark rearing (after 2 weeks, 3.29 mm vs. 3.18 mm; after 6 weeks, 4.44 mm vs. 3.97 mm)[79]. These effects are quite different to what we observe here. In the current study, where chick eyes recovered in the darkness for only 2 to 3 days, hyperopic shifts after minus lens wear were accompanied by a decrease in eye length, and although corneal power was not measured, the anterior chamber appeared unaffected (refer to Figs. A1.1 and A1.2 and Table A1.1). Additionally, dark rearing effects are reported after longer periods than in the current study whereby changes occurred after only a few days. Certainly, they are unlikely to explain the opposite changes that we observe with positive versus negative prior lens wear.

Perhaps more relevant, dark rearing also has a small effect on the diurnal rhythm in axial length in untreated chicks[171]: While the rhythm in axial length still persisted while the chicks were kept in constant darkness for 4 days, the peak of the rhythm occurs slightly earlier than that of eyes in the normal 14:10 hour light:dark cycle, and the growth rate of the eye became significantly higher than normal eyes (117 vs. 72 μm / day). It is possible that such enhanced growth rates (predicted to be 117 μm over 24 hours versus 83 μm that we observe) may support the recovery from induced hyperopia after positive lens wear removal. However, if true, it suggests that the inhibited growth rate observed after negative lens-wear removal are an underestimate of the actual strength of the underlying inhibitory signal. Therefore, it is unlikely that chick eyes recovered from prior negative lens wear in darkness because of the effect dark rearing may have on the diurnal rhythm.

Regardless of the above effects, the fact that opposite changes in the direction of growth appropriate for the eye to regain a matched eye length with its untreated fellow eye, in the absence of any visual input, suggests that the eye growth control system has access to some kind of intrinsic homeostatic mechanism that either "knows" the expected absolute size of the eye for a particular age of development or is sensitive to differences between the two eyes.

3.6.2 Comparison between recovery rates in the light and dark

The absolute amount of recovery observed 2 days after lens removal in the light was similar for positive and negative lenses for refractive error, axial length, and vitreous chamber depth (Relative change, $\Delta X - \Delta N$, group 1 vs. group 3, $p > 0.05$, unpaired, 2-tailed, *Student*'s t-test), except for choroidal thickness ($\Delta X - \Delta N$, group 1 vs. group 3, $+0.18 \pm 0.02$ mm vs. -0.08 ± 0.02 mm, $p < 0.01$ for the absolute amount). On the other hand, in the absence of light, the absolute amount of recovery observed 3 days after lens removal was similar for positive and negative lenses (group 2 vs. group 4) for refractive error, axial length, and choroidal thickness, except for vitreous chamber depth ($\Delta X - \Delta N$, group 2 vs. group 4, $+0.15 \pm 0.04$ mm vs. -0.06 ± 0.02 mm, $p < 0.05$ for the absolute amount).

In the current study, recovery from myopia and hyperopia in the dark appeared less than recovery in the light. One limitation was the difference in starting age (e.g., chicks in groups 3 and 4 were 1 and 7 days old when the lens treatment started, respectively), the difference in the lens treating duration (e.g., chicks in groups 1 and 2 wore +7 D lenses for 7 and 4 days, respectively). However, it is unlikely that this limitation had a big impact on the results since the chick eyes developed approximately the same amount of refractive error along with changes in various ocular components before lens removal. Another limitation was the difference in the recovery period under light (2 days) and in darkness (3 days). But it is unlikely that these differences were the causes of the substantial differences in the amount of recovery in light vs. in darkness because, should a longer recovery period cause more recovery, chick eyes recovered in darkness would show more recovery, which is not what was discovered. Therefore, even though there are limitations in the experimental design, the results are valid in showing that chick eyes recovered more from prior lens treatment when recovered in light compared with in darkness.

3.6.3 Comparison with previous studies

Findings in this chapter agree with previous results in guinea pigs: McFadden *et al.* have shown that guinea pigs can recover from form deprivation myopia after 3 days of darkness[237]. Norton *et al.*[244], on the other hand, showed that tree shrew eyes that were myopic

as a result of prior lens treatment became more myopic after the animals were kept in constant darkness for 10 days. There was a consistent myopic shift in both the treated and the untreated fellow eyes, suggesting that a visual signal is necessary for recovery, i.e., a size-factor alone cannot guide recovery or emmetropization. These different results could be attributed to differences in the age of animals used in these two experiments: The guinea pigs used the McFadden study were very young (13 days after birth) when the eyes were still actively emmetropizing (guinea pig eyes emmetropize since birth until at least 30 days of life [245]; while the tree shrews used in the Norton study were older (around 48 days old on average) when they were placed in darkness, and were in a relatively late stage of their emmetropization process at this age[244]. Note that tree shrew eyes generally open at 3 weeks of age, and actively emmetropize from 2 weeks to 5 weeks after normal eye opening[215, 244]. It is also possible that the intrinsic-factor is weaker in tree shrews than in guinea pigs[244].

3.6.4 Possible mechanisms responsible for maintaining organ size or shape

The normal size of a tissue or organ is maintained through a highly coordinated regulatory process of cell growth, proliferation, and apoptosis[246]. It has been suggested that the size of a tissue or organ itself is regulated, independent of the cell size, cellular growth rate, and the environment in which the tissue or organ is grown, i.e., the size information is intrinsic to the tissue itself (a more detailed review can been seen in Crickmore and Mann (2008)[247]). Cell competition is an example of this intrinsic mechanism. The Hippo pathway has been shown to be a master regulator for size-determining purposes[246]. In addition, several candidate processes and signals have also been suggested to be potential contributors, such as Transforming Growth Factor-beta 1[248]. While it is not clear exactly what parameters are used to determine final organ size, previous studies argue against the simple "cell counting" or "amount of time spent growing" models of size regulation[247]. When it comes to the eye, even though the non-visual, intrinsic mechanism was referred to as a "size-factor" in this thesis, it is important to note that the actual mechanism does not necessarily only detect axial length to determine the correct eye size. Indeed, it may use any physical or chemical signals, such as pressure and temperature within the tissue.

3.6.5 The effect of starting lens treatment at different ages

One of the limitations of this experiment is that chicks started wearing lenses at different days of age: While chicks in groups 1, 2, and 4 started lens wear at 7 days of age, chicks in group 3 started lens wear at 1 day of age. Since untreated chicks emmetropize most rapidly within the first 3 days of life, and only minor, nonsignificant changes in refractive error occur thereafter[76], it is possible that chicks that started lens wear at 7 days of age (groups 1, 2, and 4) compensated less than those started at 1 day of age (group 3). In fact, however, chicks in group 3 and 4 showed similar amounts of compensation for –7 D lenses before recovery, in terms of refractive error (relative change during the course of –7 D lens wear, $\Delta X - \Delta N$, group 3 vs. group 4, –4.79 ± 0.45 D vs. –4.19 ± 0.48 D, Mean ± SEM; $p = 0.44$, 2-tailed, unpaired, Student's t-test), axial length (0.20 ± 0.04 mm vs. 0.12 ± 0.04 mm; $p = 0.23$), and choroidal thickness (–0.04 ± 0.03 mm vs. –0.06 ± 0.04 mm; $p = 0.74$). On the other hand, chicks in group 3 showed more vitreous chamber elongation compared with those in group 4 (0.34 ± 0.05 mm vs. 0.22 ± 0.03 mm; $p = 0.0178$), possibly caused by the early starting age. In addition, chicks in group 3 showed a significantly larger hyperopic shift and more vitreous chamber inhibition compared with those in group 4, facilitated by choroidal thickening during recovery from prior negative lens wear under light (group 3, Fig. 3.2). It is possible that this difference in response was caused by different starting ages. Finally, it has been shown that untreated chick eyes grow in a linear fashion by an average of 72 μm per day[75]. To correct for the change in axial length in the fellow, untreated eyes, interocular difference (X – N) was used for analyses in the thesis.

3.6.6 Conclusions

An intrinsic, non-visual, homeostatic mechanism, e.g., a size-factor, seems to exist in chick eyes to guide the eye regain its normal size when the defocus-factor is absent.

4. The Effect of Eye Size on Monocular Lens Compensation in Chicks

4.1 Forward

This series of experiments studied the relative importance of the defocus-factor compared to non-visual-factor(s) in the control of ocular growth by generating situations in which the size- and defocus-factors in the eye would be required to work in opposite directions for the lens-wearing eye to regain the same size as the fellow, untreated eye. This was achieved by first exposing one eye first to a weak defocus signal and after a brief adaptation period, the same eye was then exposed to a stronger defocus signal of the same sign under monocular conditions.

The following hypothesis is proposed:

A size-factor reduces lens compensation when it is working in the opposite direction of the defocus-factor. If a size-factor exists to help maintain the "right" eye size, it should prevent the eye from growing too long or short when the eye is experiencing hyperopic or myopic defocus and tend to otherwise compensate for it, and therefore, reduce lens compensation.

Some of these results have been presented in abstract form (Zhu X, *et al*., *Invest. Ophthal. Vis. Sci.* 2012, E-Abstract 3441; Zhu X, Wallman J, and McFadden SA, *Invest. Ophthal. Vis. Sci.* 2016, E-Abstract 3791).

4.2 Abstract

Purpose: It has been shown in the previous chapter that a size-factor seems to exist in chick eyes to regulate eye growth in the absence of the defocus-factor. The size- and defocus-factors can work in either the same or opposite directions. This chapter tests whether the visual mechanism entirely dominates compensation for spectacle lenses or whether the size-factor is also operative.

Methods: All chicks wore lenses over one eye, and the fellow eye was left untreated. To study the effects of the size- and the defocus-factors when they were working in opposite directions, chicks first wore either +7 D (n = 4) or –7 D (n = 25) lenses on one eye for up to 7 days then +15 or –15 D lenses for another 4 to 11 days (from 6 to 19 days of age). The size- and defocus-factors would compete in opposite directions at the time of the lens power increase (step-up). This experiment was also repeated with weaker-powered positive (first +5 D then +10 D lens wear, n = 6) and negative (first –5 D then –10 D lens wear, n = 9) lenses, respectively. The size-factor was further investigated in recovery versus lens compensation: The change in refractive error and ocular dimensions two days after a positive lens step-up (from +7 D to +15 D, n = 4) was compared to that produced two days after recovery from –7 D lens wear (n = 5) when both groups were experiencing similar amounts of myopic defocus, with the main difference being the eye size or length. The equivalent comparison was made after a negative lens step-up (from –7 D to –15 D, n = 11) and a group recovering from +7 D lens wear (n = 8), when both groups were experiencing similar amounts of hyperopic defocus, with the main difference, again, being the eye size or length. This experiment was also repeated with weaker-powered positive (from +5 D to +10 D lens wear, n = 6, versus recovery from –5 D lens wear, n = 7) and negative (from –5 D to –10 D lens wear n = 9, versus recovery from +5 D lens wear, n = 6) lenses. Refractive error and ocular dimensions were measured before and after each treatment, and repeatedly at various intervals during treatment with a Hartinger refractometer and A-scan biometry, respectively.

Results: Chick eyes fully compensated for +15 D lenses after they had compensated for +7 D lenses, despite having an abnormally reduced axial length at the time of lens-switching, suggesting that myopic defocus dominated any potential adaptive signals from a reduced eye

size or length. In contrast, while chick eyes could fully compensate for –15 D lenses if they wore them from the beginning, chick eyes did not fully compensate for –15 D lenses after having compensated for –7 D lenses, suggesting that some kind of signal sensitive to abnormally perturbed, or asymmetric, eye-size or length (the so-called size-factor) dominated the eyes response to hyperopic defocus. Similar findings were discovered with weaker-powered lenses. It was also discovered that chick eyes in the positive lens step-up group reduced their rate of ocular elongation more than those in the group recovering from prior negative lens wear, confirming the dominance of the defocus-factor in response to myopic defocus. On the other hand, eyes recovering from prior positive lens wear developed a greater myopic shift compared with negative lens-wearing eyes after the step-up, confirming the influence of a size-factor in response to hyperopic defocus.

Conclusions: An intrinsic, homeostatic mechanism influences lens-compensation in chicks in the case of negative lens compensation: Abnormally long eyes seem to be influenced by this intrinsic mechanism, whereas abnormally short eyes are not. This intrinsic mechanism is probably sensitive to eye length or size. The implications for myopia treatments are discussed.

4.3 Introduction

It has been demonstrated in the previous chapter that some intrinsic, non-visual mechanism(s) seem to exist in chick eyes to guide the eyes to regain their normal size or length after they had been made too long (after compensating for negative lenses) or too short (after compensating for positive lenses) without any visual stimuli (chick kept darkness). However, normally the visual and non-visual processes would be both present simultaneously. Therefore, it is of interest to know how these two processes may interact when the animals are kept in the light.

In the introductory summary of the various visual cues that the eye may use to discern the magnitude and direction of defocus, the array of negative results when each of these visual cues was tested in isolation suggests that the eye growth system may have multiple mechanisms to detect defocus, and when one is absent, others can be effective. Similarly, visual and intrinsic factors may interact. Although elimination of one (such as removal of the visual factor by recovering animals in darkness) allows the other to be exposed, the incomplete nature of its effectiveness suggests that perhaps one dominates the other under some circumstances.

There is a great deal of evidence to suggest that the eye responds differently to myopic and hyperopic defocus. In terms of the temporal nature of these signals, it has been discovered that retina weighs myopic defocus more than hyperopic defocus when presented separately. Indeed, it has been shown that a short period of "normal vision" (viewing without any lens or occluder on the eye) each day cancels myopia from wearing negative lenses or occluders during the rest of the day in chicks[136, 137]; by contrast, it takes a much longer period of normal vision to cancel out hyperopia from wearing positive lenses during the rest of the day in chicks[137], tree shrews[138] and monkeys[139-141]. Furthermore, when positive and negative lenses are worn alternately, the eye is more responsive to myopic defocus and develops hyperopia, rather than averaging out the defocus of the opposite signs in chicks[142-144] and tree shrews[145]. In addition, Zhu and Wallman showed that the long-lasting effect of myopic defocus is possibly because axial inhibition caused by positive lens-wear lasts longer than axial elongation caused by negative lenses[146].

The retina also weighs myopic defocus more than hyperopic defocus when these two signals are presented simultaneously. Presenting chick eyes with simultaneously competing myopic and hyperopic defocus, using either mixed astigmatic (toric) lenses with opposite lens powers on the two perpendicular meridians[93], lens-cone devices with two target planes[111], multi-zone contact lenses with alternating powers[147], or dual-power lenses that had different combinations of lens powers[148], caused hyperopia. In addition, projecting myopic defocus onto the peripheral retina (while allowing the central retina to receive clear images) slows myopia progression in chicks[149].

The studies mentioned above fitted the optical device only over one eye and left the contralateral eye untreated, which raises the question: Could this be due to an uneven contribution of visual and non-visual factors to each type of defocus? Furthermore, is a non-visual factor(s) still operative in the presence of visual stimuli or defocus.

Therefore, this chapter was designed to study the interaction of the size- and defocus factors in monocular lens compensation. The defocus- and size-factors can work either in the same or opposite directions in regulating eye growth. For example, during recovery from wearing a negative lens (axial length already too long after compensation), the defocus-factor (myopic defocus) will act to reduce the rate of ocular elongation until emmetropization is restored (Fig. 4.1A). The size-factor will also reduce the rate of ocular elongation until the normal, age-appropriate eye size or length is restored. On the other hand, when a normal eye is wearing a negative lens, the defocus-factor (hyperopic defocus caused by the negative lens) will increase the rate of ocular elongation, while the size-factor will tend to decrease this "abnormal" increase in the rate of ocular elongation to keep the eye size or length normal (Fig. 4.1B).

It was discovered that the size-factor influences lens-compensation: Specifically, abnormally long eyes seem more influenced by the size-factor than abnormally short eyes.

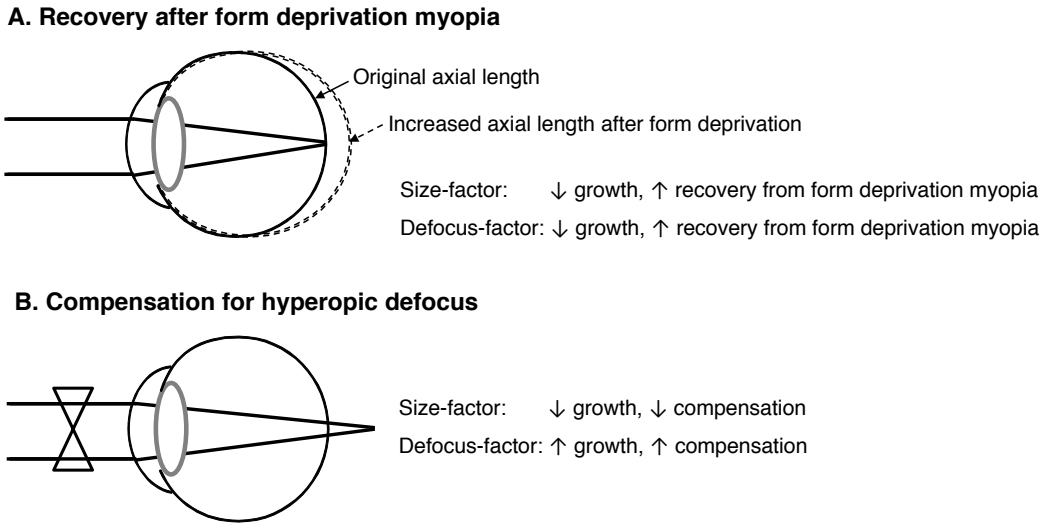

Figure 4.1. Schematics for the interactions between the hypothesized size- and defocus-factors.

4.4 Methods

4.4.1 Animals

White Leghorn chicks (n = 135) were obtained and housed in described in the General methods (Section 2.1). Lighting was the same as that described in the General methods (Section 2.1).

4.4.2 Experimental procedures

All chicks wore a lens over one eye, and the other eye was left as an untreated control. Two different types of experimental comparisons were undertaken. In both, the stimuli related to defocus and eye size were manipulated so that the correct response to each were pitted in opposite or similar directions. This was achieved by using a change in the magnitude of the imposed lens power after a period of compensation. In the first experiment, comparison was made to animals that viewed through the final (stepped-up) lens power continuously (Experiment 4.1, Constant vs. stepped lens powers). In the second experiment, comparison was made to animals recovering from induced abnormal ocular length and refractive changes after lens compensation (Experiment 4.2, Stepped lens powers vs.

recovery). The treatment details, length of lens-wear and age, and sample sizes are summarized in Table 4.1.

Glass lenses of powers –5, –7, –10, –15, +5, +7, +10, or +15 D were used as described in General Methods (Section 2.2).

Measurements of refractive error and ocular dimensions were measured repeatedly (approximately every 2 days during the experiment) while the chicks were anesthetized with 1.5% of isoflurane as detailed in the General Methods (Section 2.3).

Table 4.1. Summary of treatment details, the effects of the proposed size- and defocus-factors, and sample sizes (n) in Exp. 4.1 and 4.2

Exp name	Group #	Lens type	Details (age in days)	Size- vs. defocus- factor direction during recovery or after step up*	n
4.1. Constant vs. stepped lens powers	5	Plus	+15 D lens wear for 5 days (6-11)		9
	6	Plus	+7 D lens wear for 5 days (1-6), then +15 D lens for 11 days (6-17)	S: ↑ growth; D: ↓ growth	4
	7	Plus	+10 D lens wear for 11 days (7-18)		6
	8	Plus	+5 D lens wear for 4 days (7-11), then +10 D lens for 7 days (11-18)	S: ↑ growth; D: ↓ growth	6
	9	Minus	–15 D lens wear for 11 days (7-18)		10
	10	Minus	–7 D lens wear for 7 days (7-14), then –15 D lens for 4 days (14-18)	S: ↓ growth; D: ↑ growth	11
	11**	Minus	–7 D lens wear for 7 days (6-13), then –15 D lens for 6 days (13-19)	S: ↓ growth; D: ↑ growth	4
	12**	Minus	–7 D lens wear for 4 days (3-7), then –15 D lens for 4 days (7-11)	S: ↓ growth; D: ↑ growth	10
	13	Minus	–10 D lens wear for 11 days (7-18)		10
	14	Minus	–5 D lens wear for 7 days (7-14), then –10 D lens for 4 days (14-18)	S: ↓ growth; D: ↑ growth	9
4.2. Stepped lens powers vs. recovery	6***	Plus	+7 D lens wear for 5 days (1-6), then +15 D lens for 11 days (6-17)	S: ↑ growth; D: ↓ growth	4
	3***	Minus	–7 D lens wear for 5 days (1-6), then recovery in normal light for 11 days (6-17)	S: ↓ growth; D: ↓ growth	5
	8***	Plus	+5 D lens wear for 4 days (7-11), then +10 D lens for 7 days (11-18)	S: ↑ growth; D: ↓ growth	6
	15	Minus	–5 D lens wear for 4 days (7-11), then recovery in normal light for 7 days (11-18)	S: ↓ growth; D: ↓ growth	7
	10***	Minus	–7 D lens wear for 7 days (7-14), then –15 D lens for 4 days (14-18)	S: ↓ growth; D: ↑ growth	11
	1***	Plus	+7 D lens wear for 7 days (7-14), then recovery in normal light for 4 days (14-18)	S: ↑ growth; D: ↑ growth	8
	14***	Minus	–5 D lens wear for 7 days (7-14), then –10 D lens for 4 days (14-18)	S: ↓ growth; D: ↑ growth	9
	16	Plus	+5 D lens wear for 7 days (7-14), then recovery in normal light for 4 days (14-18)	S: ↑ growth; D: ↑ growth	6

* S: Size-factor; D: Defocus-factor.

** Repetitions for group 10. Chicks were measured right before lens step up and repeatedly afterward.

*** Groups that were mentioned more than once the in the table for comparison purposes. Groups 3 and 4 are from Chapter 3.

4.4.2.1 Exp. 4.1: Constant vs. Stepped Lens Powers

To investigate the direction of eye growth when the size- and defocus-factors predict opposite directions of eye growth for correct adaptation, in one set of experiments, chicks wore positive lenses to investigate the response to myopic defocus, while in the second set, chicks wore negative lenses to investigate the response to hyperopic defocus.

Myopic Defocus - Positive Lens Step-up:

To investigate if chick eyes could compensate for myopic defocus when the size- and defocus-factors predict opposite directions of growth, the time course of lens compensation for +7 D lenses then stepping up to +15 D lenses (group 6) was compared with a control group that wore +15 D from the beginning of the experiment (group 5, Table 4.1).

Note that after the step-up of the positive lens power, the size- and defocus- factors predict opposite directions of growth: A size-factor should act to increase eye growth and prevent further compensation for the +15 D lenses, since the lens-wearing eye was already shorter than normal; whereas the defocus-factor would act to further induce reduction in eye growth and compensation for the +15 D lenses, since +15 D lenses superimposed myopic defocus (8 D) in front of the retina of eyes that were +7 D hyperopic (Fig. 4.2A).

This experiment was also repeated with lower-powered positive lenses (+10 D vs. +5 D stepped to +10 D, groups 7 and 8, Table 4.1).

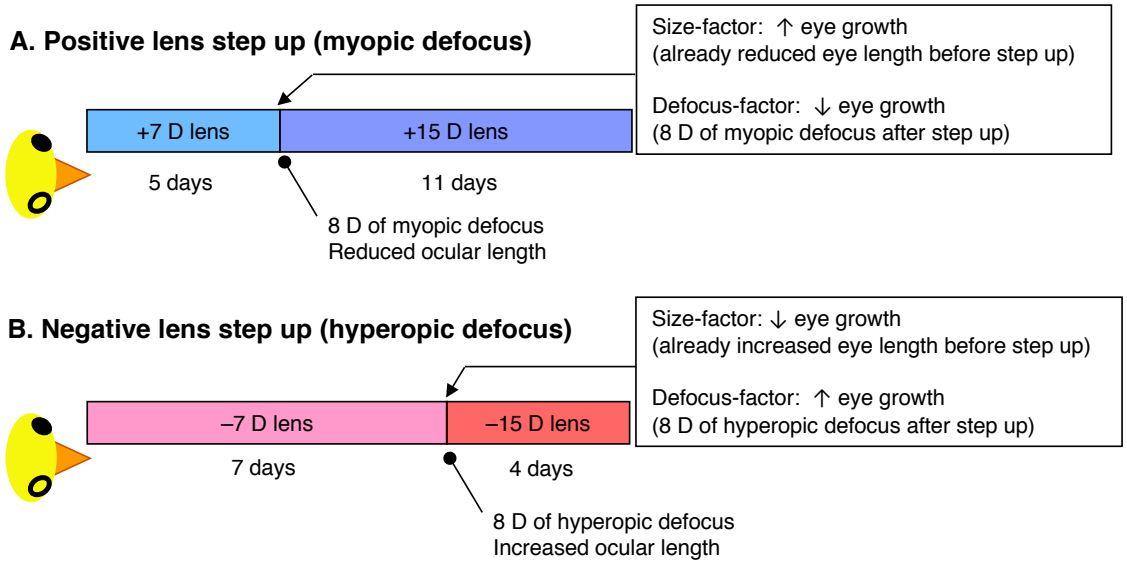

Figure 4.2. Schematics for lens-wearing paradigms and the proposed effects of the size- and defocus-factors at lens step-up. Text boxes labelled with the round arrowheads show the actual states of defocus and axial length at the time of lens change, and text boxes labelled with the pointy arrowheads show the hypothesized effects of the size- and defocus-factors.

Hyperopic Defocus - Negative Lens Step-up:

In case of negative lens wear, the time course of lens compensation for –7 D lenses then stepping up to –15 D lenses (group 10) was compared with a control group that wore –15 D from the beginning (group 9, Table 4.1).

Note that after the step-up of the negative lens power, the size- and defocus- factors would, again, predict opposite directions of growth: Any size related-factor would now act to reduce eye growth and decrease compensation for the –15 D lenses, since the lens-wearing eye was already longer than normal; whereas the defocus-factor would acted to further induce increase in eye growth and compensation for the –15 D lenses, since –15 D lenses superimposed hyperopic defocus (8 D) behind the retina of eyes that were –7 D myopic (Fig. 4.2B). Since our results showed that chick eyes did not further compensate for –15 D lenses after first compensating for –7 D lenses, the same experiment was repeated but with different compensation periods and starting age (groups 11 and 12, Table 4.1).

The first version of the above experiment was also repeated with lower-powered negative lenses (–10 D Vs. –5D stepped to –10 D, groups 13 and 14, Table 4.1).

4.4.2.2 Exp. 4.2: Stepped Lens Powers vs. Recovery

To compare the ocular growth response between the two groups when they both experienced the same sign and amount of defocus but the size-factor provided growth cues that were in the opposite direction to the defocus-factor in one group and in the same direction with the defocus-factor in the other, a direct comparison of compensation for myopic defocus occurred between groups 6 and 3 for myopic defocus, and between 10 and 1 for hyperopic defocus (Table 4.1).

Myopic Defocus - Positive Lens Stepped Up vs. Negative Lens Recovery:

To study if the size-factor could reduce compensation for myopic defocus when it provided growth cues in the opposite direction to the defocus cues, lens compensation for +15 D lenses after stepping up from +7 D lenses (group 6) was compared to with recovery from –7 D lens wear (group 3, Table 4.1).

Note that after the step-up in positive lens power, the size- and defocus- factors predict growth in opposite directions: A size-factor should act to increase eye growth and prevent further compensation for the +15 D lenses; whereas the defocus-factor would act to further induce reduction in eye growth and compensation for the +15 D lenses (Fig. 4.2A). During recovery from –7 D lens wear, on the other hand, both the size- and defocus-factors would be working in the same direction to restore the eye's normal size and to compensate for the 7 D of myopic defocus caused by the removal of –7 D lenses.

This experiment was also repeated with a smaller amount of myopic defocus (+5 D stepped to +10 D vs. recovery from –5 D lens wear, groups 8 and 15, Table 4.1).

Hyperopic Defocus - Negative Lens Stepped Up vs. Positive Lens Recovery:

In the case of hyperopic defocus, lens compensation for –15 D lenses after stepping up from –7 D lenses (group 10) was compared to with recovery for +7 D lens wear (group 1, Table 4.1).

Similarly, after the step-up of the negative lens power, the size- and defocus- factors provided opposing growth cues: A size-factor should act to decrease eye growth and prevent further compensation for the –15 D lenses; whereas the defocus-factor would act to further increase eye growth and compensation for the —15 D lenses (Fig. 4.2B). During recovery from +7 D lens wear, on the other hand, both the size- and defocus-factors would be working in the same direction to restore the eye's normal size and to compensate for the 7 D of hyperopic defocus caused by the removal of +7 D lenses.

Again, the same comparison was repeated with a smaller amount of hyperopic defocus (–5 D stepped to –10 D vs. recovery from +5 D lens wear groups 14 and 16, Table 4.1).

4.4.3 Analyses

Data was analyzed in the following three ways. First, the experimental and fellow eyes were compared at each measurement time using a Two-Way Mixed Measures Analysis of Variance (ANOVA) with Post-hoc comparisons adjusted for familywise error using the Holm-Sidak method. This analysis was repeated for refractive error and each ocular dimension. The resulting *p* values are reported in Tables 4.2 and 4.3.

Second, inter-ocular differences (X – N) at each measurement age were compared with One-Way Repeated Measures ANOVA with the Holm-Sidak method for comparisons between different time points (for example, before and after lens-step-up), and Two-Way Mixed Measures ANOVA with the Holm-Sidak method for comparisons at any particular time point between different groups. The data for these inter-ocular differences (X – N) in refractive error and each ocular distance are shown as the Mean ± SEM in Tables 4.2 (Exp. 4.1) and 4.3 (Exp. 4.2)**.**

Finally, the relative changes (change in the experimental eyes over a specified time period minus the matched change in the untreated eyes, $\Delta X - \Delta N$) from two groups were compared using 2-tailed, unpaired *Student*'s t-tests.

The detailed values for refractive error and each ocular distance for each age are shown as Mean ± SEM in Table A2.1 in Appendix 2.

4.5 Results

4.5.1 Exp. 4.1: Constant vs. stepped lens powers

Briefly, when the size– and defocus–factors provided growth cues in opposite directions, the defocus-factor dominated in the case of myopic defocus, and the size-factor prevented the eye from further elongating in the case of hyperopic defocus.

Table 4.2. Summary of inter-ocular difference (X – N, Mean ± SEM) for ocular dimensions, refractive error, and *p* values for Exp. 4.1

Group	n	Age	Anterior chamber depth (mm)	*p*	Lens thickness (mm)	*p*	Vitreous chamber depth (mm)	*p*	Choroidal thickness (mm)	*p*	Axial length (mm)	*p*	Refractive error (D)	*p*
5: +15 D control	9	6	0.01 ± 0.01	0.580	-0.01 ± 0.01	0.617	0.00 ± 0.01	0.983	0.00 ± 0.01	0.938	0.01 ± 0.02	0.816	0.51 ± 0.41	0.618
		7	0.02 ± 0.02	0.366	-0.04 ± 0.01	**0.001**	-0.15 ± 0.03	**0.007**	0.15 ± 0.03	**≤ 0.001**	-0.03 ± 0.03	0.450	5.42 ± 0.96	**≤ 0.001**
		9	-0.01 ± 0.01	0.705	-0.09 ± 0.01	**≤ 0.001**	-0.45 ± 0.07	**≤ 0.001**	0.26 ± 0.05	**≤ 0.001**	-0.29 ± 0.04	**≤ 0.001**	13.85 ± 1.33	**≤ 0.001**
		11	-0.04 ± 0.03	0.059	-0.09 ± 0.01	**≤ 0.001**	-0.50 ± 0.07	**≤ 0.001**	0.20 ± 0.03	**≤ 0.001**	-0.42 ± 0.06	**≤ 0.001**	16.89 ± 1.07	**≤ 0.001**
6: +7 --> +15 D	4	1	0.01 ± 0.01	0.805	0.00 ± 0.00	0.820	-0.01 ± 0.04	0.695	0.01 ± 0.01	0.577	0.00 ± 0.06	0.996	-0.49 ± 0.29	0.569
		4	-0.05 ± 0.00	**0.040**	-0.02 ± 0.02	0.173	-0.22 ± 0.04	**≤ 0.001**	0.04 ± 0.02	0.108	-0.26 ± 0.04	**≤ 0.001**	8.09 ± 0.68	**≤ 0.001**
		6	-0.08 ± 0.01	**0.008**	-0.04 ± 0.01	**0.007**	-0.20 ± 0.02	**≤ 0.001**	0.00 ± 0.02	0.919	-0.32 ± 0.05	**≤ 0.001**	8.86 ± 0.82	**≤ 0.001**
		8	-0.12 ± 0.02	**≤ 0.001**	-0.07 ± 0.01	**≤ 0.001**	-0.44 ± 0.04	**≤ 0.001**	0.11 ± 0.03	**≤ 0.001**	-0.50 ± 0.03	**≤ 0.001**	16.85 ± 1.36	**≤ 0.001**
		11	-0.16 ± 0.02	**≤ 0.001**	-0.02 ± 0.01	0.099	-0.43 ± 0.02	**≤ 0.001**	0.06 ± 0.04	**0.029**	-0.56 ± 0.05	**≤ 0.001**	16.15 ± 1.00	**≤ 0.001**
		13	-0.17 ± 0.03	**≤ 0.001**	-0.03 ± 0.01	0.051	-0.42 ± 0.04	**≤ 0.001**	0.05 ± 0.02	0.061	-0.56 ± 0.03	**≤ 0.001**	16.14 ± 0.42	**≤ 0.001**
		17	-0.22 ± 0.04	**≤ 0.001**	-0.05 ± 0.02	**0.004**	-0.55 ± 0.04	**≤ 0.001**	0.00 ± 0.02	0.951	-0.81 ± 0.04	**≤ 0.001**	18.83 ± 0.77	**≤ 0.001**
7: +10 D control	6	7	0.01 ± 0.02	0.721	-0.01 ± 0.01	0.401	0.02 ± 0.02	0.471	-0.02 ± 0.01	0.375	0.00 ± 0.04	0.988	1.16 ± 1.10	0.213
		11	-0.02 ± 0.02	0.328	-0.03 ± 0.01	**0.023**	-0.37 ± 0.03	**≤ 0.001**	0.18 ± 0.03	**≤ 0.001**	-0.25 ± 0.06	**≤ 0.001**	9.05 ± 1.16	**≤ 0.001**
		13	-0.04 ± 0.02	0.145	-0.01 ± 0.01	0.637	-0.38 ± 0.02	**≤ 0.001**	0.18 ± 0.02	**≤ 0.001**	-0.24 ± 0.04	**≤ 0.001**	9.54 ± 0.99	**≤ 0.001**
		15	-0.02 ± 0.02	0.356	0.00 ± 0.02	0.871	-0.38 ± 0.02	**≤ 0.001**	0.16 ± 0.01	**≤ 0.001**	-0.24 ± 0.05	**≤ 0.001**	10.55 ± 0.62	**≤ 0.001**
		18	-0.03 ± 0.03	0.218	-0.01 ± 0.02	0.673	-0.38 ± 0.03	**≤ 0.001**	0.12 ± 0.02	**≤ 0.001**	-0.30 ± 0.05	**≤ 0.001**	11.01 ± 0.43	**≤ 0.001**
8: +5 --> +10 D	6	7	0.00 ± 0.01	0.885	0.01 ± 0.02	0.652	-0.03 ± 0.05	0.547	0.00 ± 0.02	0.887	-0.01 ± 0.05	0.876	0.31 ± 0.50	0.653
		11	0.01 ± 0.01	0.519	-0.03 ± 0.01	0.098	-0.23 ± 0.05	**≤ 0.001**	0.08 ± 0.03	**0.027**	-0.18 ± 0.05	**0.024**	5.40 ± 0.60	**≤ 0.001**
		13	-0.01 ± 0.01	0.349	-0.02 ± 0.02	0.208	-0.38 ± 0.03	**≤ 0.001**	0.18 ± 0.04	**≤ 0.001**	-0.24 ± 0.07	**0.006**	9.11 ± 0.53	**≤ 0.001**
		15	-0.01 ± 0.02	0.647	-0.04 ± 0.02	**0.031**	-0.46 ± 0.03	**≤ 0.001**	0.26 ± 0.04	**≤ 0.001**	-0.26 ± 0.08	**0.004**	8.32 ± 0.90	**≤ 0.001**
		18	0.00 ± 0.01	0.815	-0.02 ± 0.02	0.225	-0.48 ± 0.04	**≤ 0.001**	0.19 ± 0.04	**≤ 0.001**	-0.32 ± 0.07	**0.001**	10.04 ± 0.77	**≤ 0.001**
9: –15 D control	10	7	0.00 ± 0.01	0.825	0.00 ± 0.01	0.706	-0.02 ± 0.03	0.465	0.02 ± 0.02	0.917	0.00 ± 0.02	0.596	0.30 ± 0.46	0.804
		9	0.00 ± 0.01	0.883	-0.05 ± 0.03	0.213	0.21 ± 0.05	**0.004**	-0.10 ± 0.02	**≤ 0.001**	0.06 ± 0.05	0.246	-4.27 ± 1.03	**≤ 0.001**
		11	-0.04 ± 0.02	0.405	-0.03 ± 0.01	0.724	0.33 ± 0.03	**≤ 0.001**	-0.06 ± 0.02	**≤ 0.001**	0.19 ± 0.04	**0.037**	-6.66 ± 0.60	**≤ 0.001**
		14	-0.07 ± 0.02	0.190	0.01 ± 0.01	0.293	0.48 ± 0.04	**≤ 0.001**	-0.10 ± 0.02	**0.001**	0.32 ± 0.07	**0.009**	-9.73 ± 0.40	**≤ 0.001**
		16	-0.05 ± 0.03	0.381	0.02 ± 0.01	0.269	0.65 ± 0.07	**≤ 0.001**	-0.03 ± 0.02	**0.001**	0.57 ± 0.07	**0.002**	-12.61 ± 0.35	**≤ 0.001**
		18	-0.06 ± 0.04	0.242	0.01 ± 0.03	0.243	0.80 ± 0.08	**≤ 0.001**	-0.09 ± 0.01	**≤ 0.001**	0.65 ± 0.10	**0.001**	-14.40 ± 0.85	**≤ 0.001**
10: –7 --> –15 D	11	7	0.00 ± 0.01	0.779	0.00 ± 0.01	0.376	0.01 ± 0.01	0.900	-0.01 ± 0.01	0.830	0.00 ± 0.01	0.838	-0.47 ± 0.44	0.686
		9	-0.02 ± 0.02	0.217	-0.04 ± 0.01	**0.009**	0.21 ± 0.02	**≤ 0.001**	-0.06 ± 0.01	**0.009**	0.08 ± 0.02	0.135	-4.01 ± 1.00	**≤ 0.001**
		11	-0.04 ± 0.01	**0.015**	-0.04 ± 0.01	**0.041**	0.33 ± 0.03	**≤ 0.001**	-0.05 ± 0.02	0.188	0.19 ± 0.03	**0.004**	-5.10 ± 0.40	**≤ 0.001**
		14	-0.10 ± 0.02	**≤ 0.001**	-0.01 ± 0.01	0.059	0.41 ± 0.03	**≤ 0.001**	-0.04 ± 0.02	0.100	0.25 ± 0.04	**≤ 0.001**	-7.69 ± 0.61	**≤ 0.001**
		16	-0.09 ± 0.02	**≤ 0.001**	-0.02 ± 0.01	0.188	0.40 ± 0.06	**≤ 0.001**	-0.01 ± 0.02	0.376	0.27 ± 0.04	**≤ 0.001**	-5.32 ± 0.91	**≤ 0.001**
		18	-0.08 ± 0.03	**≤ 0.001**	-0.01 ± 0.01	0.740	0.42 ± 0.07	**≤ 0.001**	0.04 ± 0.03	**0.010**	0.37 ± 0.06	**≤ 0.001**	-4.48 ± 0.78	**≤ 0.001**
11: –7 --> –15 D	4	13	-0.07 ± 0.06	0.265	-0.01 ± 0.04	0.863	0.30 ± 0.08	**0.016**	-0.02 ± 0.03	0.685	0.20 ± 0.06	**0.013**	-7.04 ± 1.76	**≤ 0.001**
		14	-0.10 ± 0.05	0.167	-0.02 ± 0.02	0.495	0.33 ± 0.08	**0.012**	0.00 ± 0.04	0.927	0.21 ± 0.06	**0.011**	-6.74 ± 0.33	**0.001**
		16	-0.09 ± 0.06	0.202	-0.03 ± 0.03	0.415	0.29 ± 0.08	**0.019**	0.04 ± 0.04	0.376	0.21 ± 0.05	**0.012**	-3.09 ± 1.79	0.073
		19	-0.10 ± 0.05	0.163	-0.03 ± 0.03	0.375	0.33 ± 0.10	**0.011**	0.01 ± 0.06	0.853	0.20 ± 0.04	**0.013**	-3.44 ± 1.86	**0.049**
12: –7 --> –15 D	10	7	-0.05 ± 0.02	**0.036**	-0.02 ± 0.01	0.133	0.25 ± 0.04	**≤ 0.001**	-0.03 ± 0.02	**0.037**	0.15 ± 0.04	**0.004**	-4.15 ± 1.06	**≤ 0.001**
		9	-0.05 ± 0.02	**0.027**	-0.01 ± 0.01	0.580	0.36 ± 0.04	**≤ 0.001**	-0.05 ± 0.01	**0.001**	0.24 ± 0.05	**≤ 0.001**	-5.72 ± 0.93	**≤ 0.001**
		11	-0.06 ± 0.02	**0.006**	-0.02 ± 0.01	0.140	0.37 ± 0.03	**≤ 0.001**	-0.03 ± 0.01	0.062	0.25 ± 0.04	**≤ 0.001**	-5.49 ± 1.12	**≤ 0.001**
13: –10 D control	10	7	-0.01 ± 0.01	0.752	0.01 ± 0.02	0.752	0.00 ± 0.03	0.922	0.02 ± 0.02	0.204	0.03 ± 0.03	0.453	0.53 ± 0.28	0.176
		11	-0.04 ± 0.01	0.146	0.00 ± 0.02	0.997	0.22 ± 0.03	**≤ 0.001**	-0.04 ± 0.01	**0.022**	0.13 ± 0.03	**0.003**	-4.07 ± 0.21	**≤ 0.001**
		14	-0.06 ± 0.03	**0.028**	0.02 ± 0.01	0.392	0.35 ± 0.05	**≤ 0.001**	-0.05 ± 0.02	**0.006**	0.23 ± 0.05	**≤ 0.001**	-7.50 ± 0.45	**≤ 0.001**
		16	-0.06 ± 0.04	**0.036**	0.02 ± 0.02	0.363	0.43 ± 0.05	**≤ 0.001**	-0.04 ± 0.02	**0.045**	0.34 ± 0.05	**≤ 0.001**	-9.60 ± 0.49	**≤ 0.001**
		18	-0.06 ± 0.03	**0.031**	0.02 ± 0.01	0.191	0.48 ± 0.05	**≤ 0.001**	-0.02 ± 0.01	0.212	0.41 ± 0.03	**≤ 0.001**	-9.21 ± 0.40	**≤ 0.001**
14: –5 --> –10 D	9	7	0.00 ± 0.02	0.980	-0.01 ± 0.02	0.715	0.00 ± 0.03	0.972	-0.01 ± 0.01	0.424	-0.03 ± 0.03	0.581	-0.14 ± 0.23	0.783
		11	-0.04 ± 0.03	0.361	-0.04 ± 0.01	**0.042**	0.14 ± 0.03	**0.013**	-0.02 ± 0.02	0.276	0.04 ± 0.04	0.482	-4.12 ± 0.39	**≤ 0.001**
		14	-0.06 ± 0.04	0.143	-0.02 ± 0.01	0.384	0.22 ± 0.05	**≤ 0.001**	-0.08 ± 0.02	**≤ 0.001**	0.06 ± 0.05	0.339	-7.32 ± 0.46	**≤ 0.001**
		16	-0.10 ± 0.04	**0.021**	0.03 ± 0.02	0.155	0.32 ± 0.07	**≤ 0.001**	-0.07 ± 0.02	**≤ 0.001**	0.18 ± 0.08	**0.006**	-5.66 ± 0.57	**≤ 0.001**
		18	-0.12 ± 0.05	**0.005**	0.04 ± 0.03	0.085	0.39 ± 0.05	**≤ 0.001**	-0.09 ± 0.02	**≤ 0.001**	0.22 ± 0.07	**0.002**	-5.14 ± 0.70	**≤ 0.001**

p: The mean values in the experimental and fellow eyes were compared at different time points measured using Two-Way Mixed Measures Analysis of Variance (ANOVA), with the Holm-Sidak adjustment method. See Table A2.1 in Appendix 2 for the mean values in the experimental and fellow eyes on various days. *p* values of statistical significance are underlined and bold.

Refer to Table 4.1 above for group definitions.

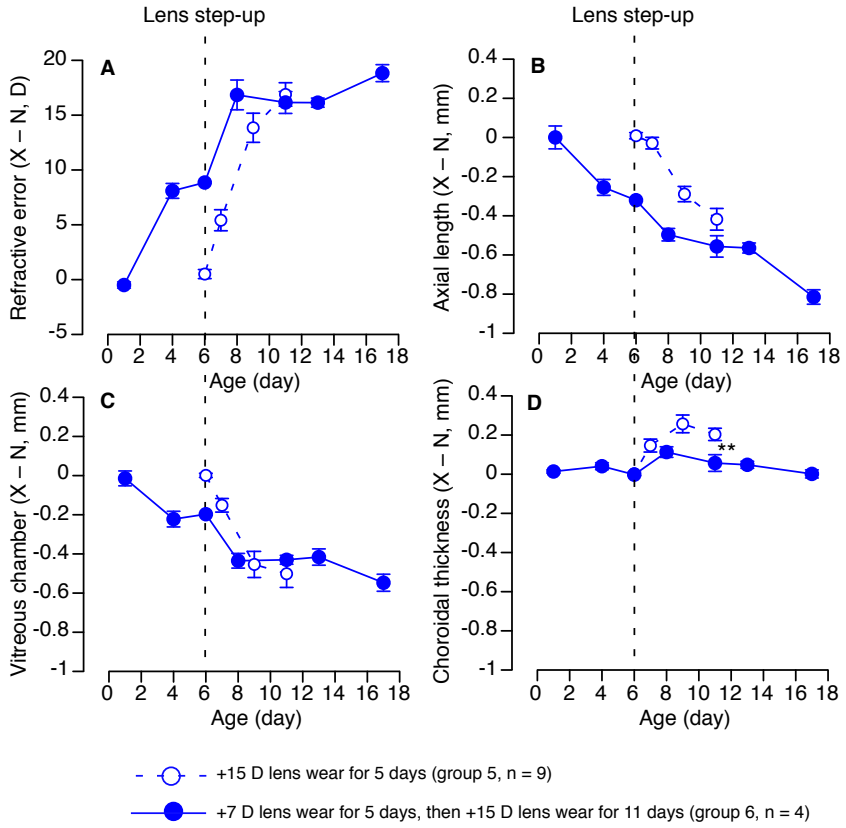

Figure 4.3. Time course of compensation for +15 D lenses and for first +7 D then +15 D lenses.
Chicks either wore +15 D lenses over one eye for 5 days (group 5, open circles), or +7 D lenses for 5 days (on the left side of the dashed line) then stepping to +15 D lenses for another 11 days (on the right side of the dash line, group 6, filled blue circles). Data is shown as the inter-ocular difference (X – N, Mean ± SEM) for (A) refractive error, (B) axial length, (C) vitreous chamber depth, and (D) choroidal thickness. Comparison of the inter-ocular difference between these two groups only yielded a significant difference for choroidal thickness on day 11 (**: $p < 0.01$, Two-Way Mixed Measures ANOVA).

4.5.1.1 Myopic Defocus - Positive Lens Step-up

Eyes wearing +15 D lenses (from 6 to 11 days of age, group 5) fully compensated for the imposed defocus after 5 days (relative difference between the two eyes in refractive error changes between 6 and 11 days of age, ΔX – ΔN, Mean ± SEM, +16.38 ± 1.05 D; X – N on day 11 vs. day 6, $p < 0.001$, Table 4.2, Fig. 4.3A, open symbols), with significantly reduced axial lengths (ΔX – ΔN from days 6 to 11, –0.43 ± 0.06 mm; X – N on day 11 vs.

day 6, $p < 0.001$, Fig. 4.3B), vitreous chamber depths (ΔX – ΔN from days 6 to 11, –0.50 ± 0.07 mm; X – N on day 11 vs. day 6, $p < 0.001$, Fig. 4.3C), and thickened choroids (ΔX – ΔN from days 6 to 11, +0.20 ± 0.03 mm; X – N on day 11 vs. day 6, $p < 0.001$, Fig. 4.2D).

Wearing a +7 D lens over one eye for 5 days (from 1 to 6 days of age, group 6) also resulted in full compensation (ΔX – ΔN from days 1 to 6, +9.35 ± 1.02 D; X – N on day 6 vs. day 1, $p < 0.001$, Fig. 4.3A, filled symbols). This robust hyperopic shift at 6 days of age was accompanied by a significant reduction in axial length (ΔX – ΔN from days 1 to 6, –0.32 ± 0.01 mm; X – N on day 6 vs. day 1, $p < 0.001$, Fig. 4.3B) and reduction in vitreous chamber depth (ΔX – ΔN from days 1 to 6, –0.18 ± 0.03 mm; X – N on day 6 vs. on day 1, $p < 0.001$, Fig. 4.3C). However, there was no choroidal thickening before lens step-up on day 6 (ΔX – ΔN from days 1 to 6, –0.02 ± 0.02 mm; X – N on day 6 vs. day 1, $p > 0.05$, Fig. 4.3D).

Increasing the lens power from +7 D to +15 D lenses on day 6 (group 6) induced an average of +7.4 D of myopic defocus in the lens-wearing eye since the experimental eyes had responded by becoming 7.6 D hyperopic on average (see Table A2.1 in Appendix A2 for details). These positive lens-wearing eyes fully compensated for +15 D lenses only 2 days after the step-up (ΔX – ΔN from 6 to 8 days of age, Mean ± SEM, +7.99 ± 1.04 D; X – N on day 8 vs. day 6, $p < 0.001$, Fig. 4.3A). The level of hyperopia found in these eyes at 11 days of age was very similar to that found in the control group (X – N on day 11, group 6 vs. group 5, +16.15 D vs. +16.89 D, $p > 0.05$, Table 4.2). Specifically, stepping the lens power up to +15 D for just 2 days caused a further reduction in axial length compared to the fellow, untreated eyes (ΔX – ΔN from 6 to 8 days of age, –0.18± 0.03 mm; X – N on day 8 vs. day 6, $p < 0.01$, Fig. 4.3B), despite the significant inhibition already induced by the eye compensating for the +7 D lenses. This inhibition in axial elongation kept increasing for another 9 days, becoming an extraordinary 800 μm shorter than that in their fellow eyes by 17 days of age (ΔX – ΔN from 8 to 17 days of age, –0.32 ± 0.03 mm; X – N on day 17 vs. day 6, $p < 0.001$, Fig. 4.3B). That is, despite the lens-wearing eye being shorter than its fellow eye, this size-factor seemed to have no influence on the eye's subsequent growth, and instead the eye continued to respond to the additional myopic defocus.

Stepping up to +15 D lenses for 2 days also caused further reduction in vitreous chamber depth (ΔX – ΔN from 6 to 8 days of age, –0.24 ± 0.04 mm; X – N on day 8 vs. day 6, $p < 0.001$; Fig. 4.3C) that was long lasting (ΔX – ΔN from8 to 17 days of age, –0.11 ± 0.04 mm; X – N on day 8 vs. day 17, $p < 0.001$, Fig. 4.3C). There was a small increase in choroidal thickness 2 days after the lens step-up (ΔX – ΔN from 6 to 8 days of age, +0.07 ± 0.03 mm; X – N on day 6 vs. day 8, $p = 0.062$, Fig. 4.3D), but this dissipated thereafter. Comparison of the inter-ocular difference between groups 5 and 6 only revealed a significant difference in choroidal thickness on day 11 (X – N on 11 days of age, group 5 vs. group 6, +0.20 ± 0.03 mm vs. +0.06 ± 0.04 mm, $p < 0.01$, Fig. 4.3D). Therefore, it would appear that the source of the hyperopic shift in response to the additional myopic defocus arose primarily from a genuine inhibition in eye growth resulting in a reduction in the vitreous chamber elongation, while choroidal changes were small and transient.

See Table A2.1 and Fig. A2.1 in Appendix 2 for changes in anterior chamber depth and lens thickness.

Similar results were found when the experiment was repeated with weak-powered positive lenses (+10 D lens wear from the beginning vs. +5 D stepping to +10 D lenses, groups 7 and 8, Table 4.1): Chick eyes fully compensated for +10 D lenses after the step-up, regardless of the size-factor. This robust hyperopic shift was caused by reduction in axial length and in vitreous chamber depth, and by choroidal thickening. See Table A2 and Fig. A2.2 in Appendix 2 for details.

These findings provide good evidence that the defocus-factor dominates in the case of myopic defocus and that the size-factor was not able to prevent further compensation to myopic defocus to maintain the eye's original size.

4.5.1.2 Hyperopic Defocus - Negative Lens Step-up

A very different pattern of results was observed when an eye was exposed to an increased amount of hyperopic defocus by stepping-up the magnitude of negative lens power.

Chick eyes fully compensated for –15 D lenses after wearing them for 11 days (group 9, ΔX –Δ N for refractive error from 7 to 18 days of age, Mean ± SEM, –14.55 ± 1.09 D; X – N on day 7 vs. day 18, $p < 0.001$, Table 4.2, Fig. 4.4A, open symbols), with a significant increase in axial length (ΔX – ΔN from 7 to 18 days of age, +0.66 ± 0.12 mm; X – N on day 7 vs. day 18, $p < 0.001$, Fig. 4.4B) and vitreous chamber depth (ΔX – ΔN from 7 and 18 days of age, +0.82 ± 0.09 mm; X – N on day 7 vs. day 18, $p < 0.001$, Fig. 4.4C). This compensatory response was approximately linear over the 11 days of the experiment (refractive error: $y = –1.31x – 9.79$, $r^2 = 0.89$; axial length: $y = 0.06x + 0.39$, $r^2 = 0.67$; vitreous chamber depth: $y = 0.07x + 0.51$, $r^2 = 0.78$; $p < 0.0001$ for each regression).

Wearing a –7 D lens over one eye for 7 days (7 to 14 days of age) also resulted in full compensation (group 10, ΔX – ΔN from 7 to 14 day of age, Mean ± SEM, –7.22 ± 0.76 D; X – N on day 7 vs. day 14, $p < 0.001$, Fig. 4.4A, red filled circles), with a significantly elongated relative axial length (ΔX – ΔN from 7 to 14 days of age, +0.26 ± 0.05 mm; X – N on day 7 vs. day 14, $p < 0.001$, Fig. 4.4B) and relative vitreous chamber depth (ΔX – ΔN from 7 to 14 days of age, +0.41 ± 0.04 mm; X – N on day 7 vs. day 14, $p < 0.001$, Fig. 4.4C). The inter-ocular difference (X – N) for refractive error, axial length, and vitreous chamber depth at 14 days of age was not significantly different from those in group 9 at 14 days of age (X – N on 14 days of age, group 9 vs. group 10, refractive error: –9.73 D vs. –7.69 D; axial length: +0.32 mm vs. +0.25 mm; vitreous chamber depth: +0.48 mm vs. +0.41 mm; $p > 0.05$ for each comparison, Table 4.2).

Surprisingly, unlike the response to continuous -15 D lens wear in group 9, after the step-up of negative lens power from –7 D to –15 D on 14 days of age, there was **no** further refractive compensation 2 days later. Instead, the –15 D lens-wearing eyes developed a small hyperopic shift relative to the fellow eyes (ΔX – ΔN between days 14 and 16, +2.36 ± 0.70 D). As a result, these eyes were significantly more hyperopic than the –15 D lens-wearing eyes in group 10 (X – N on 16 days of age, group 9 vs. group 10, –12.61 D vs. –5.32 D, $p < 0.001$, Fig. 4.4A; $p = 0.97$, power analysis). The lack of further compensation in refractive error was supported by the lack of further increase in axial length (ΔX – ΔN from 14 to 16 days of age, +0.01 ± 0.04 mm) and the lack of further increase in vitreous chamber depth (ΔX – ΔN from 14 to 16 days of age, –0.02 ± 0.05 mm) 2 days after the step-up. As a result,

there was a significant difference between the control group (group 9) and the step-up group (group 10) for both axial length (X – N on 16 days of age, group 9 vs. group 10, +0.57 mm vs. +0.27 mm, $p < 0.001$, Fig. 4.4B) and vitreous chamber depth (X – N on day 16, group 9 vs. group 10, +0.65 mm vs. +0.40 mm, $p < 0.01$, Fig. 4.4C) 2 days after the step-up. Similarly, no further compensation was observed 4 days after the step-up (Table 4.2 and Fig. 4.4).

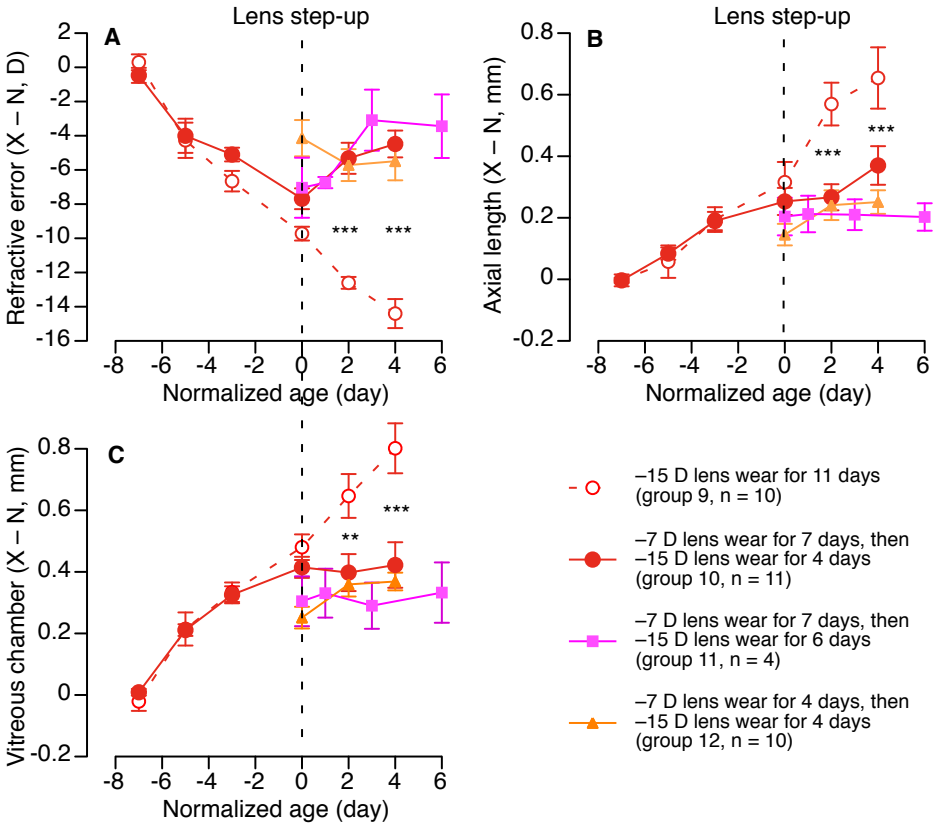

Figure 4.4. Time course of compensation for –15 D lenses and for –7 D then –15 D lenses. Chicks wore either –15 D lenses over one eye for 11 days (group 9, open symbols), or –7 D lenses for 4 to 7 days then stepping to –15 D lenses for another 4 to 6 days (groups 10 to 12, filled symbols). Data is shown as the inter-ocular difference (X-N, Mean ± SEM) for (A) refractive error, (B) axial length, and (C) vitreous chamber depth. Note that eyes were not measured until the end of the −7 D lens wear for groups 11 and 12. Also note that ages have been normalized so the day of lens step-up is represented by zero on the X-axes (see Table 4.1 for details), e.g., day 0 in the figure correspond to 14 days of age for groups 9 and 10, respectively. Asterisks indicate statistical significant between group 9 and groups 10 to 12 after the negative lens step-up on various days (**: $p < 0.01$, ***: $p < 0.001$, Two-Way Mixed Measures ANOVA).

The above negative lens step-up experiment was repeated twice using the same lenses, either with a longer observation period after lens step-up (chick eyes measured up to 6 days after lens step-up, group 11, magenta squares in Fig. 4.4), or with younger chicks (treatment started when chicks were 3 days old, group 12, orange triangles in Fig. 4.4). Neither group showed further compensation for –15 D lenses after the lens power was increased (Table 4.2 and Fig. 4.4). Take group 11, for example: The –7 D lens-wearing eyes fully compensated for the negative lenses after wearing them for 7 days (X – N on 13 days of age, –7.04 ± 1.76 D, Table 4.2, purple squares in Fig. 4.4). Six days after stepping to –15 D lenses, there was no further compensation to –15 D lenses in refractive error (ΔX – ΔN from 13 to 19 days of age, +3.60 ± 2.70 D, Fig. 4.4A), axial length (ΔX – ΔN from 13 to 19 days of age, 0.00 ± 0.50 mm, Fig. 4.4B), or vitreous chamber depth (ΔX – ΔN from 13 to 19 days of age, +0.03 ± 0.07 mm, Fig. 4.4C), similar to findings in groups 10 and 12.

See Table A2.1 and Fig. A2.3 in Appendix 2 for changes in choroidal thickness, anterior chamber depth, and lens thickness in groups 9 to 12.

The above experiment was also repeated with weaker-powered negative lenses (groups 13 and 14) with similar results:

As expected, chick eyes fully compensated for –10 D lenses after wearing them for 11 days (group 13, ΔX –Δ N for refractive error from 7 to 18 days of age, Mean ± SEM, –9.74 ± 0.7 D; X – N on day 7 vs. day 18, $p < 0.001$, Table 4.2, Fig. 4.5A, open symbols), with a significant increase in axial length (ΔX – ΔN from 7 to 18 days of age, +0.38 ± 0.05 mm; X – N on day 7 vs. day 18, $p < 0.001$, Fig. 4.5B) and vitreous chamber depth (ΔX – ΔN from 7 to 18 days of age, +0.49 ± 0.05 mm; X – N on day 7 vs. day 18, $p < 0.001$, Fig. 4.5C). This compensatory response was also approximately linear over the 11 days of the experiment (refractive error: $y = –0.95x + 6.63$, $r^2 = 0.87$; axial length: $y = 0.03x – 0.23$, $r^2 = 0.55$; vitreous chamber depth: $y = 0.04x – 0.29$, $r^2 = 0.65$; $p < 0.0001$ for each regression).

Wearing a –5 D lens over one eye for 7 days (from 7 to 14 days of age) caused full compensation in refractive error (group 14, ΔX – ΔN from 7 to 14 days of age, Mean ± SEM, –7.19 ± 0.57 D; X – N on day 7 vs. day 14, $p < 0.001$, Fig. 4.5A, filled circles), with slightly elongated relative axial length (ΔX – ΔN from 7 to 14 days of age, +0.09 ± 0.04 mm; X – N

on day 7 vs. day 14, $p > 0.05$, Fig. 4.5B) and significantly elongated relative vitreous chamber depth (ΔX – ΔN from 7 to 14 days of age, +0.22 ± 0.04 mm; X – N on day 7 vs. day 14, $p < 0.001$, Fig. 4.5C). (The lack of significant axial elongation on day 14 was caused by reduction in anterior chamber depth and lens thickness. See Table A2.1 and Fig. A2.4 in Appendix 2 for details.) The inter-ocular difference (X – N) on day 14 was not significantly different from those in group 13 for refractive error and vitreous chamber depth (X – N on day 14, group 13 vs. group 14, refractive error: –7.50 D vs. –7.32 D; vitreous chamber depth: +0.35 mm vs. +0.22 mm; $p > 0.05$ for each comparison, Table 4.2), but was significantly different for axial length (X – N on day 14, group 13 vs. group 14, +0.23 mm vs. 0.06 mm; $p < 0.05$).

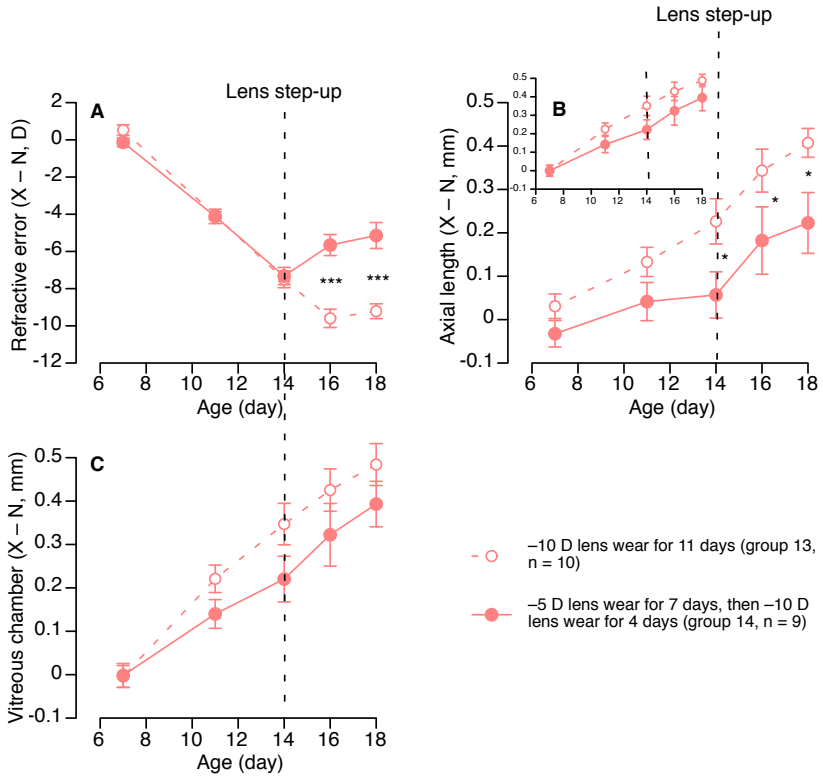

Figure 4.5. Time course of compensation for –10 D lenses and for –5 D then –10 D lenses. Chicks either wore –10 D lenses over one eye for 11 days (group 13, open circles) or –5 D lenses for 7 days then stepping to –10 D lenses for another 4 days (group 14, filled circles). Data is shown as the inter-ocular difference (X – N, Mean ± SEM) for refractive error (A), axial length (B), and vitreous chamber depth (C). Insert in Fig. 4.4B is the data on axial length with the initial values normalized to zero. Asterisks indicate statistical significant between groups 13 and 14 after the negative lens step-up on various days (*: $p < 0.05$, ***: $p < 0.001$, Two-Way Mixed Measures ANOVA).

After the step-up of negative lens power from –5 D to –10 D at 14 days of age, similar to results found with higher-powered negative lenses (groups 10 to 12), there was **no** further myopic compensation for –10 D lenses 2 days later (group 14). Instead, the –10 D lens-wearing eyes also developed a small hyperopic shift relative to the fellow eyes (ΔX – ΔN from 14 to 16 days of age, +1.67 ± 0.93 D, Table 4.2, Fig. 4.5A). As a result, these eyes were significantly more hyperopic than the –10 D lens-wearing eyes in group 13 (X – N on day 16, group 13 vs. group 14, –9.60 D vs. –5.66 D, $p < 0.001$, Fig. 4.5A). There was no significant increase in axial length (ΔX – ΔN between 14 and 16 days of age, +0.13 ± 0.04 mm; X – N, day 14 vs. day 16, $p = 0.074$, Fig. 4.5B) or vitreous chamber depth (ΔX – ΔN between days 14 and 16, +0.10 ± 0.04 mm; X – N, day 14 vs. day 16, $p = 0.076$, Fig. 4.45C). Therefore, there was a significant difference between the control group (group 13) and the step-up group (group 14) in the inter-ocular difference in axial length 2 days after the step-up (X – N on 16 days of age, group 13 vs. group 14, +0.34 mm vs. +0.18 mm, $p < 0.05$, Fig. 4.5B) and 4 days after the step-up (X – N on 18 days of age, group 13 vs. group 14, +0.41 mm vs. +0.22 mm, $p < 0.05$). On the other hand, there was no significant difference in the inter-ocular difference in vitreous chamber depth between the control and step-up groups (X – N on day 16, group 13 vs. group 14, +0.43 mm vs. +0.32 mm, $p > 0.05$, Fig. 4.5C). Similarly, there was no significant difference in the inter-ocular difference in choroidal thickness, anterior chamber depth, or lens thickness between the two groups (X – N on day 16, group 13 vs. group 14, choroidal thickness: –0.04 mm vs. –0.07 mm, anterior chamber depth: –0.06 mm vs. –0.10 mm, lens thickness: +0.02 mm vs. +0.03 mm, $p > 0.05$ for each comparison, Table 4.2 and Fig. A2.4 in Appendix 2).

See Table A2.1 and Fig. A2.4 in Appendix 2 for changes in choroidal thickness, anterior chamber depth, and lens thickness in groups 13 and 14.

Taken together, these findings suggest that some factors not related to the current defocus state, prevents the eye from further elongating to become more myopic. One such factor is that the eye is already abnormally elongated relative to its fellow eye.

4.5.2 Exp. 4.2: Stepped lens powers vs. recovery

Similar to the findings in Exp. 4.1, when the size– and defocus–factors predicted opposite directions of growth, the defocus-factor dominated in the case of myopic defocus, and the size-factor still prevented the eye from further elongating in the case of hyperopic defocus.

Table 4.3. Summary of inter-ocular difference (X – N, Mean ± SEM) for ocular dimensions, refractive error and *p* values for Exp. 4.2

Group	Age	Anterior chamber depth (mm)	*p*	Lens thickness (mm)	*p*	Vitreous chamber depth (mm)	*p*	Choroidal thickness (mm)	*p*	Axial length (mm)	*p*	Refractive error (D)	*p*
15: recovery from –5 D	7	0.01 ± 0.01	0.215	-0.01 ± 0.01	0.397	-0.01 ± 0.02	0.916	-0.01 ± 0.01	0.366	-0.02 ± 0.03	0.799	0.55 ± 0.32	0.133
	11	0.01 ± 0.01	0.408	-0.01 ± 0.02	0.425	0.10 ± 0.05	0.202	-0.10 ± 0.02	**≤ 0.001**	-0.01 ± 0.07	0.908	-4.46 ± 0.42	**≤ 0.001**
	13	0.01 ± 0.01	0.116	0.00 ± 0.01	0.866	-0.11 ± 0.06	0.148	0.06 ± 0.02	**≤ 0.001**	-0.05 ± 0.06	0.551	-0.27 ± 0.46	0.454
	15	0.00 ± 0.00	0.926	0.06 ± 0.01	**≤ 0.001**	0.00 ± 0.11	0.954	0.05 ± 0.01	**0.004**	0.10 ± 0.10	0.198	0.56 ± 0.27	0.129
	18	0.01 ± 0.01	0.362	0.02 ± 0.01	0.108	0.04 ± 0.09	0.613	0.01 ± 0.01	0.396	0.08 ± 0.09	0.321	0.31 ± 0.29	0.389
16: recovery from +5 D	7	0.00 ± 0.02	0.875	0.00 ± 0.01	0.966	0.01 ± 0.02	0.856	-0.01 ± 0.04	0.874	0.01 ± 0.03	0.755	0.70 ± 0.66	0.228
	14	-0.04 ± 0.02	0.067	-0.04 ± 0.02	**0.020**	-0.26 ± 0.04	**≤ 0.001**	0.19 ± 0.05	**≤ 0.001**	-0.14 ± 0.07	**0.003**	6.31 ± 0.52	**≤ 0.001**
	16	-0.05 ± 0.02	**0.013**	0.03 ± 0.02	0.055	-0.06 ± 0.02	0.109	0.01 ± 0.02	0.865	-0.05 ± 0.03	0.213	-0.12 ± 0.64	0.828
	18	-0.05 ± 0.02	**0.030**	0.05 ± 0.02	**0.003**	0.01 ± 0.04	0.801	0.07 ± 0.02	0.065	0.08 ± 0.02	0.063	0.40 ± 0.35	0.487

p: The mean values in the experimental and fellow eyes were compared at different time points measured using Two-Way Mixed Measures Analysis of Variance (ANOVA), with the Holm-Sidak adjustment method. See Table A2.1 in Appendix 2 for the mean values in the experimental and fellow eyes on various days. *p* values of statistical significance are underlined and bold.

Refer to Table 4.1 above for group definitions.

4.5.2.1 Myopic Defocus - Positive Lens Stepped Up vs. Negative Lens Recovery

The experimental eyes in the two groups that experienced myopic defocus by either stepping from +7 D to +15 D lenses (group 6, Table 4.1) or recovery from prior –7 D lens wear (group 3) developed a hyperopic shift at a similar rate. For the group with a positive lens step-up (group 6), the lens-wearing eyes were 7.62 D hyperopic on average just before the step-up (see Table A2.1 in Appendix 2 for details), and experienced 7.38 D of myopic defocus at the step-up (15 – 7.62 = 7.38). For the group that recovered from prior –7 D lens treatment (group 3), the treated eyes were –5.78 D myopic right before the recovery (Table A2.1 in Appendix 2), and experienced 5.78 D of myopic defocus after the lenses were

removed, similar to that in the group 6. Any effects of the eye size or length, however, would be in opposite directions for these two groups: If eye size was a factor contributing to eye growth, the eye should increase its growth for the step-up group but decrease its growth for the recovery group. However, two days after the step-up or recovery, both groups developed a similar amount of hyperopia (relative change 2 days after the step-up or recovery, $\Delta X - \Delta N$, group 6 vs. group 3, +7.99 D vs. +5.93 D, $p > 0.05$, Figs. 4.6A and B, refer to Tables 4.2 and 4.3). Furthermore, the step-up group did not have enhanced growth but rather showed significantly more axial inhibition than the recovery group 2 days after the step-up or recovery ($\Delta X - \Delta N$, group 6 vs. group 3, –0.18 mm vs. –0.04 mm, $p < 0.01$, Figs. 4.6C and D, refer to Table 4.2), supporting the dominance of the defocus-factor in the case of myopic defocus. Both groups showed similar amounts of reduction in vitreous chamber depth (–0.24 mm vs. –0.26 mm, $p > 0.05$, Fig. 4.6E and F) and choroidal thickening (+0.12 mm vs. +0.18 mm, $p > 0.05$, Fig. 4.6G and H).

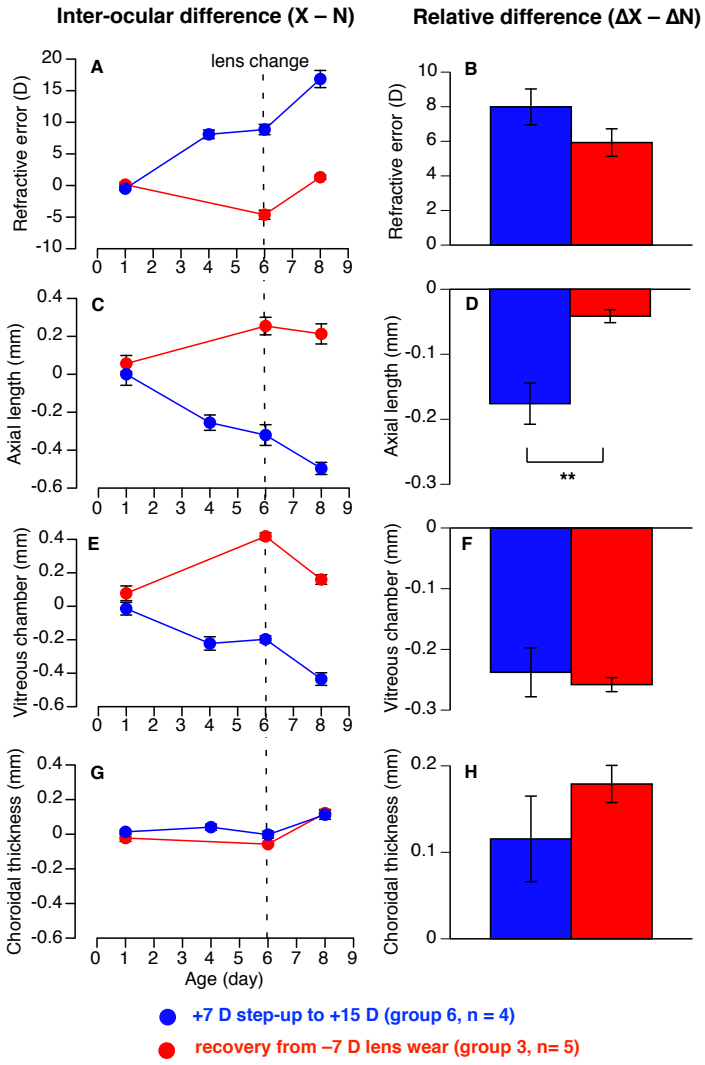

Figure 4.6. Comparisons between positive lens step-up and recovery from negative lens wear. Chicks either wore +7 D lenses then stepping to +15 D lenses (group 6, blue circles or bars), or –7 D lenses then recovery (group 3, red circles and bars). Line scatter plots show the inter-ocular difference (X – N, Mean ± SME) for the time course (up to 2 days after the step-up or recovery), and bar charts show the relative change (ΔX – ΔN, Mean ± SEM) within the first 2 days after the step- up or recovery, in (A and B) refractive error, (C and D) axial length, (E and F) vitreous chamber depth, and (G and H) choroidal thickness. Asterisks show the level of statistical significance for comparisons between the step-up group and recovery group for the relative change (***: $p < 0.001$, 2-tailed, unpaired, Student's t-test).

Similar findings were discovered in the groups of +5 D step-up (group 8) and of –5 D recovery (group 15, relative change 2 days after the step-up or recovery, ΔX – ΔN, group 8 vs. group 15, refractive error: +3.71 D vs. +4.19 D; axial length: –0.06 mm vs. –0.04 mm; vitreous chamber depth: –0.15 mm vs. –0.21 mm; choroidal thickness: +0.10 mm vs. +0.16 mm; $p > 0.05$ for each parameter; Table 4.2 and Fig. A2.5 in Appendix 2).

4.5.2.2 Hyperopic Defocus - Negative Lens Stepped Up vs. Positive Lens Recovery

The experimental eyes in groups 1 and 10 experienced hyperopic defocus by either stepping from a –7 D to a –15 D lens (group 10, Table 4.1) or recovery from prior +7 D lens wear (group 1). While the recovery group developed a myopic shift as expected (group 1), the negative lens step-up group did not show any further myopic development (group 10), as previously shown. Specifically, the negative lens-wearing eyes in the lens step-up group were –7.21 D myopic on average prior to the step-up (see Table A2.1 in Appendix 2), and experienced 7.79 D of hyperopic defocus (15 – 7.21 = 7.79) at the step-up. The treated eyes in the recovery group were 6.58 D hyperopic right before the recovery, and experienced 6.58 D of hyperopic defocus after the positive lenses were removed, similar to that in the step-up group. Any effects of the eye size, however, would be in opposite directions for these two groups: If eye size was a factor controlling eye growth, the eye should decrease its growth for the step-up group but increase its growth for the recovery group.

Two days after the step-up or recovery, the negative lens wearing eyes in the step-up group did not develop further myopia. Instead, the treated eyes in this group showed a small hyperopic shift relative to the fellow eyes, whereas the experimental eyes in the recovery group fully recovered from prior positive lens wear (relative change 2 days after the step-up or recovery, ΔX – ΔN between days 14 and 16, group 10 vs. group 1, +2.37 D vs. –6.07 D, $p < 0.001$, Figs. 4.7A and B). Compared with the recovery group, there was no further elongation in axial length (ΔX – ΔN, +0.01 vs. +0.10 mm, $p > 0.05$, Figs. 4.7C and D) or vitreous chamber depth (–0.02 mm vs. +0.24 mm, $p < 0.001$, Fig. 4.7E and F, refer to Tables 4.2 and 4.3) in the step-up group, suggesting that some non-defocus factor, possibly related to eye size, dominated in this case.

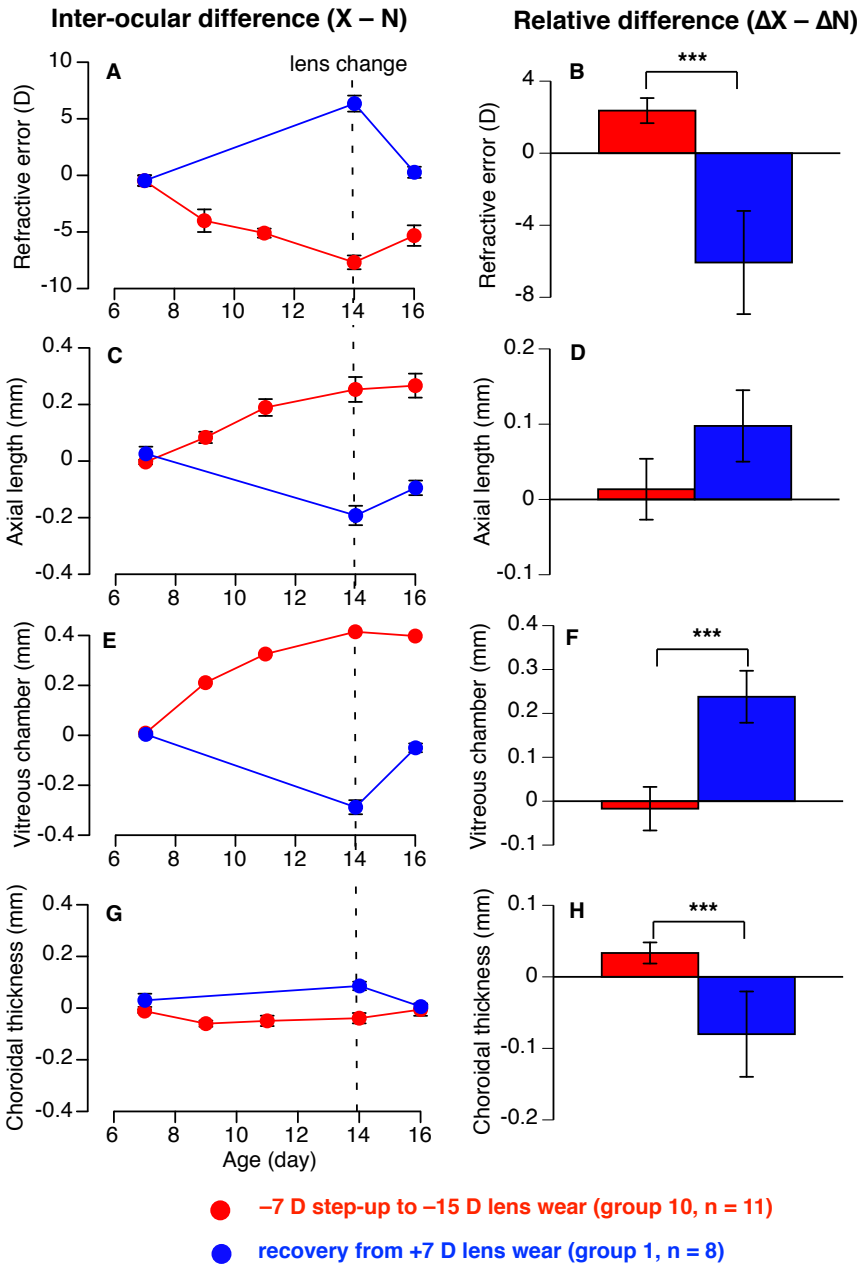

Figure 4.7. Comparisons between negative lens step-up and recovery from positive lens wear. Chicks either wore –7 D lenses then stepping to –15 D lenses (group 10, red circles or bars), or +7 D lenses then recovery (group 1, blue circles and bars). Line scatter plots show the inter-ocular difference (X – N, Mean ± SME) for the time course (up to 2 days after the step-up or recovery), and bar charts show the relative change (ΔX – ΔN, Mean ± SEM) within the first 2 days after the step- up or recovery, in (A and B) refractive error, (C and D) axial length, (E and F) vitreous chamber depth, and (G and H) choroidal thickness. Asterisks show the level of statistical significance for comparisons between the step-up group and recovery group for the relative change (***: $p < 0.001$, 2-tailed, unpaired, Student's t-test).

Similar findings were discovered for the groups of –5 D step-up (group 14) and of +5 D recovery (group 16, relative change 2 days after the step-up or recovery, $\Delta X - \Delta N$, group 14 vs. group 16, refractive error: +1.67 D vs. –6.44 D, $p < 0.001$; axial length: +0.13 mm vs. +0.09 mm, $p > 0.05$; vitreous chamber depth: +0.10 mm vs. +0.20 mm, $p > 0.05$; choroidal thickness: +0.10 mm vs. –0.18 mm, $p < 0.001$; Fig. A2.6 in Appendix 2, refer to Table A2.1 in Appendix 2).

4.6 Discussion

The most striking finding in the current chapter is that the visual input did not seem to be sufficient to promote further ocular elongation to compensate for a strong negative lens after the eyes had compensated for the weak negative lens, suggesting that there is some unknown mechanism not related to the current defocus state that is involved in the regulation of eye growth. First, there is a pronounced lack of sensitivity to the magnitude of hyperopic defocus in terms of the eyes initial growth response. Second, the eye growth controller is insensitive to a sudden change in the magnitude of hyperopic defocus. It is possible that the constraining factor is related to a reversion to some intrinsic growth mechanism related to the expected eye size, when confronted with a sudden change in the magnitude of hyperopic defocus, or that there is a default response to inhibit growth when exposed to a sudden change.

4.6.1 Summary of results

4.6.1.1 The Effects of Visual Mechanism(s) on Eye Growth

The effects of visual mechanism(s) on both positive and negative lens step-up are illustrated in Fig. 4.8. For instance, in the group that wore higher-powered positive lenses (group 6, +7 D to +15 D), the lens-wearing eyes were 7.62 D hyperopic (without the +7 D lens in place) or 0.62 D hyperopic (with the +7 D lens in place) right before the step-up (Table A2.1 in Appendix 2), and experienced 7.38 D of myopic defocus (15 – 7.62 = 7.38, the functional defocus) when the positive lens power stepped from +7 D to +15 D (with the lens in place, the dark blue diamonds in Fig. 4.8). If these treated eyes fully compensated for

the functional defocus, the symbols should all fall on the line of equality. While all symbols of positive lens step-up groups (dark and light blue symbols in Fig. 4.8) fall on the line of equality, indicating that the visual mechanism (mostly likely defocus) dominated in these cases, the symbols for negative lens step-up groups (dark and light red diamonds) are not on the line of equality, indicating the defocus alone was not sufficient in guiding compensation for negative lenses and that something prevented the eyes from further elongating.

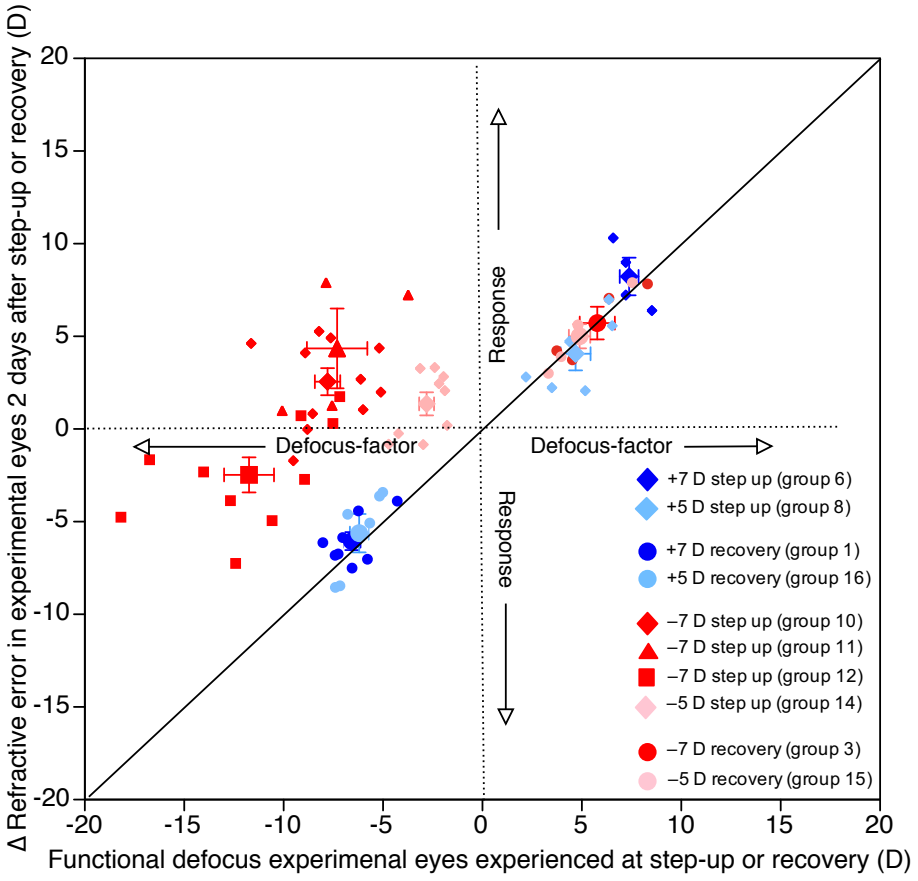

Figure 4.8. A Scatter plot of change in refractive error after lens step-up or recovery. Data is shown as Mean ± SEM. The magnitude of functional defocus the treated eyes were experiencing at the step-up or recovery is naturally a measure of the defocus-factor (stimuli, labeled with the dashed line on the X axis) and the actual change in refractive error in these eye is the measure of these eyes' response to the defocus-factor (labeled with the dashed line on the Y axis). If these treated eyes fully compensated for the functional defocus, the symbols should all fall on the line of equality. While all symbols of positive lens step-up and recovery and negative lens recovery fall on the line of equality, indicating that the defocus-factor dominated in these cases, the symbols for negative lens step-up are not on the line of equality, indicating the defocus-factor alone was not sufficient in guiding compensation for negative lenses.

4.6.1.2 The Effects of Possible Non-Visual Mechanism(s) on Eye Growth

The potential role of a possibly non-visual mechanism(s), such as the eye size or length, as a preventive factor in controlling eye growth is illustrated in Fig 4.9: The inter-ocular difference in axial length (X – N) at the time of lens step-up is a natural measure of a pre-determined size-factor, and the change in axial length in the experimental eyes (ΔX) after the lens step-up could represent the eye's response to such a size-factor. For groups with a positive lens step-up (groups 6 and 8, dark and light blue diamonds in Fig. 4.9), the experimental eyes were abnormally shorter than normal at the step-up (X – N on the day of lens step-up, Mean, –0.32 mm for group 6, and –0.18 mm for group 8), but they still further reduced eye growth after the step-up to compensate for the strong positive lenses (ΔX two days after the step-up, –0.18 mm and –0.06 mm for groups 6 and 8, respectively).

For groups with negative lens step-up (groups 10, 11, 12, and 14), in contrast, there was little further axial elongation after the step-up. Indeed, there was a weak, negative correlation between the change in axial length after the step-up and the inter-ocular difference in axial length ($y = –0.25x + 0.11$, $r^2 = 0.09$, $p > 0.05$), suggesting that the more the experimental eyes elongated before the step-up (the larger the effect of the non-visual mechanism or the proposed size-factor), the less the experimental eyes elongated after the step-up.

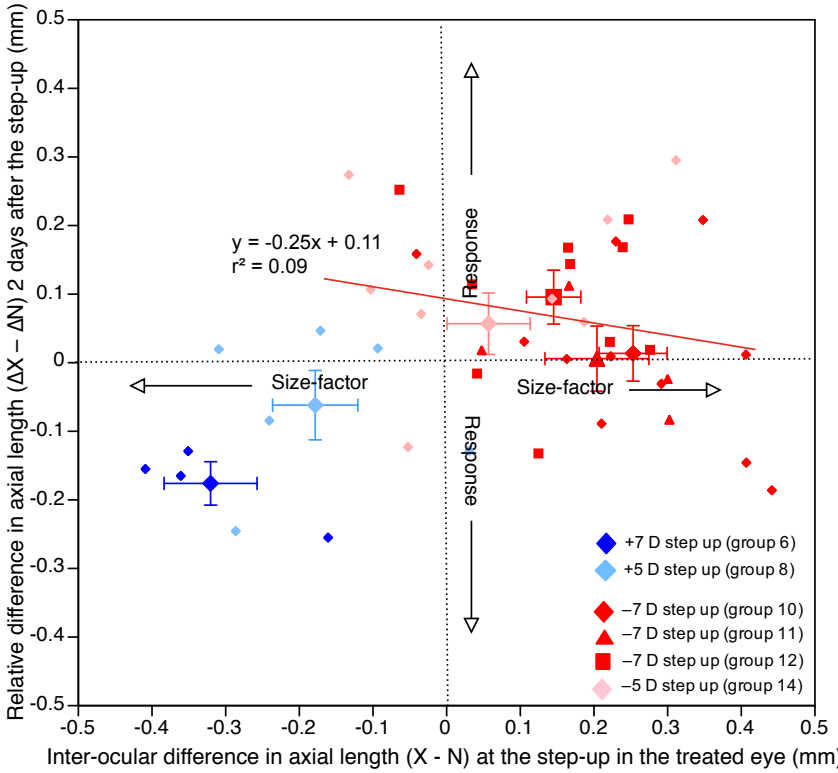

Figure 4.9. A scatter plot of change in axial length after lens step-up. The inter-ocular difference in axial length in the treated eyes right before step-up is a natural measure of the size-factor (stimuli, labeled with the dashed line on the X axis) and the change in the inter-ocular difference in axial length is the measure of these eyes' response to the size-factor (labeled with the dashed line on the Y axis). Clearly that groups with positive lens step-up (groups 6 and 8) further reduced eye growth to compensate for the strong positive lenses after the step-up (relative change in axial length below zero on the Y axis) even though these eyes were already shorter than the fellow eyes after compensating for the weak positive lenses (relative change in axial length below zero on the X axis). For groups with negative lens step-up (groups 10, 11, 12, and 14), in contrast, there was little further axial elongation after the step-up. Indeed, there was a weak, negative correlation between change in axial length 2 days after the step-up and the inter-ocular difference in axial length at the step-up, suggesting that the more the experimental eyes elongated before the step-up (the larger the effect of the size-factor is), the less the experimental eyes elongated after the step-up.

4.6.2 The effects of recovery

A previous study showed that the response to myopic defocus during recovery from form deprivation (that causes longer axial length than normal) is more robust than the

response to myopic defocus caused by positive lenses (normal axial length), suggesting that the difference in response is related to eye size[249].

Results in the current study, however, show the opposite: Eyes that have already compensated for +7 D lenses with shorter axial length (group 6) further reduced axial elongation to compensate for the +15 D lenses (against any size-related mechanism to restore normal eye length), significantly more than the amount of axial inhibition seen in the eyes recovering from prior –7 D lens wear (group 3, Fig. 4.6D). It is also worth noting that the experimental eyes in the recovery group (group 3) did not develop a greater hyperopic shift compared with those in the step-up group (group 6), even though both the visual and non-visual mechanisms would have been working in the same direction for group 3. It implies that there is no effect of eye size when myopic defocus in present.

4.6.3 Possible reasons why chick eyes cannot compensate for the strong negative lens after the step-up

It is not clear why chick eyes cannot compensate for the strong negative lenses (–10 and –15 D) after the step-up, while these negative lens powers are certainly within the compensation capacity range as shown in the control groups (groups 9 and 13). The first possibility is a possible limitation in *de novo* scleral Glycosaminoglycan synthesis associated with increased eye growth seen in negative lens wear[227, 250]. However, the fact the treated eyes in the control group fully compensated for the higher-powered negative lenses rules out this possibility. The second possibility is that there might be a delay for these myopic eyes to further compensate for the strong negative lenses, and the further compensation did not start before the experiment was terminated. To rule out this possibility, the chicks were observed 4 to 6 days after the step-up of negative lens power, which should be long enough to discover any further increase in axial elongation. A third possibility is that maybe the size-factor normally remains dormant in chick eyes and the defocus-factor has a dominant effect in regulating eye growth, hence the chicks in the control groups were able to fully compensate for the strong negative lenses (groups 9 and 13), and that the sudden step-up in the negative lens power activates an alternative response not related to the current defocus-factor to prevent the already elongated eyes from further elongating. Perhaps this alternative

factor signifies a limitation in the emmetropization mechanism in that chick eyes cannot detect an increase in the magnitude of the hyperopic defocus once the eye is already on a particular growth trajectory with a particular set point.

While our data here supports the existence of some kind of factor that refrains the eye from further elongating, it might be thought that it could be due to asymmetry between the two eyes, in terms of both the perceived defocus and/or the eye size. However, it is unlikely that an inability to cope with the progressively increasing asymmetry caused the eye to stop responding to the visual stimuli. Regarding the perceived defocus, it is clear that chick eyes have the capacity to compensate for myopic defocus after the step-up even though there was an asymmetry in perceived defocus up to approximately 8 D (Fig. 4.8). In terms of the asymmetry in the eye size, Fig. 4.9 shows that larger asymmetry between paired eyes (greater inter-ocular difference in axial length) tends to reduce the eye's ability to further elongate to compensate for the strong negative lenses after step-up. Or, it could be a combination of the asymmetry between the paired eyes and a sudden change in the visual input (e.g., defocus).

4.6.4 Contradictory results from early studies

Although the current study suggests that a size-factor may refrain the eye from further elongating, some previous studies showed opposite results. Troilo and Wallman discovered that the vitreous chamber elongated for a week after constant dark rearing to compensate for hyperopia caused by corneal flattening, even though the eyes had become excessively longer than normal after constant dark rearing[188]. Since chicks used in that study were a couple of months old, much older than the chicks used in the present study, it is possible that the phenomena we observe only operates when the animal is young, and gradually loses its effectiveness after the animal becomes older. Alternatively, as discussed above, maybe a size-factor normally remains dormant and needs to be activated by a sudden change in hyperopic defocus to exert its effect. Chicks eyes elongated excessively after constant dark rearing, and they further elongated to compensate for hyperopic defocus, just as chicks in the present study fully compensated for the initial negative lenses (–7 and –5 D lenses). Chick eyes might need a "step-up" in negative lens power for the size-factor to exert

its effect in reducing axial elongation. It would be interesting to study if these older chicks could compensate for the strong negative lenses after the step-up. Nevertheless, the fact that chick eyes made hyperopic after constant dark rearing with excessively elongated axial length and flattened corneas and shallow anterior chambers were able to resume their normal, age-appropriate eye size, even in eyes with severed optic nerve, strongly supports the contribution of a local, intrinsic mechanism controlling eye size[188]. Similarly, Whal, Li, and Howland discovered that chick eyes kept elongating for 1 week after they were exposed to constant light which already induced axial elongation[251]. Other than the two reasons mentioned above, it is also possible that constant light caused photo toxicity in the eye[252] and the size-factor is no longer available to refrain the eye from further elongating. Furthermore, Wildsoet and Schmid[253] showed that myopia induced by form deprivation can be maintained by negative lenses with the appropriate power. Similar results have also been found in tree shrews[254]. These results are in line with the possibility that the size-factor does not exert its effect until it is activated by a sudden change in hyperopic defocus after the eye is already on a trajectory to negative lens compensation.

4.6.5 Conclusions

The defocus-factor dominates in the case of eyes which are inhibiting their growth in response to myopic defocus from positive lens wear or recovery from negative lens wear, whereas there is a surprising limit in the ability of the eye to use defocus cues to guide enhancements in ocular growth. In particular, the avian eye seems unable to respond to a sudden change in the magnitude of imposed hyperopic defocus, even though it is clear that it can detect this degree of hyperopic defocus. In the face of a defocus change, it seems that the eye is constrained from further axial elongation as if it becomes sensitive to its preferred size or the asymmetry between the two eyes.

5. Chick Eyes Can Shorten to Compensate for Myopic Defocus

5.1 Forward

Data from chapter 4 showed that chick eyes can further compensate for a stronger positively-powered lens worn monocularly after the rate of ocular elongation has already been reduced (from wearing a weaker positive lens), supporting the idea that the defocus-factor dominates in the case of positive lens compensation. The next logical step is to study to what extent eyes can compensate for myopic defocus, i.e., whether eyes can shrink axially to facilitate positive lens compensation, effectively going against the size-factor. This chapter consists of an analysis of data from previous experiments conducted at Josh Wallman's laboratory at the City College of the City University of New York, some of which have been published in different contexts[82, 142, 144].

The following hypotheses are proposed:

(1) Chick eyes can shorten axially to facilitate compensation for myopic defocus, and (2) axial shortening is caused mostly by a reduction in vitreous chamber depth which was greater than and could not be accounted for by choroidal expansion or measurement error.

Some of the results from this chapter have previously been presented in an abstract form (Zhu X. and Wallman J., *Invest. Ophthalmol. Vis.* 2009, E-Abstract 3929) and as a published peer reviewed paper (Zhu X. *et al., Invest. Ophthalmol. Vis. Sci.* 2013)[255].

5.2 Abstract

Purpose: It has been shown in the previous chapter that some unknown non-visual mechanism(s) influences negative lens-compensation in chicks by refraining the eyes from becoming too long. On the other hand, positive lens compensation is not affected by this non-visual mechanism and chick eyes can further reduce the rate of ocular elongation to compensate for positive lenses. This chapter tests whether young chick eyes can shorten in the axial dimension to facilitate compensation for myopic defocus.

Methods: (1) One-week-old chicks wore positive lenses over one eye for 3 days and axial dimensions were measured with ultrasound biometry 3 days apart. (2) A control group of normal, untreated chicks were measured 3 days apart on the same days as chicks in the previous group. (3) Another group of chicks wore various lenses on one eye and had the fellow control eyes measured by ultrasound biometry repeatedly within 1 hour.

Results: Chick eyes wearing positive lenses reduced their rate of ocular elongation by two-thirds, including 38.5% of eyes in which the axial length became shorter than before (mean change in axial length over the course of the experiment, experimental vs. fellow eyes, 40 vs. 171 μm). The axial shortening was caused mostly by the reduction in vitreous chamber depth.

Conclusions: Chick eyes can shorten axially when presented with myopic defocus created by wearing positive lenses. This eye shortening facilitates compensation for the imposed myopia. If the same is true in humans, implications for human myopia control are significant.

5.3 Introduction

Many animal studies have shown that eyes can compensate for imposed defocus by changing both choroidal thickness and the rate of ocular elongation, above or below that found in normal untreated growing eyes. For instance, when wearing a positive lens that puts the focal plane in front of the photoreceptors, the eye decreases its rate of ocular elongation and increases choroidal thickness thereby pushing the retina forward to meet the focal plane; the opposite happens in the case of wearing a negative lens. Among the various animal species used, chick eyes have been shown to be able to compensate for the widest range of defocus[78].

It is usually assumed that, when eyes compensate for myopic defocus imposed by positive lenses, their rate of ocular elongation is reduced, so the eye either elongates at a slower rate than normal or, at the most, stops its growth. Even though it seems more natural that an eye in a growing animal should elongate rather than actually shorten (reduced length from the front of cornea to the back of sclera), there seems no obvious reason why an eye experiencing myopic defocus cannot axially shorten through a mechanism such as extracellular matrix remodeling of the sclera, thereby further facilitating compensation. Given that tissues are continuously remodeled under a homeostatic control, it is curious why should axial shortening be more implausible than growth.

Previous studies have shown that organ size can fluctuate drastically under physiological conditions. In Burmese pythons, which typically feed once every a couple of months, the heart, lungs, liver, intestinal mucosa, and kidneys all alternate between a large and a small size: After a large meal, the increase in mass of these organs ranges between 50-150% (as percentage of fasted mass)[183]. In many seasonally breeding birds, the gonads can shrink by 87% when the day-length decreases from 13 to 12 hours (e.g., spotted antbirds[182]). If other organs can fluctuate in size, perhaps eyes as well can shrink when needed. In this chapter, it is demonstrated that chick eyes, too, can axially shorten in response to myopic defocus when wearing positive lenses.

5.4 Methods

5.4.1 Animals

White Leghorn chicks were obtained from either Cornell University (Cornell K-strain; Ithaca, NY) or Truslow Farms (Hyline-W98-strain; Chestertown, MD). Chicks were housed in a heated, sound-attenuated chamber (76 x 61 cm), with a 14:10 hour light:dark cycle as described in the General Methods (Section 2.1). These chicks were used for previous experiments conducted at Josh Wallman's laboratory at the City College of the City University of New York.

5.4.2 Experimental procedures and axial biometry measurements

Glass lenses of –8.6, –7, –6, +6, +7, or +10 D were used as described in General Methods (Section 2.2). The majority of chicks wore a lens over one eye for 3 days: Some chicks wore positive lenses (+6, +7, or +10 D) either continuously (with or without a weak diffuser) or for various durations (specifically, 20 seconds per 20 minutes, 5 seconds per 5 minutes, 2 minutes per 10 minutes or hour, 5 minutes per 4 hours, and 30 minutes per 2, 4, or 12 hours) with darkness between episodes (groups 17 to 27, n = 195, Table 5.1). All of the above chicks were measured by ultrasound biometry (as described in General Methods, Section 2.3) before and after 3 days of treatment on 7 and 10 days of age. A control group of untreated, normal chicks were also measured on days 7 and 10 (group 28, n = 48). Another set of chicks wore various lenses on one eye and had the fellow control eyes measured by ultrasound biometry repeatedly within 1 hour (group 29, n = 145), and change in axial length and vitreous chamber depth was used the estimate the measure error of A-scan biometry. The starting age of all chicks was one-week-old in all the experiments. See Table 5.1 for treatment details and sample sizes for each treatment.

All chicks were measured within a 4-window during the morning of their day cycle (between 10 am and 2 pm) to help minimize the effect of circadian rhythm on axial length and choroidal thickness[75].

Table 5.1. Summary of treatment details and sample size (n)

Group number	Measurement	Lens type	Treatment	n
17	3 days apart (at 7 and 10 days of age)	Plus	+6 or +7 D lens, continuous	36
18			+7 D lens with a weak diffuser, continuous	13
19			+6 D lens, 5 seconds every 5 minutes	10
20			+6 D lens, 20 seconds every 20 minutes	9
21			+7 D lens, 2 minutes every 10 minutes	7
22			+6 D lens, 2 minutes every hour	14
23			+10 D lens, 5 minutes every 4 hours	6
24			+6 D lens, 30 minutes every 2 hours	6
25			+6 or +10 D lens, 30 minutes every 4 hours	76
26			+6 D lens, 30 minutes every 12 hours	6
27		Plus and minus	+6 and –6 D lenses, each worn alternately for 15 minutes every 4 hours	12
28		None	Normal, untreated	48
29	Repeatedly within an hour	Plus and minus	+10 or –8.6 D lens for 10 minutes	145

5.4.3 Analyses

Data are presented as the Mean ± standard deviation (SD). Three different statistical methods were used to compare the number of eyes that axially shortened vs. the number of eyes that did not in the positive lens-treated eyes:

(1) The change axial length in the positive lens-wearing eyes over the course of the experiment (between days 7 and 10, groups 17 to 27) was compared to that found in the right eyes from untreated, normal chicks (group 28) over the same duration with unpaired, 2-tailed *Student*'s t-test.

(2) The number of treated eyes that shortened while wearing positive lenses vs. those that did not (groups 17 to 27) was compared to the number eyes from untreated chicks that shortened vs. those that did not (group 28, only the right eyes from group 28 were used) with chi-square test.

(3) The measurement error of A-scan ultrasonography was estimated by reviewing data from experiments in which the control eyes were measured twice within 1 hour, during which their fellow eyes wore various lenses (group 29, n = 145). Since very little change in axial length was expected within an hour, the standard deviation of the change in axial length

between two consecutive measurements was used as an index of the measurement error. The percentage of treated eyes that shortened that could be explained by measurement error vs. the actual percentage encountered was compared with Fisher's exact test. The 95% confidence intervals for A-scan ultrasonography measures of axial length was utilized to assess whether the observed axial shortening in response to myopic defocus could be accounted for by measurement error. The 95% confidence intervals were calculated from control eyes (n = 145, groups 17 to 27) that were measured twice within an interval of one hour, during which time their fellow eyes wore various lenses.

5.5 Results

As expected, positive spectacle lenses decreased the rate of ocular elongation (Fig. 5.1): Eyes wearing positive lenses for 3 days (groups 17 to 27) elongated approximately a quarter as much as the right eyes of untreated, normal chicks (group 28, change in axial length between days 7 and 10, mean ± SD, groups 17 to 27 pooled vs. group 28, +40 ± 105 μm vs. +188 ± 94 μm, $p < 0.001$, Figs. 5.1A and B). In chicks wearing positive lenses (groups 17 to 27), 75 out of 195 positive lens-wearing eyes became shorter than at the start of the experiment (mean shortening: –63 ± 49 μm, mean ± SD, Fig. 5.1B), whereas only 1 out of 48 normal eyes shortened (group 28, –121 μm, Fig. 5.1B). The frequency of eye shortening in the positive lens-wearing eyes and the frequency of eye shortening in the normal eyes was significantly different (chi-square = 20.547, $p < 0.001$).

The 95% confidence interval for change in axial length was estimated from repeated measures on 145 fellow eyes (group 29), each measure separated by 1 hour. This provided a standard deviation (SD) of 26 μm, resulting in 95% confidence intervals of ± 51 μm. The standard deviation of these measurements (SD = 26 μm) overestimated the measurement error because it was based on a heterogeneous sample of experimental animals measured at different times of day. Using this standard deviation and supposing the changes in the length of individual positive-lens-wearing eyes approximated a normal distribution (Fig. 5.1C), a zero change in axial length in eyes wearing positive lenses would be 1.54 standard deviations below the mean (40 μm). Therefore, if measurement error were the only cause, it would be

expected that 6.2% of these 195 eyes (12 eyes) to have shortened, rather than 38.5% (75 eyes) that were encountered ($p < 0.0001$, Fisher's exact test).

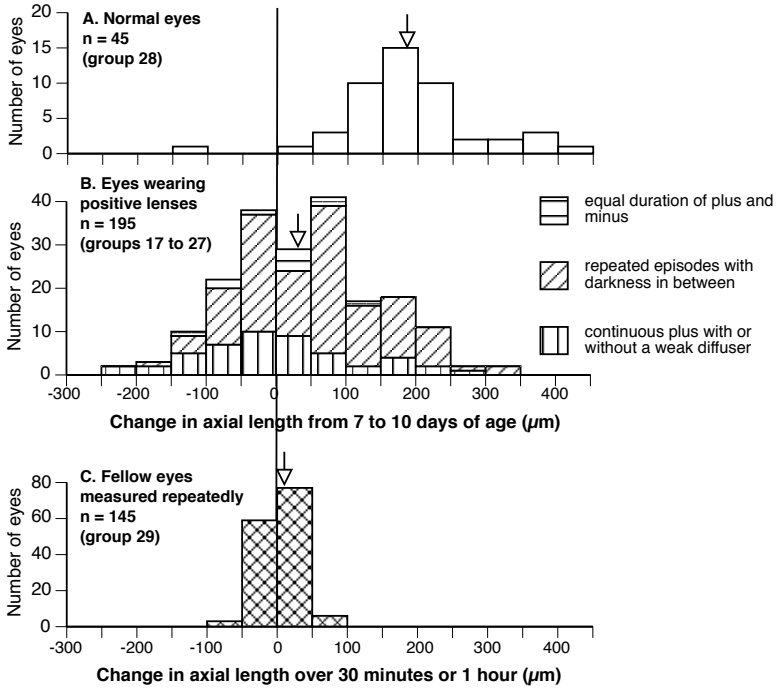

Figure 5.1. The frequency distributions of change in axial length. (A) Untreated normal eyes, (B) positive lens-wearing eyes, and (C) fellow eyes of lens-wearing eyes. Arrows in each panel indicate the average of each group.

Not surprisingly, the shortening in axial length also resulted in a decrease in the depth of the vitreous chamber (Fig. 5.2): While the mean vitreous chamber depth in untreated, normal eyes elongated by +24 μm over 3 days (Fig. 5.2A), the mean vitreous chamber depth in positive lens-wearing eyes decreased by –84 μm over the same duration ($p < 0.001$, Fig. 5.2B). Furthermore, significantly reduced axial enlargement in anterior chamber depth and lens thickness was also found in positive lens-wearing eyes, although to a smaller degree (groups 17 to 27 pooled vs. group 28, anterior chamber depth, positive-lens-wearing eyes vs. normal eyes: +1 vs. +34 μm, $p < 0.001$; lens thickness, +89 vs. 121 μm, $p < 0.001$). The choroids in positive-lens-wearing eyes slightly thickened more than those in the untreated normal eyes (+32 μm vs. +6 μm, $p = 0.09$). This change, however, only caused a reduction in vitreous chamber depth without changing axial length. Indeed, the shortening in the

vitreous chamber cannot be fully explained by choroidal thickening (mean change in vitreous chamber depth vs. mean change in choroidal thickness: –84 μm vs. +32 μm, i.e., choroidal thickening only accounts for less than 50% of the reduction in vitreous chamber depth), but is a consequence of the reduced axial length. No significant change was found in retinal or scleral thickness during the short course of experiments.

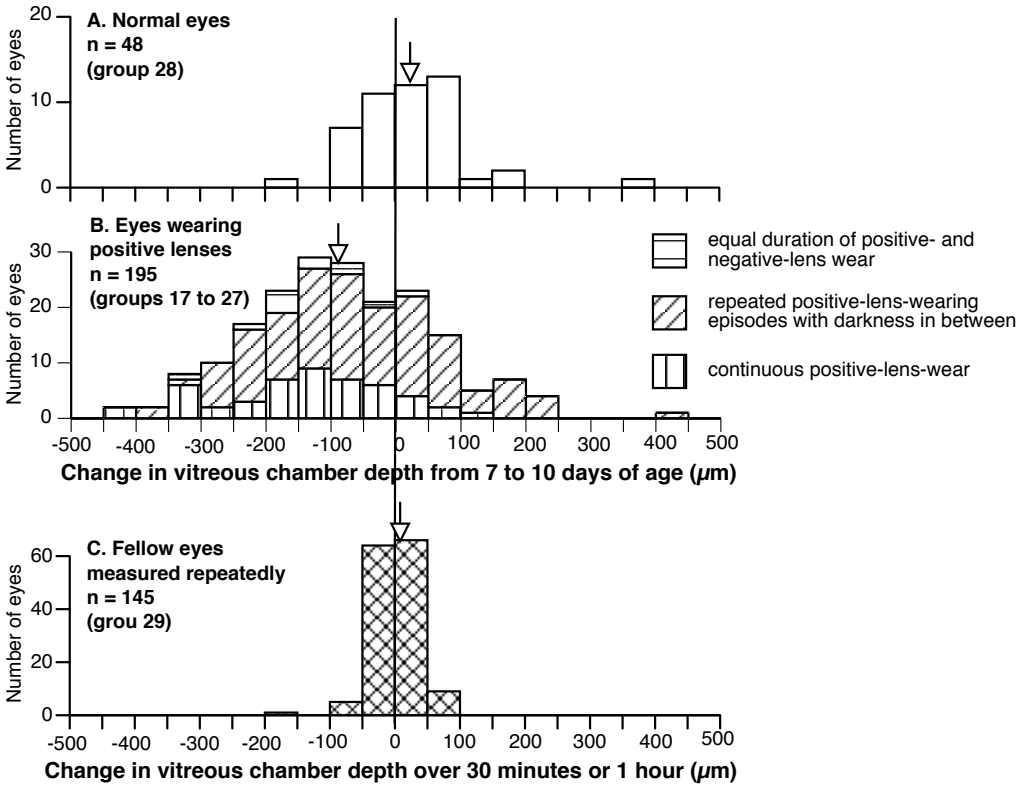

Figure 5.2. The frequency distributions of change in vitreous chamber depth.
(A) Untreated normal eyes, (B) positive lens-wearing eyes, and (C) fellow eyes of lens-wearing eyes. Arrows in each panel indicate the average of each group.

To rule out the possibility of abnormal growth in the chicks whose positive lens-wearing eyes shortened, the axial growth of the fellow eyes in these chicks was compared to the rest of the fellow eyes in positive lens-wearing chicks, since a systemic pathological condition would have reduced eye growth not only in the lens-wearing eye, but also in the fellow eye. For the 75 out of 195 positive-lens-wearing eyes that shortened over the 3-day period (38.5%), only 9 out of these 75 fellow eyes shortened (12%, $p < 0.0001$, Fisher's exact

test, Fig. 5.3). Therefore, it is unlikely that the axial shortening in the positive lens-wearing eyes was caused by some systemic pathological condition.

In summary, wearing positive lenses caused the eyes to axially shorten over a wide range of paradigms, suggesting that axial shortening of positive lens-wearing eyes in chicks was not the result of pathology, but was the product of an active compensatory mechanism for superimposed monocular myopic defocus.

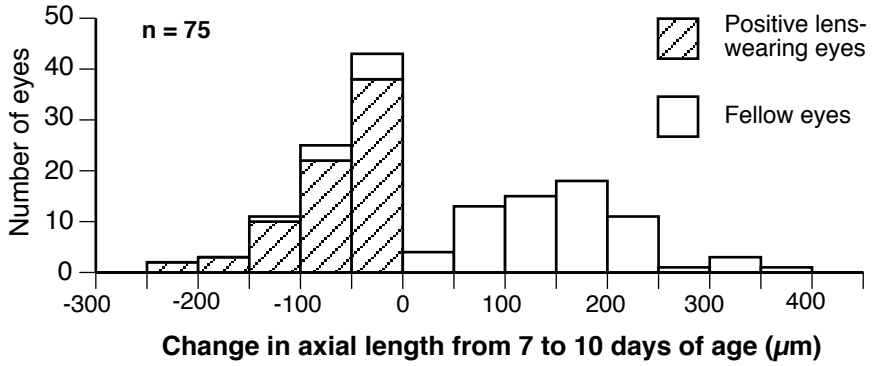

Figure 5.3. The frequency distributions of change in axial length in eyes that shrank after wearing positive lenses and their fellow eyes.

5.6 Discussion

Our data provide evidence that avian eyes can axially shorten to compensate for myopic defocus. It has been shown in humans that eye length can reduce by 0.04 ± 0.08 mm after overnight orthokeratology[256], although in this study, measurement errors were not incorporated.

It might be considered that it would be more difficult for chick eyes to shorten than to reduce growth because the chick sclera has a more rigid cartilaginous layer composed of chondrocytes, whereas the outer layer of mammalian eyes only has fibrous sclera composed of fibroblasts and myofibroblasts[257] which theoretically should make it easier to remodel. However, an earlier study by Kusakari *et al.* found evidence of scleral remodeling in the

posterior sclera during induced myopia in chicks[258]. Specifically, after deprivation-induced myopia, the boundary between the cartilaginous and fibrous sclera became indistinct, and some spindle-shaped transitional mesenchymal cells that showed morphological features of both fibroblasts and chondrocytes were discovered between the two layers, suggesting possible transformation of the two cell types during altered eye growth. These findings support the possibility of remodeling during compensation that could lead to actual eye shortening in chicks.

Eye growth is controlled by local retinal mechanisms, as demonstrated by the fact that after the eye and the brain are disconnected, either by optic nerve section in chicks[69] and guinea pigs[259], or by blocking the action potentials of retinal cells by tetrodotoxin[201, 260] chick and tree shrew eyes still develop deprivation-induced myopia. Chick eyes have also been shown to maintain the ability to compensate for positive or negative spectacle lenses after optic nerve section, albeit with some variation[81, 261], although different results have been found in mammals[262]. These results suggest that the avian retina can modulate eye growth in response to altered visual stimuli without significant input from the brain. In addition, this local mechanism can selectively alter eye growth within a limited region or quadrant of the eye when diffusers[263] or lenses[94] degrade the retinal image in that part of the eye, while leaving the rest of the retinal image relatively intact. It seems highly likely that active axial shortening of eyes, as reported in this study, is also controlled by local ocular mechanisms. In contrast, some early studies suggest that the brain may be involved in the control of eye growth: (1) Chick eyes recovering from form deprivation myopia over-shoot emmetropia after optic nerve section[188, 264], (2) untreated chick eyes were smaller and more hyperopic than normal after optic nerve section[69, 81], and (3) compensation for hyperopic defocus caused by negative lenses is greatly reduced by optic nerve section[81].

In summary, an analysis of data from eyes in young, rapidly growing chicks demonstrates that some chick eyes can shorten axially to facilitate compensation for myopic defocus. It would be interesting to determine if this phenomenon also exists in children or adolescents, since older adult human eyes have been shown to shorten axially, possibly in response to the increased refractive power of both the cornea and the lens[265]. In particular,

can applying myopic defocus cause the developing human eye to axially shorten or shrink? If this was the case then strategies involving myopic defocus for preventing or reducing the axial elongation of the eye that results in high myopia in human, and subsequent ocular pathology, might be feasibly used.

5.7 Conclusions

Chick eyes can shorten axially when presented with myopic defocus created by wearing positive lenses. This eye shortening facilitates compensation for the imposed myopia. If the same is true in humans, implications for human myopia control are significant.

6. Interaction between Paired Eyes: Symmetrical Growth, Yoking, and Anti-Yoking

6.1 Forward

This Chapter will be dedicated to specifically study the inter-ocular interactions in the control of eye growth, namely, symmetrical growth, yoking and anti-yoking. As mentioned in Chapter 1 (Section 1.7.3), yoking is defined as the situation when monocular treatment causes the fellow eye to change in the same direction (e.g., treated eyes of positive lens-wearing animals reduce elongation and become hyperopic, while their fellow eyes also become more hyperopic with shorter axial length compared with age-matched normal animals, albeit to a lesser extent than in treated eyes), and anti-yoking is defined as the situation when monocular treatment causes the fellow eye to change in the opposite direction to the treated eye, relative to normal eye growth. Instances of yoking and phenomena consistent with anti-yoking have been reported in various species, such as chicks, tree shrews, rhesus monkeys, marmosets after various treatments (see Section 1.7.3, Tables 1.2 and 1.3 for details). However, there are no systematic investigation of these phenomena or any studies of the change in fellow eyes of animals treated with positive and negative lenses relative to untreated animals. In addition, it is not clear if there is any association between the amount of yoking/anti-yoking and the treatment duration.

The following hypotheses are proposed:

Monocular lens treatment will affect eye growth in the fellow eye, i.e., the rate of ocular growth in the fellow eye of a treated animal will be significantly different compared with that in the eye of untreated, normal chicks. Furthermore, the amount of yoking or anti-yoking may be lens treatment duration dependent.

6.2 Abstract

Purpose: Animal research has shown that eye growth can be independently modulated between the two eyes, such that the axial length of an experimental eye can be induced to grow at a different rate from that in its fellow eye, producing an inter-ocular difference in both refractive state and ocular dimensions, as well as the expression of mRNA of certain proteins and genes. This is particularly true in young chicks, that demonstrate substantial independence between the two eyes. However, there are also instances reported of interactions between the two eyes, in that the treatment in the experimental eye sometimes influences the untreated fellow eyes to grow either in the same (yoking) or opposite (anti-yoking) direction compared with untreated eyes. In this chapter, such yoking and anti-yoking effects were investigated in young chicks in response to monocular lens wear.

Methods: (1) Data from a large group of untreated chicks from the Wallman database (n = 2960) measured from 1 to 17 days of age were reviewed to generate a normal growth curve for untreated eyes, and (2) other groups of chicks from the same source (n = 169) wore either a positive or negative lens of various powers (+/–5, +/–7, +/–10, and +/–15 D) over one eye for various durations (1 to 7 days), and ocular dimensions were measured before and after the experiment with A-scan biometry. The change in axial length in the fellow eyes was compared with the expected change in axial length in normal eyes for each experiment, and the difference between the two (the "adjusted change in axial length") were compared across all lens wearing durations and lens powers.

Results: Paired eyes in untreated chicks were significantly correlated in their axial lengths 24 h after birth (8.55 mm and 8.53 mm for the right and left eyes, respectively; $r^2 = 0.77$, $p < 0.0001$), demonstrating symmetrical size. They also continued to demonstrate symmetrical growth as they aged. While monocular lens treatment caused significant compensation in the treated eyes, there was still a significant correlation in axial length between paired eyes. Furthermore, yoking and anti-yoking, as defined by significant differences compared to growth predicted from untreated animals, were observed in approximately half of the experiments. In general, monocular lens treatment tended to cause reduced eye growth in the fellow eyes after shorter lens wearing durations (1-2 days, yoking for positive lens

treatment and anti-yoking for negative lens treatment) and increased eye growth after longer lens wearing durations (longer than 4 days, anti-yoking for positive lens treatment and yoking for negative lens treatment), and had minimal effect on the fellow eyes if the treatment duration was around 3-4 days.

Conclusions: In young chicks, growth in paired eyes was well correlated despite monocular lens treatment. Yoking and anti-yoking were discovered in only certain lens-wearing conditions and seemed to be dependent on the length of treatment. Experiments using the fellow eye as a control under conditions which may induce yoking and anti-yoking, can still be used but are conservative and may under- or overestimate the actual effect sizes by up to 27% if the lens treatment duration is around 3-4 days. Shorter and longer treatment durations, on the other hand, seem to have a larger effect on the fellow eyes (up to 89.0%). Caution should be taken when interpreting results of monocular treatment. Finally, it might be prudent to have a group of untreated animals as a control.

6.3 Introduction

There are several factors influencing eye size and eye growth. Although there is much evidence demonstrating that visual input has an important role in regulating eye growth[28], the growth rate and size of bilateral limbs and organs (including the eyes) are also well controlled and maintained throughout life by multiple molecular pathways. An earlier compilation of the allometry and scaling of the size of vertebrate eyes in terms of axial lengths and body weight found that bird eyes are 36% larger than those of vertebrates in general[197]. It has also been shown that optical treatment over one eye (lens treatment or form deprivation) can affect the untreated fellow eye, in terms of change in both refractive error and ocular dimensions (vitreous chamber depth and axial length), mechanical properties of the sclera, and mRNA expression of certain proteins (see Section 1.7.3 for more details).

Chick eyes have independent innervation[193], blood supply[194], and mostly show independent accommodation[195]. The two bony orbits are separated by an interorbital septum (an ossified partition)[196]. An artery ophthalmica interna that travels medial of the optic nerve provides blood supply for each eye[194]. The optic nerves project in a highly ordered manner onto their primary visual target areas with almost complete decussation at the chiasm[193]. Therefore, it is surprising that treatment on one eye could affect the untreated fellow eye. Never-the-less, birds in general, and chicks in particular, have a small binocular field, with consequential neural integration between the two eyes in brain pathways beyond the retina at the thalamotelencephalic level[266]. There is also evidence for convergent accommodation in pigeons[267-269] suggesting that binocular interactions may also occur in the chick.

In myopia research using chicks, animals are conventionally treated monocularly and researchers compare the change in the treated eye to that in the untreated fellow eye, with the assumption that both eyes develop largely independently and the effect of the treatment is confined to the treated eye. The purpose of inter-ocular comparison is to increase statistically sensitivity and to reduce the number of animals needed. However, yoking and anti-yoking could challenge the validity of performing inter-ocular comparisons since the fellow eye might also be affected by the treatment.

The aims of this chapter are: (1) To confirm the presence of symmetrical size and growth in untreated chicks, and (2) characterize the effects of wearing positive or negative lenses over one eye for various durations on producing yoked or anti-yoked growth in the "untreated" eye. To determine the expected growth in normal chicks, normal eye growth was plotted against age (in days) in untreated chicks. The change in axial length in the untreated fellow eyes in lens-wearing animals was then compared to the expected growth in eyes of normal chicks of the same age. The difference between these two was called the "adjusted change" in axial length. In addition, the possible mechanisms of binocular yoked and anti-yoked growth and how they may affect experimental outcomes are discussed.

6.4 Methods

An analysis of symmetrical growth, and yoked and anti-yoked ocular growth was undertaken based on a large group of chicks that were either normal and measured on various days, or wore a positive or negative lens over one eye for various durations. All treatment details are summarized in Tables 6.1 (for Exp. 6.1) and 6.2 (for Exp. 6.2).

6.4.1 Animals

White Leghorn chicks (n = 2960) were originally obtained from Cornell University (K strain, Cornell University, Ithaca, NY). Chicks were hatched and reared in the Wallman laboratory at City College of the City University of New York as described in Zhu & Wallman[146], and as described in General Methods (Section 2.1).

6.4.2 Experimental procedures and axial biometry measurements

Ocular components were measured under anesthesia (1.5% isoflurane) using A-scan ultrasound as described in the General Methods (Section 2.3). Axial length was used because of the large amount of data available from previous studies and its close relationship to the focal length of vertebrate eyes[197]. In addition, measurements were made at approximately the same time of day (2 hours after lights on), at the start and the end of the experiment, to control for circadian changes in growth[75].

6.4.2.1 Exp. 6.1: Symmetrical Size and Growth in Eyes of Untreated Chicks

Ocular dimensions in untreated, normal chicks were measured just once at various ages (from days 1 to 17, groups 30 to 46, n = 2960, Table 6.1) to study symmetrical size, i.e., to determine if the axial length between the right and left eyes were correlated. Another group of normal chicks were measured at 7 and 10 days of age, to study if the change in eye size over this 3-day period was also symmetrical in paired eyes (group 28, Table 6.1, n = 48).

Table 6.1. Summary of the measurement age, ocular dimensions (Mean ± SEM) of the left eyes, the r^2 and *p* values for axial length between paired eyes, and sample size for normal chicks (Exp. 6.1)

Group #	Age (day)	Anterior chamber depth (mm)	Lens thickness (mm)	Vitreous chamber depth (mm)	Choroidal thickness (mm)	Axial length (mm)	r^2	*p*	n
30	1	1.22 ± 0.01	1.60 ± 0.01	5.16 ± 0.03	0.23 ± 0.02	8.53 ± 0.04	0.749	< 0.0001	16
31	2	1.24 ± 0.01	1.76 ± 0.02	5.16 ± 0.02	0.15 ± 0.01	8.62 ± 0.03	0.858	< 0.0001	38
32	3	1.19 ± 0.01	1.63 ± 0.01	5.22 ± 0.03	0.21 ± 0.01	8.57 ± 0.02	0.665	< 0.0001	26
33	4	1.29 ± 0.01	1.79 ± 0.02	5.07 ± 0.02	0.21 ± 0.01	8.69 ± 0.03	0.859	< 0.0001	21
34	5	1.25 ± 0.01	1.83 ± 0.02	5.13 ± 0.02	0.18 ± 0.01	8.72 ± 0.04	0.914	< 0.0001	44
35	6	1.30 ± 0.01	1.92 ± 0.00	5.13 ± 0.01	0.20 ± 0.01	8.90 ± 0.01	0.913	< 0.0001	177
36	7	1.30 ± 0.00	1.93 ± 0.00	5.10 ± 0.00	0.19 ± 0.00	8.85 ± 0.01	0.894	< 0.0001	1234
37	8	1.31 ± 0.00	1.94 ± 0.00	5.07 ± 0.01	0.19 ± 0.00	8.84 ± 0.01	0.885	< 0.0001	667
38	9	1.33 ± 0.00	1.98 ± 0.00	5.09 ± 0.01	0.19 ± 0.00	8.93 ± 0.02	0.771	< 0.0001	193
39	10	1.36 ± 0.01	2.00 ± 0.01	5.15 ± 0.02	0.20 ± 0.00	9.04 ± 0.02	0.903	< 0.0001	105
40	11	1.35 ± 0.01	2.01 ± 0.01	5.10 ± 0.03	0.22 ± 0.01	8.99 ± 0.03	0.836	< 0.0001	35
41	12	1.37 ± 0.01	2.05 ± 0.01	5.15 ± 0.03	0.19 ± 0.01	9.08 ± 0.03	0.895	< 0.0001	14
42	13	1.44 ± 0.01	2.20 ± 0.01	5.35 ± 0.03	0.21 ± 0.01	9.54 ± 0.04	0.873	< 0.0001	26
43	14	1.41 ± 0.00	2.13 ± 0.01	5.22 ± 0.01	0.19 ± 0.00	9.29 ± 0.01	0.659	< 0.0001	269
44	15	1.45 ± 0.01	2.15 ± 0.01	5.33 ± 0.04	0.21 ± 0.01	9.47 ± 0.05	0.954	< 0.0001	57
45	16	1.46 ± 0.02	2.15 ± 0.01	5.37 ± 0.04	0.17 ± 0.01	9.48 ± 0.05	0.938	< 0.0001	25
46	17	1.46 ± 0.01	2.22 ± 0.01	5.40 ± 0.03	0.21 ± 0.01	9.64 ± 0.05	0.859	< 0.0001	13
28*	7	1.32 ± 0.01	1.91 ± 0.01	5.08 ± 0.02	0.19 ± 0.01	8.83 ± 0.02			48
	10	1.35 ± 0.01	2.03 ± 0.01	5.11 ± 0.02	0.20 ± 0.01	9.02 ± 0.03			

The r^2 values are the Coefficients derived from linear regressions for axial lengths between paired eyes at various ages, and *p* values are the statistical significance for the slopes for these linear regressions.

* Group that was mentioned in Chapter 5. The axial length at 7 and 10 days of age in this group are included in the corresponding ages above (group 36 and 39, respectively).

6.4.2.2 Exp. 6.2: The Effect of Monocular Lens Wear on Symmetrical Growth, Yoking and Anti-Yoking

Glass lenses of powers +15, +10, +7, +5, –5, –7, –10, and -15 D were used as described in General Methods (Section 2.2). In all experiments with lens treatment, chicks wore lenses over one eye for various durations (groups 47 to 63, total n = 169, Table 6.2), and the fellow eye was left untreated.

Table 6.2. Summary of lens treatment details, the change in ocular dimensions over the course of each experiment (Mean ± SEM), *p* values, and the sample size for Exp. 6.2

Group #	Lens type	Lens treatment (in days of age)			Anterior chamber depth (mm)			Lens thickness (mm)			Vitreous chamber depth (mm)			Choroidal thickness (mm)			Axial length (mm)		
		start	end	duration	ΔX	ΔN	*p*	ΔX	ΔN	*p*	ΔX	ΔN	*p*	ΔX	ΔN	*p*	ΔX	ΔN	*p*
47	+5 D	11	14	3	0.03 ± 0.01	0.06 ± 0.01	0.186	0.09 ± 0.01	0.11 ± 0.01	0.247	-0.12 ± 0.03	0.07 ± 0.02	**≤0.001**	0.12 ± 0.03	0.00 ± 0.01	**≤0.01**	0.14 ± 0.02	0.25 ± 0.02	**≤0.01**
48		7	11	4	0.10 ± 0.01	0.09 ± 0.01	0.206	0.10 ± 0.02	0.14 ± 0.02	0.083	-0.09 ± 0.02	0.12 ± 0.03	**≤0.001**	0.07 ± 0.03	0.00 ± 0.02	**≤0.05**	0.19 ± 0.03	0.35 ± 0.04	**≤0.01**
49		7	14	7	0.12 ± 0.02	0.16 ± 0.01	0.176	0.18 ± 0.03	0.22 ± 0.02	0.214	-0.07 ± 0.06	0.19 ± 0.05	**≤0.01**	0.17 ± 0.04	-0.02 ± 0.02	**≤0.05**	0.41 ± 0.06	0.56 ± 0.06	**≤0.05**
50	+7 D	1	4	3	-0.06 ± 0.03	0.00 ± 0.01	**≤0.05**	0.18 ± 0.02	0.20 ± 0.01	0.444	-0.26 ± 0.06	-0.06 ± 0.05	**≤0.01**	0.01 ± 0.02	-0.02 ± 0.05	0.356	-0.11 ± 0.08	0.14 ± 0.06	**≤0.01**
51		7	10	3	-0.03 ± 0.02	0.03 ± 0.01	**≤0.01**	0.08 ± 0.02	0.10 ± 0.01	0.376	-0.16 ± 0.03	0.06 ± 0.02	**≤0.001**	0.09 ± 0.02	-0.01 ± 0.02	**≤0.01**	-0.02 ± 0.03	0.18 ± 0.03	**≤0.001**
52		7	11	4	-0.03 ± 0.02	0.02 ± 0.01	**≤0.05**	0.06 ± 0.02	0.11 ± 0.02	**≤0.01**	-0.15 ± 0.02	0.08 ± 0.03	**≤0.001**	0.09 ± 0.02	-0.01 ± 0.01	**≤0.001**	-0.02 ± 0.04	0.19 ± 0.03	**≤0.001**
53		7	14	7	0.17 ± 0.02	0.15 ± 0.02	0.205	0.31 ± 0.02	0.32 ± 0.02	0.476	-0.13 ± 0.02	0.16 ± 0.03	**≤0.001**	0.09 ± 0.02	0.04 ± 0.01	**≤0.05**	0.46 ± 0.04	0.68 ± 0.03	**≤0.001**
54	+10 D	7	11	4	0.03 ± 0.01	0.06 ± 0.03	0.222	0.09 ± 0.02	0.12 ± 0.02	0.260	-0.28 ± 0.04	0.10 ± 0.02	**≤0.001**	0.18 ± 0.03	-0.01 ± 0.01	**≤0.001**	0.03 ± 0.05	0.28 ± 0.05	**≤0.05**
55	+15 D	6	7	1	0.00 ± 0.01	-0.01 ± 0.01	0.702	0.03 ± 0.01	0.06 ± 0.01	**≤0.05**	-0.18 ± 0.03	-0.03 ± 0.02	**≤0.01**	0.11 ± 0.03	-0.03 ± 0.02	**≤0.01**	-0.04 ± 0.02	0.00 ± 0.02	0.209
56	–5 D	7	11	4	0.04 ± 0.02	0.06 ± 0.01	0.282	0.18 ± 0.01	0.19 ± 0.01	0.225	0.15 ± 0.02	0.03 ± 0.02	**≤0.001**	-0.01 ± 0.01	0.03 ± 0.01	**≤0.01**	0.37 ± 0.04	0.31 ± 0.04	**≤0.05**
57		7	14	7	0.08 ± 0.01	0.11 ± 0.01	**≤0.05**	0.27 ± 0.01	0.26 ± 0.01	0.756	0.33 ± 0.03	0.19 ± 0.02	**≤0.001**	0.01 ± 0.01	0.05 ± 0.01	**≤0.05**	0.70 ± 0.03	0.63 ± 0.03	**≤0.05**
58	–7 D	7	9	2	-0.01 ± 0.02	0.02 ± 0.00	0.190	0.06 ± 0.02	0.09 ± 0.01	0.182	0.16 ± 0.02	-0.05 ± 0.01	**≤0.001**	-0.06 ± 0.01	-0.01 ± 0.01	**≤0.05**	0.15 ± 0.02	0.06 ± 0.02	**≤0.05**
59		1	4	3	-0.01 ± 0.03	0.03 ± 0.03	0.163	0.14 ± 0.01	0.15 ± 0.04	0.962	0.22 ± 0.08	-0.06 ± 0.04	**≤0.01**	-0.07 ± 0.04	-0.04 ± 0.02	0.331	0.28 ± 0.06	0.08 ± 0.04	**≤0.01**
60		7	10	3	0.01 ± 0.02	0.01 ± 0.02	0.613	0.09 ± 0.01	0.10 ± 0.01	0.410	0.28 ± 0.02	0.06 ± 0.02	**≤0.001**	-0.06 ± 0.03	0.00 ± 0.01	0.108	0.31 ± 0.04	0.19 ± 0.02	**≤0.01**
61		7	11	4	0.02 ± 0.01	0.04 ± 0.02	0.444	0.10 ± 0.01	0.11 ± 0.01	0.528	0.27 ± 0.01	0.05 ± 0.02	**≤0.001**	-0.04 ± 0.03	0.01 ± 0.02	0.197	0.35 ± 0.04	0.23 ± 0.03	**≤0.05**
62	–10 D	7	11	4	0.02 ± 0.02	0.05 ± 0.02	**≤0.05**	0.19 ± 0.02	0.20 ± 0.02	0.806	0.20 ± 0.03	-0.03 ± 0.03	**≤0.001**	-0.06 ± 0.02	0.01 ± 0.01	**≤0.05**	0.35 ± 0.05	0.24 ± 0.05	**≤0.01**
63	–15 D	7	9	2	0.01 ± 0.01	0.01 ± 0.01	0.917	0.05 ± 0.01	0.09 ± 0.01	0.063	0.11 ± 0.03	-0.09 ± 0.02	**≤0.01**	-0.08 ± 0.02	0.01 ± 0.01	**≤0.05**	0.08 ± 0.03	0.03 ± 0.01	0.088

p: The mean change over the course of the experiment in the treated eye (ΔX) was compared with the mean change in the untreated fellow eye (ΔN) in the same animal using 2-tailed, paired *Student*'s t-tests. *p* values of significance are underlined and bold.

6.4.3 Analyses

Data are presented as Mean ± standard error (SEM). Data are also shown as the change in axial length (the change in axial length over the course of the experiment) for the lens-wearing eye (ΔX) and the fellow eye (ΔN).

6.4.3.1 Analyses of Symmetrical Size and Growth

Linear regressions were performed to determine: (1) If the axial length of the two eyes within the same chick were correlated in untreated chicks (groups 30 to 46) to demonstrate symmetrical size, and (2) if the change in axial length in paired eyes over 3 days were correlated in untreated chicks (group 29) to demonstrate symmetrical growth. Data Desk 7.0.2 (Data Description, Inc., Ithaca, NY, USA) was used to determine the significance of the regressions.

6.4.3.2 Analyses of Yoked and Anti-Yoked Growth

First, regressions were performed to determine the relationship between axial length and age (in days). The best fit was linear, and was used to predict the change in axial length over defined durations within this age range. For the analysis of yoked and anti-yoked growth with various lens treatments, the growth of the fellow eyes of positive or negative lens treated animals were compared with that in untreated animals of the same age using 2-tailed, unpaired, *Student*'s t-tests. Yoking was defined as either of the following two situations: (1) When the fellow eyes of positive lens–wearing eyes elongate less than that seen in untreated age-matched controls, and (2) when the fellow eyes of negative lens–wearing eyes elongate more than that seen in untreated age-matched controls. In contrast, anti-yoking was defined as either of the following two situations: (1) When the fellow eyes of positive lens–wearing eyes elongate more than that seen in untreated age-matched controls, and (2) when the fellow eyes of negative lens–wearing eyes elongate less than that seen in untreated age-matched controls.

Second, to demonstrate the yoked and anti-yoked changes, the mean calculated change in axial length over various durations (calculated from the linear fit in the left eyes

of untreated animals) was subtracted from the change in the fellow eyes of lens-treated animals over the experimental period. This "adjusted change" in axial length in the fellow eyes was compared to zero (two-tailed, unpaired, *Student*'s t-tests) to study whether there was yoking or anti-yoking for each experiment.

Third, Two-Way Analysis of Variance (ANOVA) was performed to see if the adjusted change in axial length in the fellow eyes was significantly different across all lens-wearing durations and lens powers. Finally, linear regressions were performed to determine if there is a correlation between the amount of yoking or anti-yoking and the duration of lens treatment.

6.5 Results

The mean values of anterior chamber depth, lens thickness, vitreous chamber depth, choroidal thickness, and axial length in untreated chicks are summarized in Table 6.1, and the r square and *p* values for linear regressions for axial length between paired eyes for either the actual values at various days of age (groups 30 to 46) or change in axial length from 7 to 10 days of age (group 28) are included in Table 6.1 (Exp. 6.1). The mean change over various lens-wearing durations for these same variables in lens-wearing chicks and *p* values are summarized in Table 6.2 (Exp. 6.2).

6.5.1 Exp. 6.1. Binocular symmetrical size and growth in untreated chicks

The two eyes were of remarkably similar size in normal chicks (groups 30 to 46 in Table 6.1) as shown by the correlation of axial length from 1 to 17 days of age between the two eyes, Fig. 6.1A). Axial length in paired eyes were highly correlated from day 1 (r^2 = 0.77, $p < 0.0001$), suggesting symmetrical eye size since birth. Furthermore, axial length in paired eyes were highly correlated on all the days they were measured until day 17 ($p <$ 0.0001 for all ages, see Table 6.1 for r^2 and *p* values for each age group). Therefore, the paired eyes in untreated chicks show symmetry in axial length during development.

The two eyes in untreated chicks also showed symmetrical growth during development. In untreated chicks that were measured at 7 and 10 days of age (group 28 in Table 6.1, Fig. 6.1B), the paired eyes grew very similarly (change in axial length over three

days, Δ right axial length vs. Δ left axial length, Mean ± SEM, +0.188 ± 0.041 mm vs. +0.202 ± 0.013 mm; $p = 0.168$) and their growth was significantly correlated ($r^2 = 0.56$, $p < 0.0001$, Fig. 6.1B), suggesting symmetrical eye growth over this 3-day period.

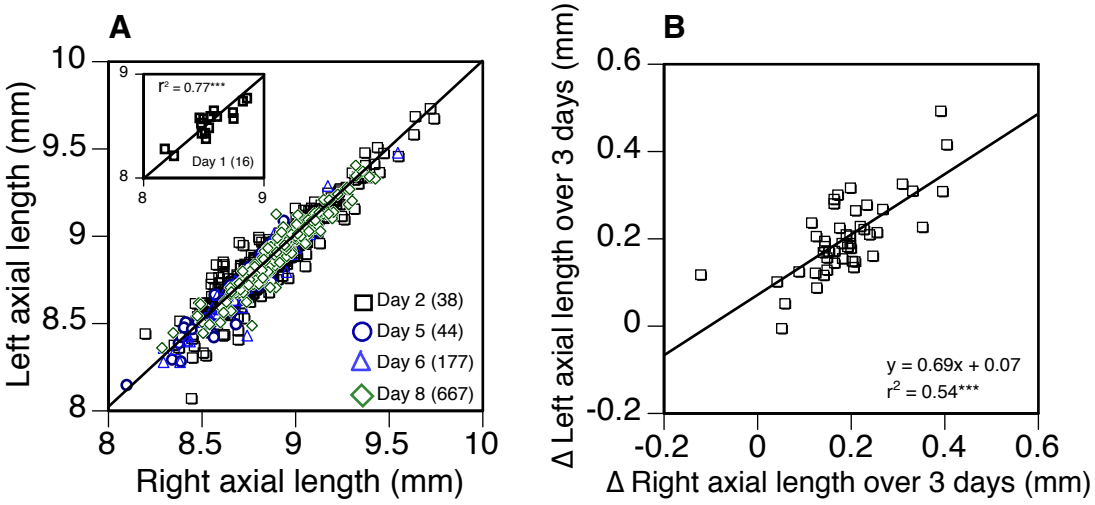

Figure 6.1. Symmetry in axial length and axial growth in paired eyes in untreated chick eyes (Exp. 6.1).
(A) Correlation of axial length in paired eyes at 2, 5, 6, and 8 days of age ages and on Day 1 (insert). See text for correlations for each age. (B) Correlation of the change in axial length over 3 days in group 28. Samples sizes for each age are shown in parentheses in (A). ***: p (for the slope) < 0.0001.

The best fit to the axial length data between 1-17 days of age of these untreated eyes (the left eyes were chosen for this analysis) yielded the equation: $y = 0.065x + 8.39$ mm (y: axial length, x: age, $r^2 = 0.36$, $p < 0.0001$; Fig. 6.2), based on Mean-Squared Error[270]. There was no advantage to using higher order or a hyperobolic fit ($y = 8.41e^{0.007x}$) over this early age range, although obviously, such a fit might be more applicable outside of the measured age range. The linear fit showed that the normal eye grew by 65 μm per day.

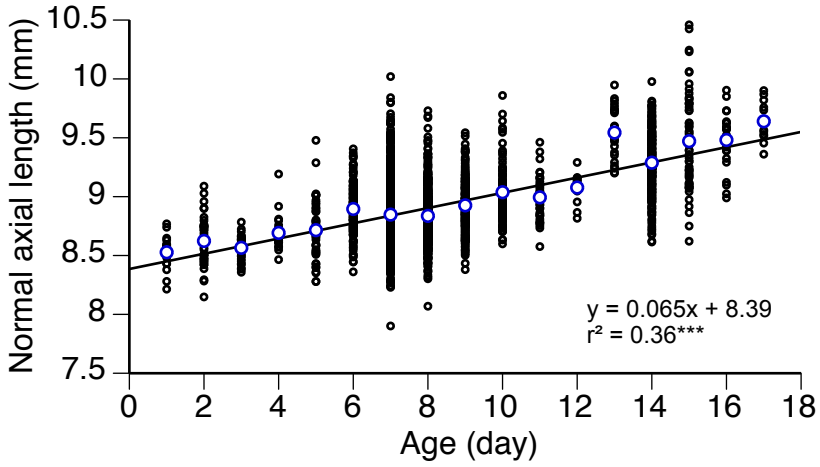

Figure 6.2. Axial length in left eyes of normal, untreated chicks from 1 to 17 days of age (Exp. 6.1).
Individual eyes (groups 30 to 46) are shown with open, black circles, and the mean axial length for each age are shown with open, blue circles. ***: *p* (for the slope) < 0.0001.

6.5.2 Exp. 6.2. The effect of monocular-lens wear on binocular symmetrical growth, yoking and anti-yoking

6.5.2.1 Disturbance in Symmetrical Growth

As expected, monocular positive and negative lens treatment resulted in inter-ocular differences in axial length, in that positive lens wear reduced the rate of axial elongation (mean change in axial length over the course of the experiment, ΔX vs. ΔN, group 47, +0.14 mm vs. +0.25 mm, $p < 0.01$, Table 6.2) and that negative lens wear increased it (group 56, +0.37 mm vs. +0.31 mm, $p < 0.05$, see Table 6.2 for the changes in each eye in each group and *p* values).

The onset of monocular positive or negative lens treatment disturbed binocular symmetrical eye growth. Unlike in untreated animals, there was little correlation between the change in paired eyes after one or two days of lens treatment (after one day of positive lens treatment: $y = 0.097x + 0.001$, $r^2 = 0.01$, $p > 0.05$, Fig. 6.3A; after two days of negative lens treatment: $y = 0.254x + 0.014$, $r^2 = 0.148$, $p > 0.05$, Fig. 6.3B). The correlation in growth between paired eyes was gradually and partially regained after longer lens treatment durations, shown by the increase in the slope of the linear regressions and the r^2 value of the

correlations (Fig 6.3): In positive lens-treated chicks, the slopes of the linear regression were 0.447 ($p < 0.0001$), 0.592 ($p = 0.002$), and 0.683 ($p = 0.0198$), after 3, 4, and 7 days of positive lens wear, respectively, approaching the slopes seen in untreated, normal animals (Fig. 6.3A). In negative lens-treated chicks, the slopes of the linear regression were 0.383 ($p = 0.0152$), 0.778 ($p < 0.0001$), and 0.511 ($p = 0.0018$), after 3, 4, and 7 days of negative lens wear, respectively (Fig. 6.3B).

6.5.2.2 Yoked Growth

Since monocular lens treatment interrupted the symmetrical eye growth observed in untreated chick eyes, the change in axial length in fellow eyes of lens-wearing chicks was subtracted from the expected change in axial length in normal untreated chicks, using the equation acquired from normal chicks measured on various days (Exp. 6.1, Fig. 6.2). This difference is referred to as the adjusted change in axial length. The adjusted change in axial length in the fellow eyes was significantly different to zero across all lens-wearing durations and lens powers ($p = 0.0003$).

Positive lenses caused yoking in the fellow eyes in 2 out of the 8 experiments (Fig. 6.4A, C and E). Specifically, wearing +15 D lenses over one eye for 1 day (6 to 7 days of age, group 55, Table 6.2, Fig. 6.4A) caused the fellow eyes to grow significantly less than that predicted for untreated normal eyes over this same age range (mean change in the fellow eyes vs. predicted change in normal eyes, 0 vs. +65 μm; $p < 0.01$, adjusted change of –65 μm, Fig. 6.4A). Wearing +7 D lenses for 4 days (7 to 11 days of age, group 52, Table 6.2) also caused the fellow eyes to grow significantly less than in the normal eyes (+194 μm vs. +260 μm, $p < 0.05$, adjusted change of –260 μm, Fig. 6.4D).

Negative lenses caused yoking in the fellow eyes in 1 out of the 8 experiments (Fig. 6.4E): Wearing –5 D lenses over one eye for 7 days (from 7 to 14 days of age, group 57, Table 6.2) caused the fellow eyes to grow significantly more than in the normal eyes (mean change in the fellow eyes vs. predicted change in normal eyes, +628 vs. +455 μm, adjusted change of –455 μm; $p < 0.001$, Fig. 6.4E). However, none of the shorter lens-wearing durations induced yoking in the fellow eyes ($p > 0.05$ in all cases).

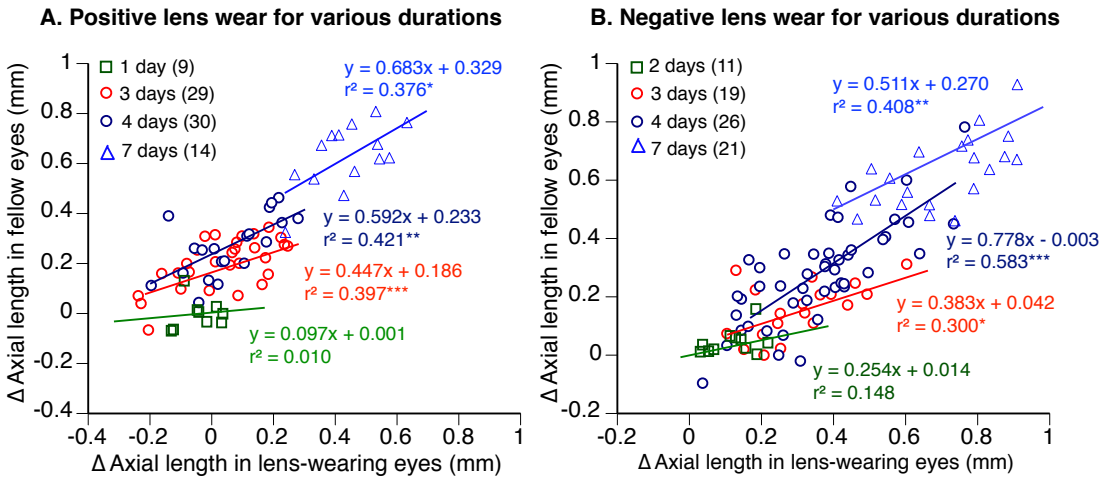

Figure 6.3. Correlation between the change in axial length in the fellow eye and that in the lens-wearing eyes for (A) positive lens treated groups and (B) negative lens treated groups, for various durations.

Data from various positive (groups 47 to 55) and negative (groups 56 to 63) lens powers were pooled. Sample size for each lens wearing duration is shown in parentheses. Sample sizes for each lens-wearing duration are shown in parentheses. *: p (for the slope) < 0.05; **: $p < 0.01$; ***: $p < 0.001$.

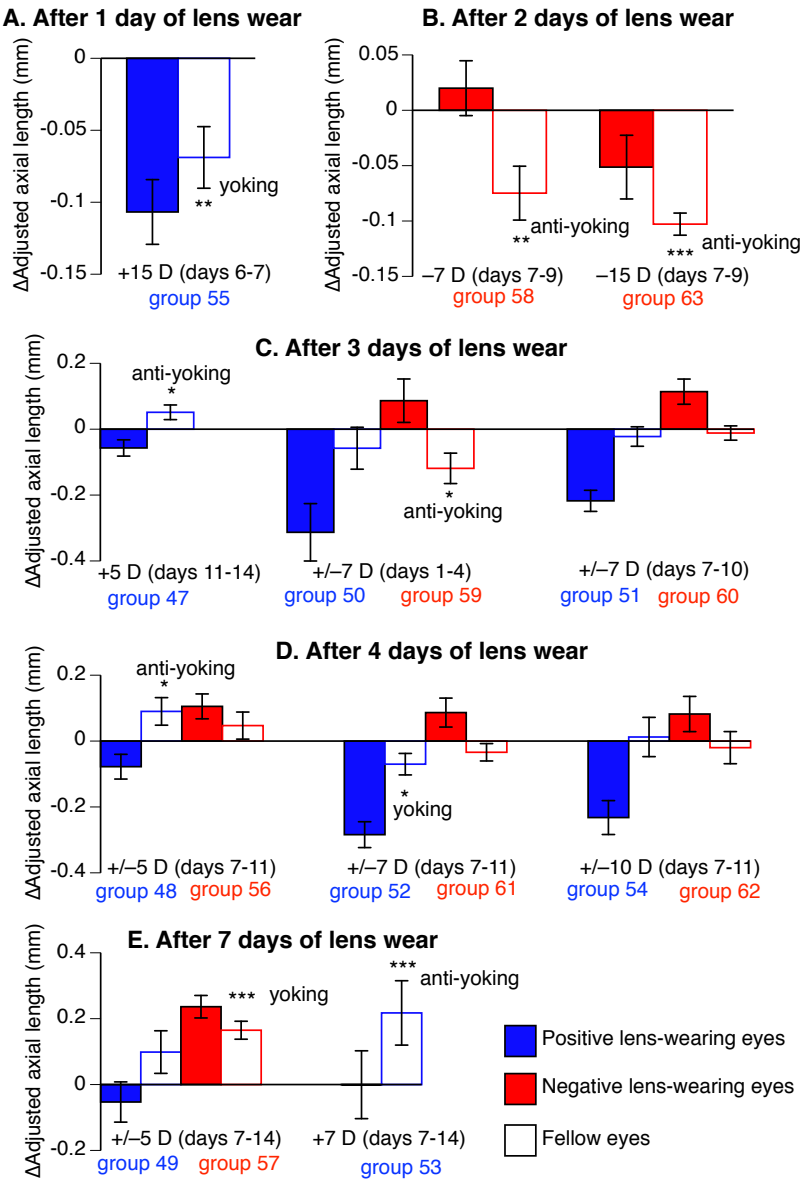

Figure 6.4. Adjusted change in axial length for both experimental and fellow eyes after either positive or negative lens wear for 1 to 7 days.
Adjusted change in axial length was calculated by subtracting the predicted mean change in normal eyes in untreated chicks (calculated from the growth fit) from the actual changes in axial length. Therefore, an adjusted change of zero indicates that the change in these eyes was the same as that found in normal eyes, while positive and negative adjusted values indicate greater or smaller changes than that in untreated animals respectively. Significant changes in the fellow eye relative to the untreated animal are indicated with * ($p < 0.05$), ** ($p < 0.01$), or *** ($p < 0.001$, unpaired, 2-tailed, *Student*'s t-test). Only changes in fellow eyes were analyzed.

6.5.2.3 Anti-yoked growth

Positive lenses caused anti-yoking in the fellow eyes in 3 out of the 8 experiments (Fig. 6.4C, D, and E). Specifically, wearing +5 D lenses over one eye for 3 days (from 11 to 14 days of age, group 47, Table 6.2) caused the fellow eyes to grow significantly more than in the normal eyes (mean change in the fellow eyes vs. predicted change in normal eyes, +250 μm vs. +195 μm; $p < 0.05$, Fig. 6.4C). Wearing +5 D lenses for 4 days (from 7 to 11 days of age, group 48, Table 6.2) also caused the fellow eyes to grow significantly more than in the normal eyes (+354 μm vs. +260 μm, $p < 0.05$, Fig. 6.4D). In addition, wearing +7 D lenses for 7 days (from 7 to 14 days of age, group 53) caused the fellow eyes to grow significantly more than in the normal eyes (+680 μm vs. +455 μm, $p < 0.001$, Fig. 6.4E).

Negative lenses caused anti-yoking in the fellow eyes in 3 out of the 8 experiments (Fig. 6.4B and C). Specifically, wearing –7 or –15 D lenses over one eye for 2 days (7 to 9 days of age, groups 58 and 63, Table 6.2) caused the fellow eyes to grow significantly less than normal (mean change in the fellow eyes vs. predicted change in normal eyes, +57 vs. +130 μm for group 58, $p < 0.01$; +29 vs. +130 μm for group 63, $p < 0.001$; Fig. 6.4B). In addition, wearing –7 D lenses over one eye for 3 days (from 1 to 4 days of age, group 59) also caused the fellow eyes to grow significantly less than in the normal eyes (+79 vs. 195 μm; $p < 0.05$, Fig. 6.4C). No anti-yoking was observed for all longer lens-wearing durations.

6.5.2.4 Correlation between amount of yoking/anti-yoking and treatment duration

There was a significant positive correlation between the adjusted change in axial length in fellow eyes (relative to untreated normal animals) and lens-wearing duration for both positive and negative lens treatments (positive lens groups pooled: $r^2 = 0.301$, $p < 0.0001$, Fig. 6.5A; negative lens groups pooled: $r^2 = 0.264$, $p < 0.0001$, Fig. 6.5B). For both positive and negative lens groups, there seemed to be reduced eye growth in the fellow eyes (yoking for positive lens groups and anti-yoking for negative lens groups) if the lens-wearing duration was 1 day, and increased growth if the lens-wearing duration was longer than 3 or 4 days (anti-yoking for positive lens groups and yoking for negative lens groups).

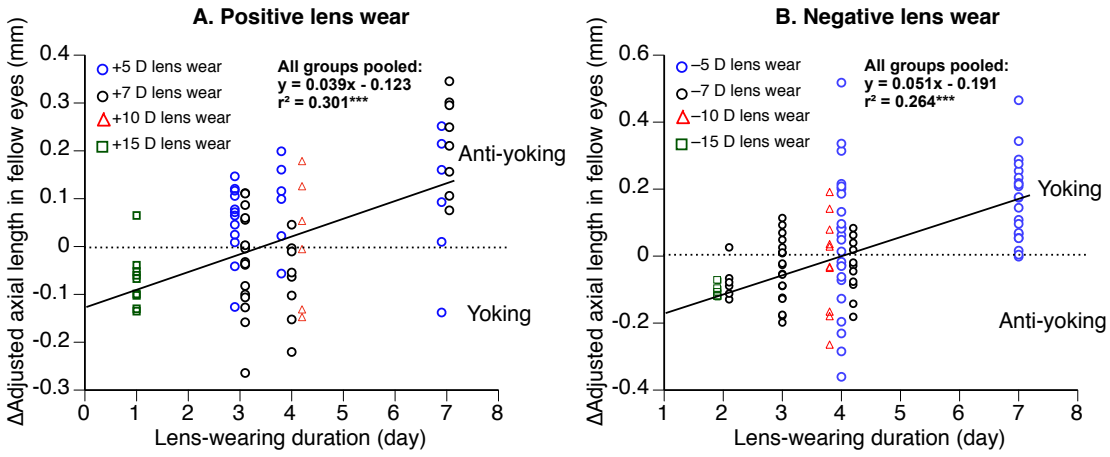

Figure 6.5. The effects of lens-wearing duration on yoking and anti-yoking.
The Y-axis shows the difference between the change in axial length in the fellow eyes and the expected change in axial length in normal eyes within the same duration, i.e., an "adjusted change" of zero, shown by the dashed line, indicates that the change in axial length in the fellow eyes was the same as the predicted change in age-matched normal eyes. (A) and (B) are changes after positive and negative lens treatment, respectively. The black lines show a positive correlation between lens-wearing duration and the adjusted change in axial length in all fellow eyes for the pooled lens treated groups within the (A) positive and (B) lens-wear conditions. The *p* value for the slope of the solid linear regression line is shown as ***, indicating a *p* value of less than 0.0001.

These was no correlation between the amount of yoking or anti-yoking and either the lens power or the starting age of lens treatment.

Within 7 days of lens treatment that was tested in this chapter, the maximum degree of yoking change in the fellow eyes was 64.5% of that in the experimental eyes after wearing +15 D lenses for 1 day (group 55, Δadjusted axial length, 69 µm of decreased eye growth in the fellow eyes below the age-matched normals vs. 107 µm of change in the experimental eyes below the age-matched normals, refer to Table 6.2), and 69.8% of that in the experimental eyes after wearing –5 D lenses for 7 days (groups 57, 165 µm of increased eye growth in the fellow eyes above the predicted change in age-matched normals vs. 236 µm of change in the experimental eyes above the age-matched normals, refer to Table 6.2), with an average maximum degree of yoking change in the fellow eyes of 67.2%. On the other hand, the maximum degree of anti-yoking change in the fellow eyes was 89.0% of that in

the experimental eyes after wearing +5 and +7 D lenses for 7 days (groups 49 and 53 pooled, Δadjusted axial length, 167 μm of increased eye growth in the fellow eyes above the predicted change in age-matched normals vs. –23 μm of change in the experimental eyes below the age-matched normals, refer to Table 6.2), and 27.1% of that in the experimental eyes after wearing –7 D lenses for 3 days, from either 1 to 4 days of age or 7 to 10 days of age (groups 59 and 60, –40 μm of decreased eye growth in the fellow eyes below the age-matched normals vs. +107 μm of change in the experimental eyes above the age-matched normals, refer to Table 6.2), with an average maximum degree of yoking change in the fellow eyes of 58.1%.

6.6 Discussion

The data in this chapter shows that axial length and growth rates were significantly correlated between the two paired eyes in untreated chicks (e.g., symmetrical growth). Symmetrical growth was interrupted after 1 or 2 days of monocular lens treatment, and gradually regained after longer lens-wearing durations. More interestingly, monocular lens treatment changed eye growth in the fellow eyes and caused either yoking or anti-yoking effects in axial length in the fellow eyes, which was dependent on the lens-wearing duration: Both positive and negative lens-wear decreased eye growth in the fellow eyes after a short lens-wearing duration (yoking for positive lens wear and anti-yoking for negative lens wear), no effects with intermediate durations, and increased eye growth in the fellow eyes after longer lens-wearing durations (significant by 7 days, anti-yoking for positive lens wear and yoking for negative lens wear). On the other hand, yoking and anti-yoking were only discovered in certain lens-wearing conditions.

6.6.1 Possible mechanisms for interactions between paired eyes

Even though the two eyes in birds are largely independent in terms of neutral projections and function, there are clearly interactions between paired eyes as shown in this chapter and there are a number of possible mechanisms that may mediate these interactions.

Firstly, although chick eyes are closely apposed near the midline, separated by a thin wall of septum with ossification[194, 196], it may be possible for growth factors or neurotransmitters to diffuse from one orbit to the other[81, 139]. This possibility is unlikely because of inconsistency in results from experiments with monocular treatment and sometimes the complete lack of yoking or anti-yoking[81, 137]. Also, given the observation that yoking also has also been observed in monkeys where the orbital separation is more substantial, argues against the possibility that this is a general mechanism across species[139].

Secondly, it has been speculated that the treatment on the experimental eye could affect the untreated fellow eye through some central mechanism regulating early eye growth[81, 106, 139, 203, 271]. Specifically, it was hypothesized that the choroidal innervation or some other crucial ocular structure has a bilateral distribution and treatment in one eye could affect the untreated fellow eye through this bilateral distribution. Choroidal innervation was considered a possible reason since choroidal compensation proceeds axial compensation[80, 81, 83-85]. Even though form deprivation[69, 79, 224, 272] and lens compensation[94] have been shown to be locally controlled within the eye, it is not contradictory to this possibility. Indeed, it has been shown that retinal ganglion cells transmit visual information to multiple targets in the brain and that efferent fibers project from the isthmo-optic nucleus (a system for polysynaptic feedback control to the retina of origin) to the retina[273-275]. It has also been shown that there is a neuronal connection between the afferent input of one eye and the motor output of the other eye[276]. On the other hand, while it is possible that the neuronal connections between paired eyes causes the observed interactions, the fact that yoking was discovered in chick eyes after optic nerve section[81] suggests that there must be other mechanisms responsible for the interactions.

It has been shown that monocular form deprivation reduced choroidal blood flow in both eyes in chicks[277], and therefore may be responsible for causing interactions between paired eyes[207]. Specifically, monocular form deprivation for 14 days reduced choroidal blood flow in the treated eyes by approximately 50%[277]. Choroidal blood flow showed a trend towards reduction in the untreated fellow eyes with only limited significance. While a reduction in choroidal blood flow might partially explain the yoking effect during form deprivation, it certainly cannot cause an anti-yoking effect where instead, choroidal blood

flow would be required to increase. At present, the change in choroidal blood flow during lens treatment (positive or negative) or during recovery is unknown. However, it is unlikely that change in choroidal blood flow can exclusively explain both yoking and anti-yoking.

Finally, since mammals such as tree shrews and monkeys have consensual accommodation, monocular lens treatment has been considered to change the fixation behavior of the fellow eyes and cause interactions between paired eyes[139, 202, 221, 278]. For example, if the infant rhesus monkey wears a weak positive lens over one eye and a plano lens (with zero power) in front of the other, the monkey will often set the accommodative level for the positive lens-wearing eye to minimize accommodative effort. Therefore, the plano lens-wearing eye will experience hyperopic defocus when looking at near objects and develop a myopic shift (Earl's Rule). This theory could explain anti-yoking in experiments using monocular positive lenses in mammals. In order to explain interactions between paired eyes in chicks, it would be necessary to show that although chick eyes have mostly independent accommodation, convergent accommodation occurs as has been proposed for pigeons, and can influence fixation states. However, it is noted that there are many experiments in chicks in which accommodation has been disabled, without major consequences for monocular lens compensation. Nevertheless, the possible change in the fellow eyes after accommodation is disabled without any treatment has not been reported.

In addition, Earls Rule cannot explain yoking or symmetrical growth, in mammals, and does not explain interactions between paired eyes after form deprivation.

6.6.2 The amount of yoking/anti-yoking depends on lens-wearing duration

The current analyses find monocular lens treatment causes reduced eye growth in the fellow eyes with shorter treatment durations and increased eye growth with longer treatment durations. Similar findings have been discovered before: Smith *et al*. reported that there was an association between the effect on the fellow eyes and the duration of treatment on the experimental eyes in rhesus monkeys: While the fellow eyes of form deprived eyes became more hyperopic with shorter vitreous chamber depth than normal, animals that were form deprived for longer periods of time every day showed larger ametropia in the untreated fellow eyes[139].

In the current study, the similar trends discovered after both positive and negative lens-wear supports the idea of a non-visual mechanism controlling this inter-ocular interaction. Although Earls' Rule may explain the anti-yoking after positive lens wear, some alternative mechanism must be the underlying cause of yoking after longer term negative lens wear. This could be related to wearing of lenses *per se* changing the growth of the cornea perhaps. If so, plano lenses should also induce increased eye growth after 7 days. It could also relate to a tendency for symmetry in growth, even under ametropic driving conditions.

6.6.3 Implications of these results for monocular experimental designs

The presence of binocular symmetry, yoking and anti-yoking, have important implications for experimental design and interpretation. Specific statistical tools have been recommended for analyzing correlated continuous data[279]. In experiments where the data are likely to be positively correlated, it is common to use the difference between the experimental eye and the fellow eye of the same animal. This approach takes into consideration the reduced variability between the two eyes of the same animal and therefore increases statistical power.

Using the fellow eye, is therefore a conservative way to identify an experimentally induced difference between the two eyes. However, it should be noted that the size of these yoking/anti-yoking effects is generally relatively small and takes some time to develop (approximately 35% on average of the change in the experimental eyes), suggesting that the results may under- or overestimate the true effect sizes. However, at least in the case of positive lens wear, effect sizes of up to 89.0% have been observed after 7 days of lens treatment. In addition, yoking and anti-yoking were only discovered in half of the experiments conducted in this chapter, the presence of which seems unpredictable.

The presence of yoking or anti-yoking, however, can confound the use of inter-ocular experimental designs by decreasing or increasing the difference between the two eyes, thereby increasing the risk of underestimating and overestimating results, respectively. For positive lens treatment (anti-yoking), wearing positive lenses for 7 days caused 89% of anti-yoking in the fellow eyes, i.e., it overestimates the treatment effect by 89%. Therefore, caution should be taken when interpreting results with a long period of positive lens

treatment. For negative lens treatment (yoking), on the other hand, wearing negative lenses for 7 days caused 70% of yoking in the fellow eyes, i.e., it underestimates the treatment effect by 70%, which makes inter-ocular comparisons a very conservative way to study the lens effect. In addition, if yoking and anti-yoking cause concern, alternative experimental designs can be used, such as binocular treatments with control groups or cross-over designs[280, 281]. It might also be helpful to use a group of untreated animals as control. However, given that yoking and anti-yoking were not reliably observed and appears to be easily overcome if lens-wear is around 3-4 days, at least in chicks, it generally is unlikely to cause a misinterpretation. An analysis of the literature shows that in chick experiments with monocular lens-wear, very few experiments are run over such short periods (e.g., reference number 80). However, it is often the case that molecular biology experiments often use shorter lens-wearing periods (eg, 40 minutes or 2 hours[208], 15, 30 and 120 minutes[209]), and in such experiments, where effect sizes are less than 65 um/day of change, it might be prudent to include untreated controls.

6.6.4 Conclusions

Binocular symmetrical growth normally occurs and facilitates a matched ocular length between the two eyes. This process is likely a combination of normal allometric growth and visual control of eye growth during emmetropisation. Normal binocular symmetrical growth can also be disrupted by modification of the visual environment for one eye. In chicks, short lens-wear periods of 2 days can lead to changes in the fellow eye that should be considered in experiments studying biochemical changes. The amount of yoking and anti-yoking increases with longer lens-wearing durations and using the fellow eye can cause an average of 39.2% overestimation of the effects of positive lens wear and underestimation of 33.4% for the effects of negative lens wear. Therefore, paradigms which induce myopia over longer periods and use the fellow eye as a control are conservative, and such designs remain a valuable research paradigm to examine the mechanisms of visually regulated eye growth control.

7. The Effect of Eye Size on Binocular Lens Compensation in Chicks

7.1 Forward

Previous chapters in this thesis provide evidence that implicate a non-visual factor in the regulation of eye growth that may relate to intrinsic growth expectations. Additionally, inter-ocular interactions (symmetrical growth, yoking and anti-yoking) could also contribute to eye growth. Specifically, factors other than local defocus can refrain the eye from further elongating in case of hyperopic defocus, eye growth in both eyes are well correlated, and treatment on one eye can affect eye growth in the untreated fellow eyes. This final experimental Chapter aims to study the interaction of recent changes in eye length/size with the traditional defocus-factor during binocular lens treatment, taking advantage of findings from the previous chapters.

The following hypothesis is proposed:

Binocular lens treatment increases lens compensation against the direction predicted by any intrinsic size-factor. Specifically, the defocus-factor dominates the size-factor in negative lens compensation when chicks experience hyperopic defocus in both eyes (in the presence of yoking).

Some of these results have been presented in an abstract (Zhu X, *et al*., *Invest Ophthal Vis Sci* 2012, E-Abstract 3441; Zhu X, Wallman J, and McFadden SA, *Invest Ophthal Vis Sci* 2016, E-Abstract 3791).

7.2 Abstract

Purpose: Animal studies have shown that post-natal eye growth is largely regulated by visual experience, which permits compensation for superimposed defocus (here referred as the "defocus-factor"). In addition, it has also been shown that eye growth in chicks can also be affected by an intrinsic homeostatic developmental mechanism, referred as the "size-factor", in which a sudden change in the magnitude of imposed hyperopic defocus functions to restrain the eye from further elongation and promote size matching between the two eyes. Specifically, when chicks wore spectacle lenses over one eye, while the defocus-factor dominated in the case of positive lens wear, this size-factor prevented the eyes from further elongating in the case of negative lens wear. Furthermore, it has been demonstrated that the growth in paired eyes in monocularly lens treated animals can be yoked in the same direction. Therefore, this chapter used a recovery type paradigm to study the response of the eye growth system when the defocus signals were equal between the two eyes but the two eyes were of unequal lengths.

Methods: To create unequal eye sizes, four groups of chicks (groups 64 to 67, n = 33) first wore either a +5 D or –5 D lens on one eye for 3 or 7 days respectively. The lens was then either stepped up in power or removed from the experimental eye (group 64: +5 D to +10 D, group 65: –5 D to –10 D, group 66: +5 D removal, group 67: –5 D removal). At the same time, the fellow eye was also treated with a lens to ensure the defocus signals at the time of lens removal or step-up were equal between the two eyes, while the size-factor was unequal between the eyes (Fellow eyes group 64: +5 D, group 65: –5 D, group 66: –5 D, group 67: +5 D). Refractive error and ocular dimensions were measured before and after each treatment, and repeatedly at various intervals during treatment with a Hartinger refractometer and A-scan biometry, respectively.

Results: Chick eyes completely compensated for +10 and –10 D lenses despite the intervening step-up when chick eyes experienced defocus of the same sign in both eyes. Similar to findings in Chapter 4, chick eyes completely compensated for +10 D lenses after the step-up. In contrast to findings in Chapter 4, chick eyes also completely compensated for

–10 D lenses after the step-up (final refractive error in the experimental eyes on day 21, –9.96 ± 0.26 D).

Conclusions: The visual mechanism dominated the intrinsic, non-visual mechanism when both eyes experienced similar amounts of defocus (of the same sign), suggesting that asymmetry in visual input in paired eyes is required to unmask or activate and the intrinsic limit to growth discovered in Chapter 4.

7.3 Introduction

Although there is undisputed evidence that the post-natal regulation of eye growth is guided by the defocus experienced by the eye, the results in Chapters 3 and 4 suggest that other intrinsic non-visual factors may also play a role. Chapter 3 showed that, after the eyes have compensated for positive and negative lenses, eye growth can recover to normal in the absence of any visual input. Although this non-visual signal is unknown, for convenience it is referred to as a "size-factor". The results in Chapter 4 showed that, when the defocus- and this proposed size-factor are in conflict and predict opposite directions for eye growth, the defocus-factor dominated in the case of myopic defocus caused by positive lenses, and resulted in eye growth appropriate for spectacle lens compensation. In contrast, in the case of hyperopic defocus caused by negative lenses worn monocularly, the eye was unable to respond appropriately to a sudden step-up in the magnitude of hyperopic defocus, despite clear evidence that it can respond to this same large magnitude if experienced continuously from a hyperopic starting point. One explanation is that the defocus factor is switched off and a default size-factor is activated by a sudden shift in defocus towards zero, and refrains the eye from further elongating.

The above interesting effect was observed under ametropic conditions. However, in Chapter 6, it was found that growth in paired eyes was well correlated despite monocular lens treatment, and eye growth interacted between a treated and non-treated eye, producing yoking or anti-yoking in certain lens-wearing conditions. Such coordination, even in relatively independent chick eyes, raises the question as to whether the observed limitation in the sensitivity to a sudden change in hyperopic defocus would occur when the defocus states between the two eyes are matched.

This final experimental chapter was designed to study the interaction of the size- and defocus-factors on binocular lens compensation. The experiments in this chapter test the hypothesis that when there is no conflict between the refractive states between the two eyes, lens compensation will be more sensitive to defocus than any intrinsic size differences between the two eyes.

One way to study this is to use a modified "recovery" paradigm. After wearing a positive lens for enough time, the eye will completely compensate for the defocus and restore emmetropia with the positive lens in place, and will therefore appear hyperopic (with the defocus behind the retina) without the lens. If the positive lens is removed, the eye will now compensate for the hyperopic defocus behind the retina by increasing its rate of ocular elongation, effectively pulling the retina backwards to meet the focal plane and regain emmetropia, and "recover" from the prior positive lens wear.

The opposite happens when wearing a negative lens that focuses images behind the retina: The eye will increase its rate of ocular elongation to compensate for the negative lens, and thus the eye will appear myopic (with the defocus in front of the retina) if the negative lens is now removed. The eye will then "recover" from the prior negative lens treatment by decreasing the rate of ocular elongation and regain emmetropia. During recovery, the visual and non-visual mechanisms presumably work in the same direction to regain emmetropia and to restore the eye size or length to normal.

In the current study, lens manipulation was designed to compare the ocular growth response between the two paired eyes in the same animal when both eyes experienced the same sign and amount of defocus but the size-factor gave growth cues that were in the opposite direction to those elicited by the defocus-factor in the experimental eye (lens power step-up) and in the same direction with the defocus-factor in the other (recovery) eye.

It is hypothesized that the defocus-factor dominates the size-factor in negative lens compensation when chicks experience hyperopic defocus in both eyes (in the presence of the yoking effect).

7.4 Methods

7.4.1 Animals

White Leghorn chicks (n = 33) were obtained and housed as described in the General methods (Section 2.1).

7.4.2 Experimental Procedures

Glass lenses of –5, –10, +5, +10 D were used. All chicks wore lenses over both eyes starting at different ages, and the eye that wore lenses first was considered the experimental eye ("X"), and the eye that wore the lens second was considered the fellow eye ("N"). All chicks were measured at the beginning of the experiment and thereafter at various intervals. Experiments started when chicks were either 7 or 11 days old. The treatment details and sample sizes are summarized in Table 7.1.

Table 7.1. Summary of the treatment details, the effects of the proposed size- and defocus-factors, and sample size (n)

Exp name	Group #	Lens type	Details (age in days)	Size- vs. defocus-factor direction during recovery or after step up for exp eyes, and in the beginning of lens wear for fellow eyes*	n
7.1 Stepped vs. constant lens powers	64	Plus	Exp eye: +5 D lens wear for 3 days (11-14), then +10 D lens wear for another 4 days (14-18)	S: ↑ growth; D: ↓ growth	6
		Plus	Fellow eye: +5 D lens wear for 4 days (14-18)	S: absent; D: ↓ growth	
	65	Minus	Exp eye: –5 D lens wear for 7 days (7-14), then –10 D lens wear for another 7 days (14-21)	S: ↓ growth; D: ↑ growth	14
		Minus	Fellow eye: –5 D lens wear for 7 days (14-21)	S: absent; D: ↑ growth	
7.2 Recovery vs. constant lens powers	66	Plus	Exp eye: +5 D lens wear for 3 days (11-14), then recovery for another 4 days (14-18)	S: ↑ growth; D: ↑ growth	6
		Minus	Fellow eye: –5 D lens wear for 4 days (14-18)	S: absent; D: ↓ growth	
	67	Minus	Exp eye: –5 D lens wear for 7 days (7-14), then recovery for another 7 days (14-21)	S: ↓ growth; D: ↓ growth	7
		Plus	Fellow eye: +5 D lens wear for 7 days (14-21)	S: absent; D: ↓ growth	

* S: Size-factor; D: Defocus-factor

7.4.2.1 Exp. 7.1: Stepped vs. Constant Lens Powers

To study whether chick eyes can compensate for positive or negative lenses after a doubling in their imposed defocus magnitude (after a step-up in power from +5 D to +10 D, n = 6, group 64; or –5 D to –10 D, n = 14, groups 65) when both eyes experience defocus of the same sign, a direct comparison of lens compensation between lens step-up (in the experimental eye) and constant lens wear without the step-up (in the fellow eye) occurred. See Groups 64 and 65 in Table 7.1 for treatment details, and in Figs. 7.1A and 7.1B for treatment schematics.

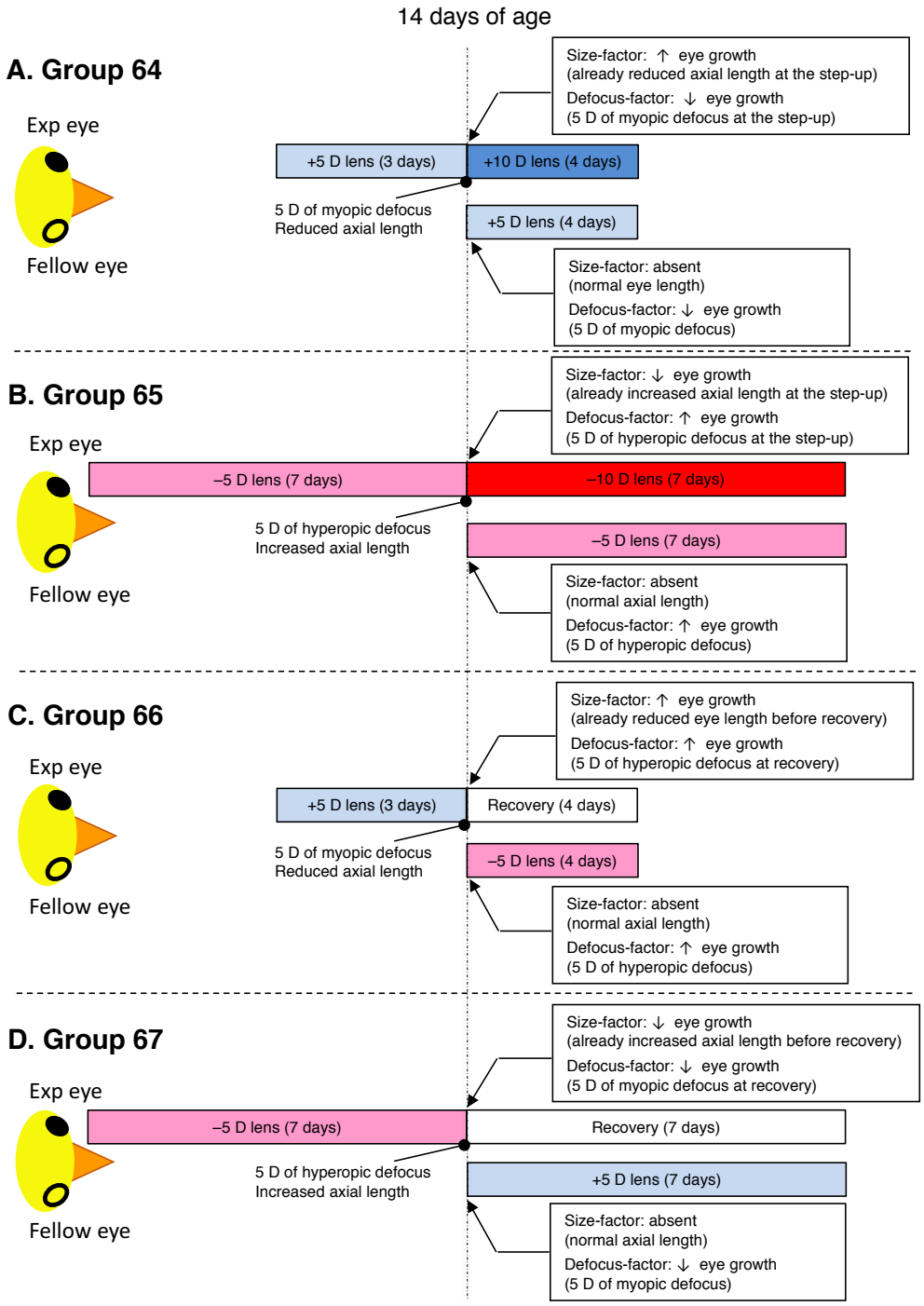

Figure 7.1. Schematic for treatment paradigms and the potential effects of the proposed size- and defocus-factors at the time of step-up or lens removal on day 14. Text boxes labeled with the round arrowheads show the actual change in defocus and axial length signals in the experimental eyes only (immediately after the lens is removed or replaced), and the text boxes labelled with the pointy arrowheads show the hypothesized effects of the size- and defocus-factors for both experimental and fellow eyes.

Positive Lens Step-up (group 64):

Six chicks wore +5 D lenses over one eye (the experimental eye) for 3 days to cause full compensation with a reduced rate of ocular elongation, then stepping to +10 D lenses for another 4 days. At the time of lens step-up on the experimental eye (14 days of age), the size- and defocus- factors would predict opposite response in eye growth: The size-factor would act to increase eye growth and prevent further compensation for the +10 D lens, since the lens-wearing eye was already shorter than normal; whereas the defocus-factor would act to further induce reduction in eye growth and compensation for the +10 D lens, since the +10 D lens superimposed myopic defocus (5 D) in front of the retina of an eye that was +5 D hyperopic. The fellow eye started to wear a +5 D lens also for 4 days when the lens power in the experimental eye was stepped on 14 days of age, so both eyes experienced a similar magnitude of myopic defocus (Fig. 7.1A).

Negative Lens Step-up (group 65):

The above experiment was repeated with negative lenses in group 65: Fourteen chicks wore –5 D lenses over one eye (the experimental eye) for 7 days to cause full compensation with an increased rate of ocular elongation, then stepping to –10 D lenses for another 7 days. At the time of lens step-up on the experimental eye on 14 days of age, the size- and defocus- factors would predict the opposite response in eye growth: The size-factor would act to decrease eye growth and prevent further compensation for the –10 D lens, since the lens-wearing eye was already longer than normal; whereas the defocus-factor would act to further increase eye growth and compensation for the –10 D lens, since the –10 D lens superimposed hyperopic defocus (5 D) in front of the retina of an eye that was –5 D myopic. The fellow eye started to wear a –5 D lens when the lens power was stepped in the experimental eye for also 7 days, so both eyes experienced approximately the same magnitude of hyperopic defocus (Fig. 7.1B).

7.4.2.2 Exp. 7.2: Recovery vs. Constant Lens Powers

To study the effect of the size-factor when it predicted growth in the same direction as the defocus-factor, the amount of recovery after prior lens treatment (in the experimental

eye) was compared to lens compensation (in the fellow eye). See Groups 66 and 67 in Table 7.1 for details and Figs. 7.1C and 7.1D for treatment schematics.

Recovery from positive lens wear (group 66):

Six chicks wore +5 D lenses over one eye (the experimental eye) for 3 days to cause full compensation with a reduced rate of ocular elongation, then the lenses were removed and eyes recovered for another 4 days. After the +5 D lenses were removed from the experimental eyes on 14 days of age, the size- and defocus- factors would predict the same response to increase eye growth since the lens-wearing eye was already shorter than normal, and superimposed with hyperopic defocus after the positive lens removal. At the time of lens removal, the fellow eye started to wear a –5 D lens for 4 days, so both eyes experienced a similar magnitude of hyperopic defocus (Fig. 7.1C).

Recovery from negative lens wear (group 67):

Seven chicks wore –5 D lenses over one eye (the experimental eye) for 7 days to cause full compensation with an increased rate of ocular elongation, then the lenses were removed and eyes recovered for another 4 days. After the –5 D lenses were removed from the experimental eyes on 14 days of age, the size- and defocus- factors would predict the same response to increase eye growth, since the lens-wearing eye was already longer than normal, and superimposed with myopic defocus after the negative lens removal. At the time of lens removal, the fellow eye started to wear a +5 D lens for 7 days, so both eyes experienced approximately the same magnitude of myopic defocus (Fig. 7.1D).

7.4.3 Measurements

Refractive error was measured with a modified Hartinger refractometer and ocular dimensions were measured using A-scan biometry while the chicks were anesthetized with 1.5% isoflurane in oxygen as previously described in the General methods (Section 2.3).

7.4.4 Analyses

Data are shown as mean ± SEM (Tables 7.2 and 7.3). Means for Anterior Chamber Depth, Lens Thickness, Vitreous Chamber Depth, Choroidal Thickness, Axial Length, and Refractive Error for the actual values at various ages are listed in Table 7.2, and the inter-ocular differences (X – N) at various ages and *p* values are listed in Table 7.3**.**

Table 7.2. Actual values in ocular dimensions and refractive error (Mean ± SEM)

Group	Age (day)	Eye	Anterior chamber depth (mm)	Lens thickness (mm)	Vitreous chamber depth (mm)	Choroidal thickness (mm)	Axial length (mm)	Refractive error (D)
64	11	X	1.37 ± 0.02	2.02 ± 0.01	5.01 ± 0.04	0.27 ± 0.02	8.98 ± 0.07	-0.26 ± 0.27
		N	1.34 ± 0.02	2.02 ± 0.01	5.01 ± 0.04	0.26 ± 0.03	8.95 ± 0.08	-0.42 ± 0.33
	14	X	1.42 ± 0.02	2.10 ± 0.02	4.95 ± 0.05	0.34 ± 0.02	9.15 ± 0.05	5.23 ± 0.86
		N	1.40 ± 0.03	2.12 ± 0.01	5.11 ± 0.06	0.26 ± 0.02	9.23 ± 0.08	0.35 ± 0.07
	16	X	1.50 ± 0.02	2.12 ± 0.02	4.85 ± 0.05	0.48 ± 0.04	9.28 ± 0.05	9.38 ± 0.60
		N	1.45 ± 0.02	2.12 ± 0.02	5.01 ± 0.08	0.40 ± 0.04	9.32 ± 0.08	3.69 ± 0.67
	18	X	1.50 ± 0.02	2.21 ± 0.01	4.85 ± 0.07	0.44 ± 0.04	9.34 ± 0.09	9.65 ± 0.86
		N	1.46 ± 0.02	2.20 ± 0.02	5.03 ± 0.07	0.38 ± 0.02	9.43 ± 0.09	4.55 ± 0.78
65	7	X	1.24 ± 0.02	1.87 ± 0.01	4.91 ± 0.05	0.19 ± 0.01	8.53 ± 0.06	-0.20 ± 0.39
		N	1.24 ± 0.02	1.88 ± 0.01	4.90 ± 0.04	0.18 ± 0.02	8.53 ± 0.07	-0.43 ± 0.31
	14	X	1.33 ± 0.02	2.13 ± 0.01	5.25 ± 0.06	0.20 ± 0.02	9.25 ± 0.08	-4.58 ± 0.30
		N	1.38 ± 0.02	2.14 ± 0.01	5.10 ± 0.06	0.23 ± 0.01	9.18 ± 0.08	-0.32 ± 0.30
	16	X	1.37 ± 0.03	2.24 ± 0.02	5.49 ± 0.09	0.18 ± 0.01	9.59 ± 0.12	-5.66 ± 0.67
		N	1.44 ± 0.02	2.17 ± 0.01	5.30 ± 0.05	0.19 ± 0.02	9.43 ± 0.08	-2.77 ± 0.67
	18	X	1.38 ± 0.03	2.27 ± 0.01	5.68 ± 0.08	0.22 ± 0.01	9.87 ± 0.09	-7.59 ± 0.51
		N	1.45 ± 0.02	2.24 ± 0.01	5.34 ± 0.07	0.23 ± 0.01	9.59 ± 0.09	-3.43 ± 0.38
	21	X	1.45 ± 0.03	2.36 ± 0.01	5.84 ± 0.09	0.19 ± 0.01	10.17 ± 0.09	-9.96 ± 0.26
		N	1.49 ± 0.02	2.34 ± 0.01	5.45 ± 0.06	0.21 ± 0.01	9.83 ± 0.09	-5.00 ± 0.28
66	11	X	1.38 ± 0.03	2.01 ± 0.02	5.20 ± 0.05	0.22 ± 0.04	9.13 ± 0.08	-0.38 ± 0.43
		N	1.38 ± 0.02	2.01 ± 0.02	5.20 ± 0.04	0.23 ± 0.02	9.12 ± 0.06	-0.75 ± 0.45
	14	X	1.40 ± 0.03	2.12 ± 0.02	5.02 ± 0.05	0.39 ± 0.05	9.25 ± 0.09	6.64 ± 0.30
		N	1.44 ± 0.02	2.13 ± 0.02	5.23 ± 0.04	0.23 ± 0.01	9.35 ± 0.07	0.11 ± 0.14
	16	X	1.40 ± 0.04	2.20 ± 0.03	5.28 ± 0.07	0.18 ± 0.02	9.40 ± 0.09	2.06 ± 0.53
		N	1.46 ± 0.03	2.17 ± 0.02	5.47 ± 0.08	0.18 ± 0.01	9.60 ± 0.09	-3.26 ± 0.56
	18	X	1.45 ± 0.04	2.29 ± 0.02	5.47 ± 0.08	0.28 ± 0.02	9.80 ± 0.12	0.16 ± 0.09
		N	1.50 ± 0.05	2.24 ± 0.02	5.66 ± 0.08	0.20 ± 0.02	9.92 ± 0.14	-3.53 ± 0.87
67	7	X	1.25 ± 0.02	1.87 ± 0.02	4.93 ± 0.07	0.18 ± 0.01	8.54 ± 0.07	-0.77 ± 0.45
		N	1.25 ± 0.03	1.88 ± 0.02	4.89 ± 0.04	0.19 ± 0.01	8.50 ± 0.05	-0.73 ± 0.42
	14	X	1.32 ± 0.02	2.12 ± 0.02	5.24 ± 0.07	0.19 ± 0.02	9.19 ± 0.08	-5.17 ± 0.88
		N	1.32 ± 0.03	2.14 ± 0.02	5.06 ± 0.06	0.25 ± 0.02	9.10 ± 0.07	-0.42 ± 0.31
	16	X	1.35 ± 0.03	2.20 ± 0.03	5.26 ± 0.11	0.26 ± 0.01	9.39 ± 0.11	-0.24 ± 0.36
		N	1.36 ± 0.02	2.14 ± 0.03	4.94 ± 0.05	0.36 ± 0.03	9.12 ± 0.08	5.55 ± 0.59
	18	X	1.40 ± 0.03	2.24 ± 0.02	5.34 ± 0.10	0.25 ± 0.01	9.55 ± 0.12	-0.59 ± 0.25
		N	1.35 ± 0.03	2.20 ± 0.02	4.99 ± 0.07	0.26 ± 0.02	9.14 ± 0.08	5.35 ± 0.51
	21	X	1.45 ± 0.02	2.31 ± 0.02	5.33 ± 0.09	0.27 ± 0.01	9.70 ± 0.08	-0.20 ± 0.27
		N	1.44 ± 0.02	2.26 ± 0.02	5.03 ± 0.07	0.29 ± 0.01	9.38 ± 0.07	5.81 ± 0.31

See Table 7.1 for group definitions.

The experimental and fellow eyes were compared at each measurement time using a Two-Way Mixed Measures Analysis of Variance (ANOVA) with Post-hoc comparisons adjusted for familywise error using the Holm-Sidak method. The *p* values are reported in Table 7.3.

Data were analyzed in the following 3 ways:

First, the experimental (X) and fellow (N) eyes were compared at each measurement time using a Two-Way Mixed Measures Analysis of Variance (ANOVA) with Post-hoc comparisons adjusted for familywise error using the Holm-Sidak method. This analysis was repeated for refractive error and each ocular dimension. The resulting *p* values are reported in Tables 7.3.

Second, inter-ocular differences (X – N) at each measurement age were compared with One-Way Repeated Measures ANOVA with the Holm-Sidak method for comparisons between different time points (for example, before and after lens-step-up).

Finally, the relative changes (change in the experimental eyes over a specified time period minus the matched change in the untreated eyes, ΔX – ΔN) from two groups were compared using 2-tailed, unpaired *Student*'s t-tests.

Table 7.3. Summary of inter-ocular difference (X – N, Mean ± SEM) for ocular dimensions and refractive error and *p* values

Group	Age (day)	Anterior chamber depth (mm)	*p*	Lens thickness (mm)	*p*	Vitreous chamber depth (mm)	*p*	Choroidal thickness (mm)	*p*	Axial length (mm)	*p*	Refractive error (D)	*p*
64	11	0.03 ± 0.02	0.280	0.00 ± 0.01	0.842	0.00 ± 0.05	0.972	0.00 ± 0.02	0.843	0.03 ± 0.04	0.546	0.17 ± 0.23	0.835
	14	0.02 ± 0.03	0.407	-0.02 ± 0.02	0.167	-0.16 ± 0.06	**0.024**	0.08 ± 0.03	**0.002**	-0.08 ± 0.04	0.139	4.87 ± 0.92	**≤ 0.001**
	16	0.05 ± 0.04	0.148	-0.01 ± 0.02	0.583	-0.16 ± 0.07	**0.026**	0.07 ± 0.02	**0.005**	-0.04 ± 0.06	0.423	5.69 ± 0.63	**≤ 0.001**
	18	0.03 ± 0.02	0.258	0.01 ± 0.01	0.430	-0.18 ± 0.05	**0.015**	0.05 ± 0.02	**0.031**	-0.08 ± 0.06	0.133	5.10 ± 1.10	**≤ 0.001**
65	7	0.00 ± 0.01	0.784	-0.02 ± 0.01	0.558	0.01 ± 0.02	0.829	0.01 ± 0.01	0.869	-0.01 ± 0.02	0.907	0.23 ± 0.29	0.892
	14	-0.04 ± 0.01	0.262	-0.01 ± 0.01	0.149	0.15 ± 0.04	**0.031**	-0.03 ± 0.02	0.180	0.07 ± 0.04	0.311	-4.26 ± 0.45	**≤ 0.001**
	16	-0.07 ± 0.01	**0.011**	0.07 ± 0.01	**≤ 0.001**	0.18 ± 0.05	**0.006**	-0.01 ± 0.02	0.543	0.16 ± 0.06	**0.011**	-2.89 ± 0.99	**≤ 0.001**
	18	-0.07 ± 0.03	**0.007**	0.03 ± 0.01	0.051	0.34 ± 0.05	**≤ 0.001**	-0.01 ± 0.02	0.185	0.28 ± 0.05	**0.002**	-4.16 ± 0.63	**≤ 0.001**
	21	-0.04 ± 0.03	**0.033**	0.02 ± 0.02	0.153	0.39 ± 0.06	**≤ 0.001**	-0.02 ± 0.02	0.296	0.34 ± 0.05	**≤ 0.001**	-4.96 ± 0.43	**≤ 0.001**
66	11	0.00 ± 0.02	0.871	0.00 ± 0.01	0.957	0.00 ± 0.02	0.935	-0.01 ± 0.04	0.871	0.01 ± 0.03	0.847	0.38 ± 0.44	0.521
	14	-0.04 ± 0.02	0.066	-0.01 ± 0.01	0.407	-0.21 ± 0.02	**≤ 0.001**	0.17 ± 0.05	**≤ 0.001**	-0.10 ± 0.05	**0.035**	6.53 ± 0.40	**≤ 0.001**
	16	-0.05 ± 0.02	**0.013**	0.03 ± 0.02	0.055	-0.18 ± 0.01	**≤ 0.001**	0.01 ± 0.02	0.871	-0.20 ± 0.03	**≤ 0.001**	5.32 ± 0.35	**≤ 0.001**
	18	-0.05 ± 0.02	**0.029**	0.05 ± 0.02	**0.004**	-0.19 ± 0.01	**≤ 0.001**	0.07 ± 0.02	0.067	-0.12 ± 0.05	**0.013**	3.69 ± 0.92	**≤ 0.001**
67	7	0.01 ± 0.01	0.725	-0.01 ± 0.01	0.311	0.04 ± 0.03	0.506	-0.01 ± 0.01	0.743	0.03 ± 0.03	0.475	-0.03 ± 0.47	0.958
	14	0.00 ± 0.02	0.993	-0.02 ± 0.02	0.255	0.18 ± 0.03	**0.009**	-0.06 ± 0.03	**0.029**	0.10 ± 0.04	0.057	-4.74 ± 0.88	**≤ 0.001**
	16	-0.01 ± 0.02	0.519	0.06 ± 0.01	**≤ 0.001**	0.32 ± 0.07	**≤ 0.001**	-0.10 ± 0.04	**≤ 0.001**	0.27 ± 0.04	**≤ 0.001**	-5.79 ± 0.61	**≤ 0.001**
	18	0.05 ± 0.01	**0.004**	0.04 ± 0.01	**0.003**	0.35 ± 0.07	**≤ 0.001**	-0.01 ± 0.03	0.818	0.42 ± 0.06	**≤ 0.001**	-5.94 ± 0.51	**≤ 0.001**
	21	0.00 ± 0.01	0.860	0.05 ± 0.01	**≤ 0.001**	0.30 ± 0.06	**≤ 0.001**	-0.02 ± 0.02	0.438	0.32 ± 0.06	**≤ 0.001**	-6.01 ± 0.57	**≤ 0.001**

See Table 7.1 for group definitions.

The experimental and fellow eyes were compared at each measurement time using a Two-Way Mixed Measures Analysis of Variance (ANOVA) with Post-hoc comparisons adjusted for familywise error using the Holm-Sidak method.

7.5 Results

Briefly, when paired eyes experienced defocus of the same sign and magnitude simultaneously, defocus alone was able to guide eye growth appropriately, even when the two eyes were of unequal lengths or sizes. Similar to the findings in Chapter 4, eyes that had become shorter than their follow eyes after fully compensating for a weak positive lens, further compensated for a stronger positive lens by keeping the rate of ocular elongation low. In contrast to findings in Chapter 4, after chick eyes compensated for the hyperopic defocus induced by a weak negative lens by becoming longer than their fellow eyes, they successfully further elongated to compensate for a stronger negative lens. Like in Chapter 4, the experimental eye experienced a sudden increase in the amount of hyperopic defocus, but unlike in Chapter 4, the fellow eye also simultaneously experienced a similar change.

The inter-ocular differences in anterior chamber depth, lens thickness, vitreous chamber depth, choroidal thickness, axial length, and refractive error are summarized in Table 7.2. Refer to Figs. A3.1 to A3.4 in Appendix 3 for changes in anterior chamber depth and lens thickness.

7.5.1 Exp. 7.1: Stepped vs. constant lens powers

7.5.1.1 Positive Lens Step-Up

Wearing a +5 D lens over the experimental eye for 3 days (11 to 14 days of age, group 64, Table 7.1) caused full compensation (X vs. N on day 14, Mean, +5.23 vs. +0.35 D, $p < 0.001$, Table 7.2, Fig. 7.2A) with an inter-ocular difference of +5.69 D (Table 7.3). This spectacle lens compensation was due primarily to a reduced vitreous chamber depth (X vs. N on day 14, 4.95 vs. 5.11 mm, $p < 0.05$; Table 7.2, Fig. 7.2E) with an inter-ocular difference of -0.16 mm (Table 7.3). Interestingly, there was no significant reduction in axial length (9.15 vs. 9.23 mm, $p > 0.05$; Fig. 7.2C). The robust hyperopic shift was also associated with increased choroidal thickness of 70 µm in the lens-wearing eye (0.34 vs. 0.26 mm, $p < 0.01$; Fig. 7.2G), with an inter-ocular difference of +0.08 mm (Table 7.3).

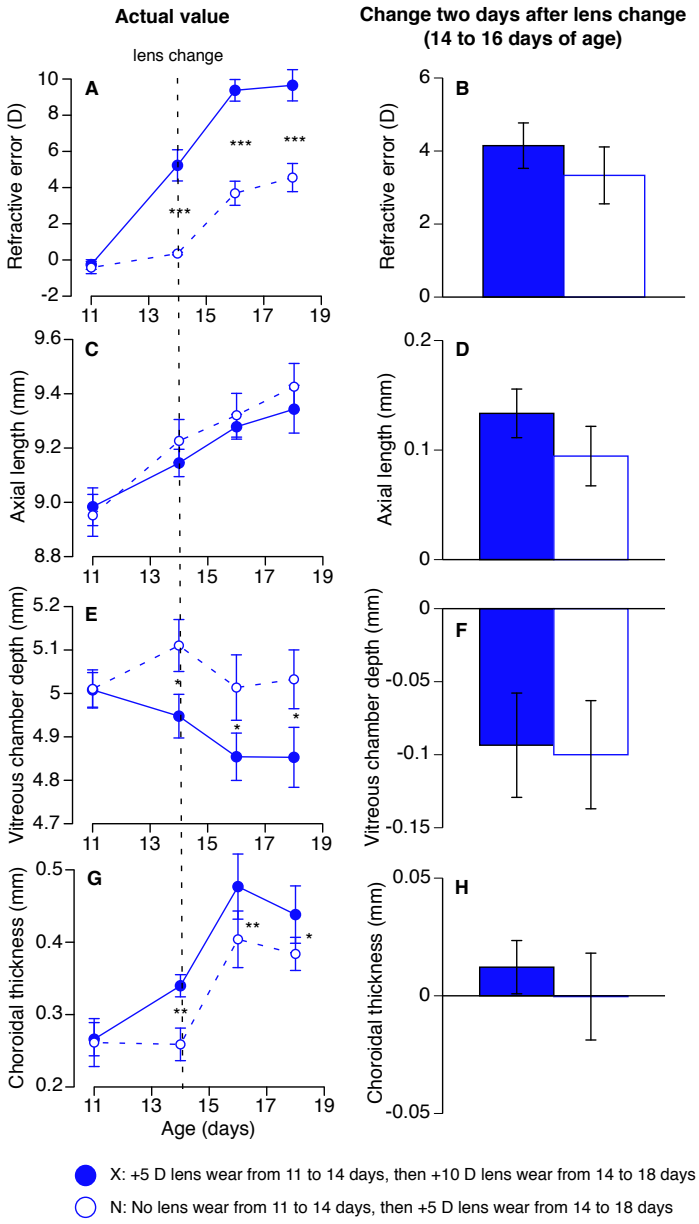

Figure 7.2. Time course of binocular positive lens treatment.
Chicks wore a +5 D lens over one eye (the experimental eye, "X", blue circles and bars) for 3 days, then stepping to a +10 D lens for another 4 days. The fellow eye ("N", white circles and bars) started wearing a +5 D lens at lens step-up for 4 days. Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 2 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The experimental eyes fully compensated for the +10 D lenses only 2 days after the step-up (X on day 16, +9.38 D; Table 7.2, Fig. 7.2A). Both experimental and fellow eyes developed approximately the same amount of hyperopia within the first 2 days after the step-up (ΔX vs. Δ N between days 14 to 16, +4.15 D vs. +3.33 D, $p > 0.05$, Fig 7.2B), since both eyes showed similar amounts of inhibition in axial elongation (ΔX vs. ΔN, +0.13 vs. +0.09 mm, $p > 0.05$, Fig. 7.2D), reduction in their vitreous chamber depths (–0.09 vs. –0.10 mm, $p > 0.05$, Fig. 7.2F), and thickening in their choroids (+0.14 vs. 0.15 mm, $p > 0.05$, Fig. 7.2H).

7.5.1.2 Negative Lens Step-Up

Wearing a –5 D lens over the experimental eye for 7 days (7 to 14 days of age, group 65, Table 7.1) caused almost full compensation (X vs. N on day 14, –4.58 D vs. –0.32 D, $p < 0.001$; Table 7.2, Fig. 7.3A) resulting in an inter-ocular difference of -4.26 D (Table 7.3). This was not associated with an elongated axial length (X. vs. N on day 14, 9.25 vs. 9.18 mm, $p > 0.05$; Fig. 7.3C), but did cause a significant inter-ocular increase in the vitreous chamber depth (X vs. N on day 14, 5.25 vs. 5.10 mm, $p < 0.05$; Fig. 7.3E; X – N of 0.15 mm, Table 7.2). The choroids thinned slightly on average (X – N of –0.03 mm, $p > 0.05$, Table 7.3), but not significantly so (X. vs. N on day 14, 0.20 vs. 0.23 mm, $p > 0.05$; Table 7.3 and Fig. 7.3G).

After the step-up of negative lens power from –5 D to –10 D in the experimental eyes and the fellow eyes started to wear –5 D lenses on 14 days of age, the experimental eyes showed further compensation in the myopic direction and fully compensated for –10 D lenses after wearing them for another 7 days (14 to 21 days of age): The experimental eyes became –9.96 D myopic at the end of the treatment on day 21 (Table 7.2, Fig. 7.3A). The fellow eyes also developed 5 D of myopia after wearing –5 D lenses for 7 days. The change in refractive error after the negative lens step-up was similar in the experimental and fellow eyes (ΔX vs. ΔN from days 14 to 21, –5.38 D vs. –4.68 D, $p > 0.05$; Fig. 7.3B). On the other hand, the experimental eyes showed significantly more ocular elongation than their fellow eyes (ΔX vs. ΔN from days 14 to 21, +0.92 mm vs. +0.65 ± 0.03 mm, $p < 0.001$, Fig. 7.3D),

mostly caused by a greater increase in their vitreous chamber depth (ΔX vs. ΔN from days 14 to 21, +0.59 mm vs. +0.35 mm, $p < 0.001$; Fig. 7.3F).

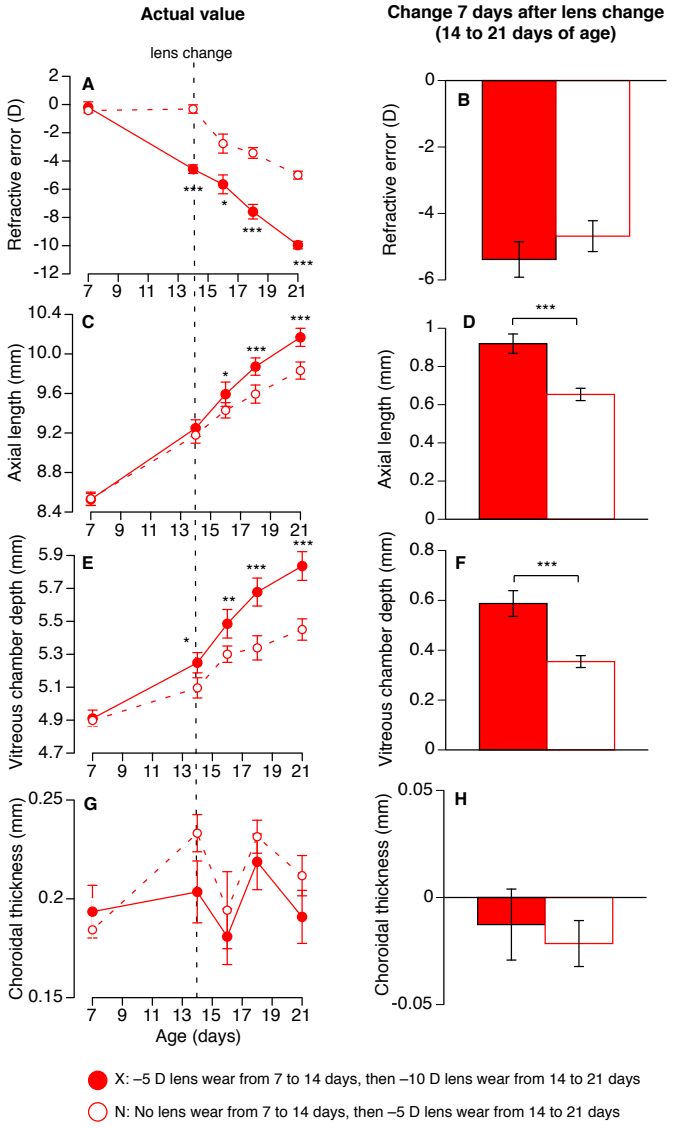

Figure 7.3. Time course of binocular negative lens treatment.
Chicks wore a –5 D lens over one eye (the experimental eye, "X", red circles and bars) for 7 days, then stepping to a –10 D lens for another 7 days. The fellow eye ("N", white circles and bars) started wearing a –5 D lens at lens step-up for 7 days. Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 7 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

There was no consistent pattern or differences between the change in the experimental and fellow eyes after the spectacle lenses were swapped in the thickness of the choroid, anterior chamber or crystalline lens (ΔX vs. ΔN from days 14 to 21; Choroid: –0.01 vs. –0.02 mm, Fig. 7.3H; Anterior Chamber: +0.12 mm vs. +0.11 mm; Crystalline lens: +0.22 mm vs. +0.20 mm; $p > 0.05$ for each parameter; Fig. A3.2 in Appendix 3).

7.5.2 Exp. 7.2: Recovery vs. constant lens powers

7.5.2.1 Positive Lens Recovery vs. Negative Lens Wear

Wearing a +5 D lens over the experimental eyes for 3 days (11 to 14 days of age, group 66, Table 7.1) caused full compensation: These eyes became 6.6 D hyperopic (X vs. N on day 14, Mean, +6.64 D vs. +0.11 D, $p < 0.001$; Table 7.2, Fig. 7.4A). This robust compensation was primarily due to a reduced axial length (X vs. N on day 14, 9.25 mm vs. 9.35 mm, $p < 0.05$; Fig. 7.4C) and vitreous chamber depth (5.02 mm vs. 5.23 mm, $p < 0.001$; Fig 7.4E). The reduction in vitreous chamber depth was partially caused by the choroidal thickening in these eyes (X vs. N on day 14, 0.39 mm vs. 0.23 mm, $p < 0.001$; Fig. 7.4G).

After the +5 D lenses were removed on day 14, these experimental eyes partially recovered from prior positive lens wear by developing a myopic shift of 4.58 D two days later (X on day 16, +2.06 D, Table 7.2 and Fig. 7.4A). The fellow eyes started wearing –5 D lenses on day 14 and developed a myopic shift 2 days later (N on day 16, –3.26 D, Table 7.2 and Fig. 7.4A). The myopic shift noted in the experimental eyes was significantly more than that found in the fellow eyes (ΔX vs. ΔN between days 14 to 16, –4.58 D vs. –3.37 D, $p < 0.05$; refer to Table 7.2, Fig. 7.4B). These rapid myopic shifts in the experimental and fellow eyes were caused by an increase in axial length (Fig. 7.4C) and vitreous chamber depth (Fig. 7.4E), and a reduction in choroidal thickness (Fig. 7.4G). Comparisons of change between paired eyes revealed that this rapid myopic shift in the experimental eyes was mostly caused by robust choroidal thinning (ΔX vs. ΔN between days 14 to 16, –0.21 mm vs. –0.05 mm, $p < 0.01$; Fig. 7.4H, refer to Table 7.2), since the increase in axial length and vitreous chamber depth was similar in both eyes (axial length: +0.15 mm vs. +0.25 mm, Fig. 7.4D; vitreous chamber depth: +0.26 mm vs. 0.23 mm, Fig. 7.4F; $p > 0.05$ for both).

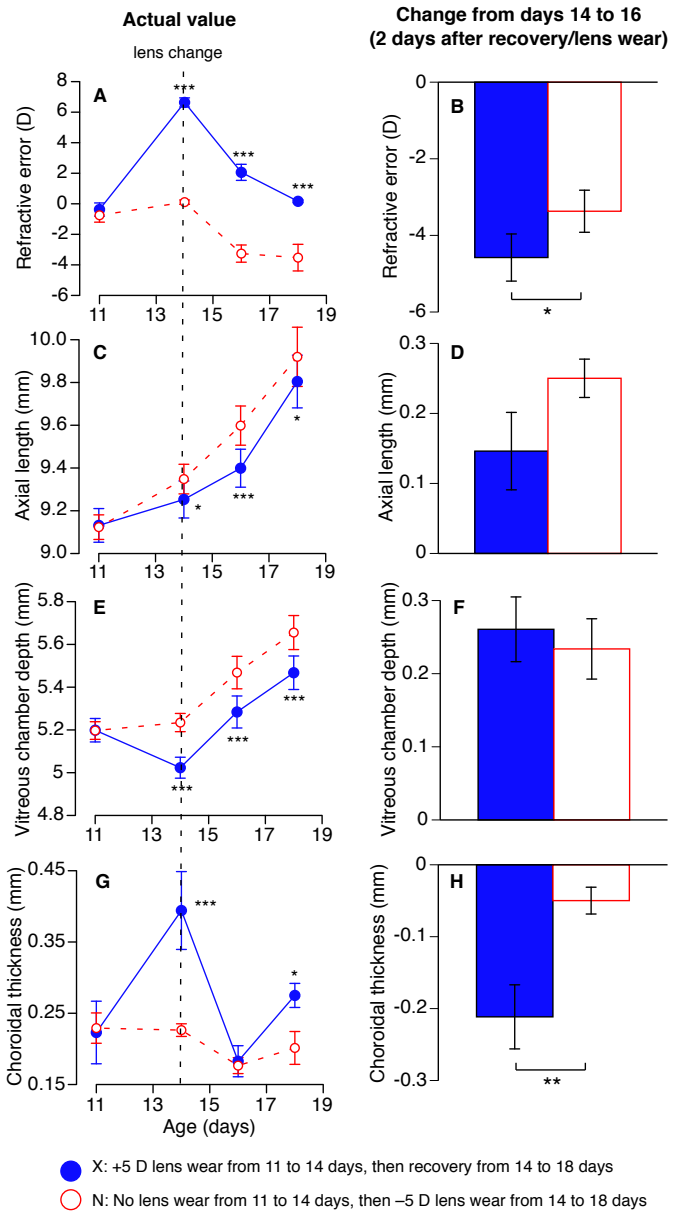

Figure 7.4. Comparison between positive lens recovery and negative lens wear. Chicks wore a +5 D lens over one eye (the experimental eye, "X", blue filled circles and bars) for 3 days, then recovered for another 4 days. The fellow eye ("N", red open circles and bars) started wearing a –5 D lens at removal for 4 days. Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 2 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Similar findings were discovered after 4 days of recovery in the experimental eyes and lens wear in the fellow eyes (ΔX vs. ΔN from 14 to 18 days of age, refractive error: –6.48 D vs. –3.63 D, $p < 0.01$; axial length: +0.55 mm vs. +0.57 mm, $p > 0.05$; vitreous chamber depth: +0.44 mm vs. +0.42 mm, $p > 0.05$; choroidal thickness: –0.12 mm vs. –0.03 mm, $p = 0.09$; refer to Table 7.2).

7.5.2.2 Negative Lens Recovery vs. Positive Lens Wear

Wearing a –5 D lens over the experimental eye for 7 days (7 to 14 days of age, group 67, Table 7.1) caused full compensation: These eyes became 5.2 D myopic (X vs. N on day 14, Mean, –5.17 D vs. –0.42 D, $p < 0.001$, Table 7.2, Fig. 7.5A). This rapid myopic shift was mainly caused by an increase in vitreous chamber depth (X vs. N on day 14, 5.24 mm vs. 5.06 mm, $p < 0.001$; Fig 7.5E). The increase in vitreous chamber depth was partially caused by choroidal thinning in these eyes (X vs. N on day 14, 0.19 mm vs. 0.25 mm, $p < 0.05$; Fig. 7.5G). On the other hand, there was no significant increase in axial length (X vs. N on day 14, 9.19 mm vs. 9.10 mm, $p > 0.05$; Fig. 7.5C)

After the –5 D lenses were removed on day 14, the experimental eyes fully recovered from prior negative lens wear by developing 4.9 D of hyperopic shift two days later (X on 16 days of age, –0.24 D, Table 7.2). The fellow eyes started wearing –5 D lenses on day 14 and developed a hyperopic shift 2 days later (N on day 16, +5.55 D, Table 7.2). The hyperopic shift found in the experimental eyes was similar to that found in the fellow eyes (ΔX vs. ΔN between 14 and 16 days of age, +4.93 D vs. +5.97 D, $p > 0.05$; refer to Table 7.2, Fig. 7.5B). Comparisons of changes between paired eyes revealed that both axial length and vitreous chamber depth in experimental eyes elongated more than those in the fellow eyes 2 days after lens change (ΔX vs. ΔN between 14 and 16 days of age, axial length: +0.20 mm vs. +0.03 mm, $p < 0.05$, Fig. 7.5D; vitreous chamber depth: +0.02 mm vs. –0.12 ± 0.02 mm, $p < 0.05$, Fig. 7.5F). Change in choroidal thickness was not significantly different between the two eyes (+0.07 mm vs. +0.11 mm, $p > 0.05$, Fig. 7.5H).

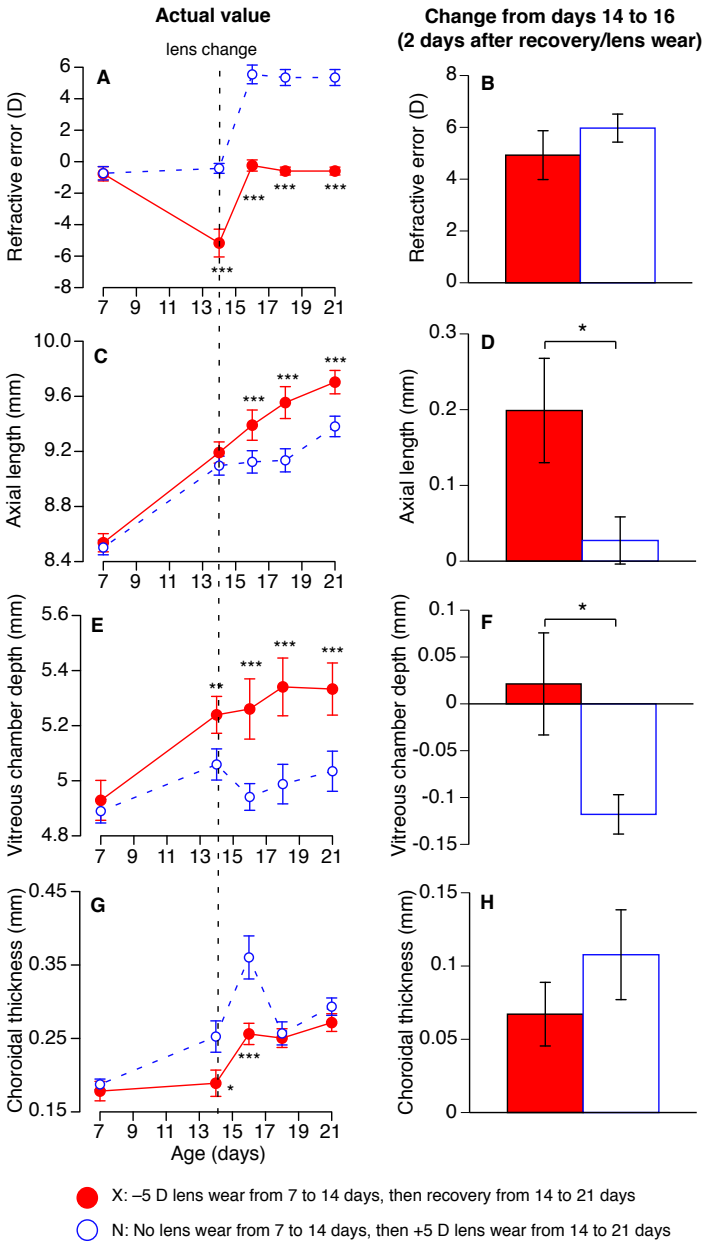

Figure 7.5. Comparison between negative lens recovery and positive lens wear. Chicks wore a –5 D lens over one eye (the experimental eye, "X", red filled circles and bars) for 7 days, then recovered for another 7 days. The fellow eye ("N", blue open circles and bars) started wearing a +5 D lens at removal for 7 days. Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 2 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Similar findings were discovered after 7 days of recovery in the experimental eyes and lens wear in the fellow eyes (ΔX vs. ΔN between 14 to 21 days of age, refractive error: +5.00 D vs. +6.24 D, $p > 0.05$; axial length: +0.51 mm vs. +0.28 mm, $p < 0.05$; vitreous chamber depth: +0.09 mm vs. –0.03 mm, $p < 0.05$; choroidal thickness: +0.08 mm vs. +0.04 mm, $p > 0.05$; refer to Table 7.2).

7.6 Discussion

Results from this chapter show that the visual mechanism dominated the proposed intrinsic non-visual mechanism when both eyes experienced defocus of the same sign and a similar magnitude.

7.6.1 Equal binocular defocus dominates eye size signals

The results from Exp. 7.1 suggest that the visual stimuli dominated the proposed intrinsic non-visual mechanism in the case of both myopic and hyperopic defocus, when chick eyes experienced defocus of the same sign and a similar magnitude in both eyes. The findings from negative lens step-up (group 65) also support the possible existence of the yoking effect between the paired eyes. These findings are illustrated in Fig. 7.6: The magnitude of the functional defocus the experimental eyes experienced at the step-up or recovery is considered a natural measure of the visual stimuli (referred as the "defocus-factor" in this thesis) and shown on the X axis, whereas the change in refractive error in the experimental eyes is considered to be these eyes' response to the visual stimuli (the defocus-factor) and shown on the Y axis. For the group with positive lens step-up (blue circle, group 64), for example, the experimental eyes were 5.2 D hyperopic on average right before the step-up on day 14 (Table 7.2), and therefore experienced 4.8 D of myopic defocus (10 – 5.2 = 4.8) right after stepping up to +10 D lenses. These eyes fully compensated for +10 D lenses by developing another 4.5 D of hyperopia from days 14 to 18 (Table 7.2). Thus, the symbol falls on the line of equality, indicating that the defocus-factor dominated in this case. For the group with negative lens step-up (red circle, group 65), the experimental eyes were –4.6 D myopic right before the step-up on 14 days of age (Table 7.2), and were experiencing 5.4 D of hyperopic defocus (10 – 4.6 = 5.4 D) after stepping up to –10 D lenses. These eyes also

fully compensated for –10 D lenses by developing another 5.4 D of myopia from 14 to 21 days of age. Thus, the symbol for this group (red circle) also falls on the line of equality. These findings indicate that the visual cues provided by imposed binocular myopic and hyperopic defocus dominated any intrinsic consequences of unequal eye sizes/lengths.

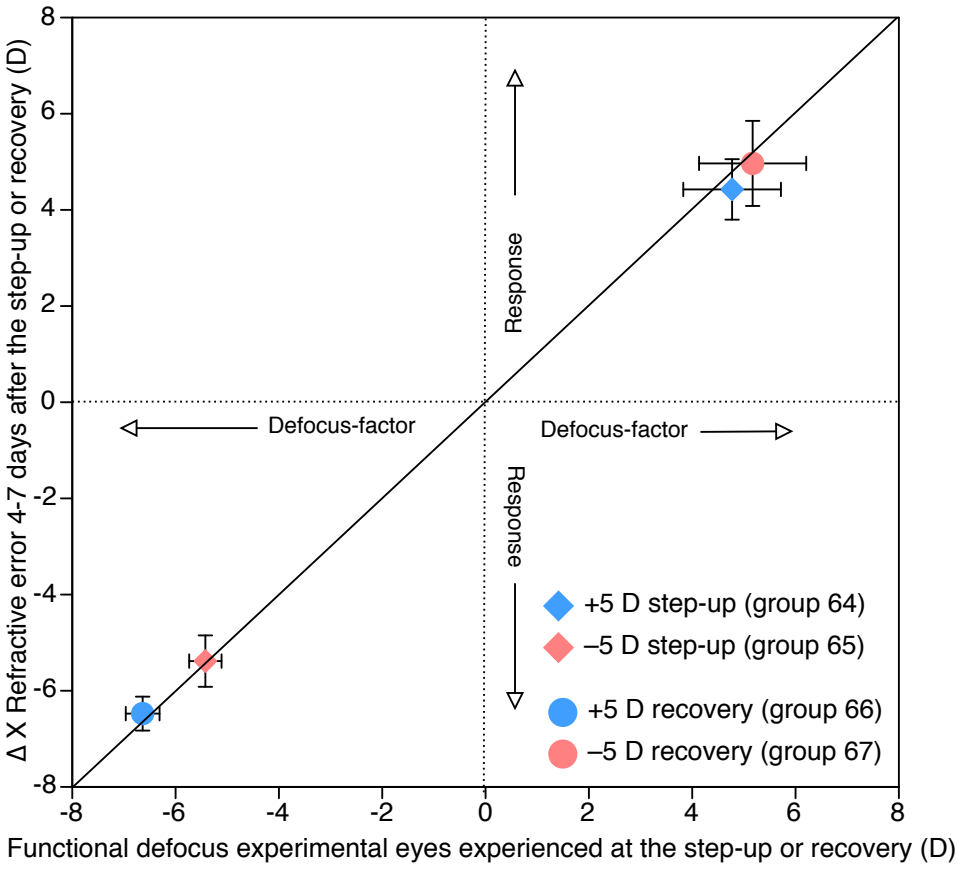

Figure 7.6. The correlation between change in refractive error and functional defocus after lens step-up or recovery.
Data is shown as Mean ± SEM. The magnitude of functional defocus the treated eyes were experiencing at the step-up or recovery is naturally a measure of the defocus-factor (stimuli, labeled with the dashed line on the X axis) and the actual change in refractive error in these eye is the measure of these eyes' response to the defocus-factor (labeled with the dashed line on the Y axis). If these treated eyes fully compensated for the functional defocus, the symbols should all fall on the line of equality. All symbols of lens step-up and recovery fall on the line of equality, indicating that the defocus-factor dominated the size-factor in the case of binocular myopic and hyperopic defocus.

7.6.2 The effect of asymmetry between paired eyes

The discrepancy between the results in Chapter 4 and the results in this chapter are likely associated with the different degrees of asymmetry between paired eyes in the monocularly treated chicks (Chapter 4) vs. binocularly treated chicks (the current Chapter). As previously speculated in Section 4.6.3, the reason that chick eyes failed to compensate for the strong negative lenses after stepping up from the weak negative lenses could be because of the asymmetry between paired eyes at the step-up: In Chapter 4, the experimental eyes that wore negative lenses were significantly more myopic with longer axial length than the fellow eyes after compensating for the weak negative lenses, and this asymmetry in both refractive error and axial length between the paired eyes disabled the experimental eyes to further compensate for the strong negative lenses after the step-up. Alternatively, the eye cannot respond to a sudden increase in the magnitude of hyperopic defocus when it does not occur in both eyes simultaneously.

In the current study, lens manipulations were designed in such a way so that both the experimental eyes and the fellow eyes experienced the same amount of magnitude of hyperopic defocus to minimize the asymmetry in refractive error. The fact that eyes further compensated for the strong negative lenses after the step-up when both eyes experienced the same amount of magnitude of defocus (i.e., minimal asymmetry in visual input) suggests that the eyes need balanced visual input to enhance eye growth. In other words, asymmetry in visual input between paired eyes may inhibit axial elongation.

In addition, it is possible that the asymmetry in axial length might not play a role as important as the asymmetry in visual input in inhibiting axial elongation. Wearing –5 D lenses for 7 days (from days 7 to 14) caused similar amounts of axial elongation in the experimental eyes in group 14 (Chapter 4, ΔX between days 7 and 14, +0.58 mm) and in group 65 (current Chapter, +0.72 mm). The only difference between these two groups is that chick eyes in group 14 in Chapter 4 experienced monocular hyperopic defocus after the step-up whereas chick eyes in group 65 in this chapter experienced binocular hyperopic defocus after the step-up. The fact that eyes in the current study further compensated for the strong negative lenses (–10 D) after the step-up, regardless of the asymmetry in axial length at the step-up, suggests that the asymmetry in axial length is less important than the asymmetry in

visual input in preventing further axial elongation. It supports the notion that visual input plays a major role in regulating eye growth, as shown by a large body of literature from animal research.

7.6.3 Conclusion

Symmetry in visual input is able to override the previously observed constraint on ocular growth that occurred when one eye experienced a sudden change in hyperopic defocus.

8. General Discussion

It has long been known that eye growth during emmetropization is particularly sensitive to, and guided by, the defocus experienced by an eye. The ultimate goal of this thesis was to examine whether there might be mechanisms, other than the local defocus signals, that may be involved in regulating eye growth. We studied this question in the chick as their early eye growth has a robust sensitivity to defocus with a large effect size. The following is a brief review of the main findings from each experimental chapter, followed by further discussion on future experiments, and implications for human myopia control.

8.1 Summary of thesis findings

8.1.1 Chapter 3

8.1.1.1 Conclusions from Chapter 3

Cues that guide eye growth based on defocus or related aspects of the visual image require visual input to be activated. If eye growth is influenced by other mechanism(s), one such mechanism may relate to intrinsic aspects of the eye not related to vision. Therefore, in this first chapter we hypothesized that there may be a non-visual mechanism(s) in chick eyes that can guide the direction of eye growth. To study this hypothesis, chick eyes that had previously compensated for +7 D and −7 D lenses were kept in darkness for 3 days to investigate if these eyes could recover from prior lens treatment without any visual input. It was discovered that all eyes changed their direction of growth in the correct direction, and partially recovered from prior lens treatment in darkness, although the speed at which they recovered was slower compared with chick eyes that recovered in normal light. These results support the hypothesis that a non-visual mechanism(s) exists in chick eyes and can initiate and guide the direction of eye growth, and eyes that are already too long (myopic) or too short (hyperopic) regain their normal size while kept in the darkness. Similar results have been found in guinea pigs: McFadden *et al.* have shown that guinea pigs can recover from form deprivation myopia after 3 days of darkness[237]. There are many possible factors that might contribute to this intrinsic ability for the eye to reverse abnormal growth patterns in

darkness. It is known that the eye mostly grows during the day rather than at night[176], but the changes in growth we observe in darkness are not due to any consistent interruption in the circadian cycle since the direction of growth is different depending on its previous state. Furthermore, the changes are not due to darkness effects per se on the anterior optics, since there was no significant change in either anterior chamber depth or lens thickness during dark rearing (Fig. A1.1 in Appendix 1, refer to Table 3.2). Instead, we propose that in the absence of visual input, darkness triggers the eye to either reverse its most recent growth state, or somehow return to its age-matched or fellow eye-matched length or size.

8.1.1.2 Study limitations from Chapter 3 and future experiments

There are three limitations of the experimental design for this chapter:

Firstly, the lens treatment started on various days of age (7 days of age for groups 1, 2, and 4; and 1 day of age for group 3, Table 3.1). Removal also took place on various days of age (14 days of age for group 1, 11 days of age for groups 2 and 4, and 6 days of age for group 3). Secondly, the lens treatment durations were different for positive and negative lens treatment. It is plausible that the difference in these parameters may potentially cause different magnitudes in both lens compensation and recovery from prior lens treatment. However, it is unlikely the case, since chick eyes in all four groups completely and accurately compensated for both +7 D and –7 D lenses, regardless of the differences. Experiments were designed so that they lasted when chicks were between 1 to 14 days of age, during which chick eyes rapidly emmetropize[74]. Taken together, it is unlikely that the different starting ages and lens treatment durations caused deviations in the results.

Thirdly, chicks may not have been observed long enough while they recovered in darkness (groups 2 and 4). Chick eyes only recovered from prior positive and negative lens treatment by 60% and 69% after 3 days of dark rearing, respectively. Since the experiments were terminated at this point, it is unknown whether chick eyes can fully recovery from prior lens treatment. Therefore, the effect of the proposed non-visual mechanism(s) in guiding the magnitude of eye growth was not studied in this thesis. It would be interesting to keep chicks in darkness for longer durations (e.g., 7 days) to study if the eyes can completely recovery from prior lens without any visual input.

8.1.2 Chapter 4

8.1.2.1 Conclusions from Chapter 4

In this chapter, we sought to determine how important the intrinsic length or size of the eye or its previous growth state is, when it is competing with the usual spectacle lens based cues related to image defocus. To study this, we firstly manipulated the ocular length of one eye with spectacle lens wear, so that the two eyes were unequal in size, as well as developing unequal defocus states. For example, in one group, chick eyes wore a weak positive lens over one eye to induce a reduced rate of axial elongation compared with their fellow eyes. The growth inhibited eye then wore a stronger positive lens. If the eye was simply sensitive to the current defocus state, it should reduce its growth even further. However, if it was sensitive to its previous growth state and the fact that it was smaller than its fellow eye, it should correct this abnormality by reversing the inhibition in growth, and failing to respond to the new stronger imposed myopic defocus. The equivalent experiment was also conducted with negative lenses. In this case, if the eye was only sensitive to the current state of defocus, it would act to cause further compensation for the stronger negative lenses lens by further increasing its rate of ocular elongation. In contrast, if the eye growth is sensitive to its prior state of growth and/or it's recently enhanced or asymmetric eye sizes, it might act to reduce further compensating and to restore the eye to its normal size. It was discovered that eye growth was dominated by the local defocus signal in the case of positive lens wear since eyes that wore positive lenses fully compensated for the subsequent stronger positive lenses, but that in the case of negative lens wear, the eye refrained from further elongation to compensate for the stronger negative lenses. These findings support the hypothesis that there are factors other than local defocus that can prevent eye from further elongating in the case of a sudden increase in hyperopic defocus arising from negative lens wear. It is possible that this arises from some intrinsic factor sensitive to the expected age-matched eye-size, or the asymmetry between the two eyes in their length or refractive state. It is unclear if it is visual or non-visual, but whatever it is, it is activated by a sudden step-up in hyperopic defocus. Interestingly, this alternative mechanism does not seem to have a potent effect in driving the eyes response to a sudden change in myopic defocus.

In order to understand if this constraint on eye elongation was related to anismetropia between the eyes in terms of asymmetric defocus or whether it was more related to asymmetric eye-lengths, the response of the eye to a change in defocus was investigated using the recovery model. Here the change in refractive error and ocular dimension two days after a positive lens step-up was compared to that after two days after recovery from negative lens wear when both groups were experiencing a similar amount of myopic defocus, with the main difference being the eye length (shorter experimental eyes after wearing positive lenses in the step-up group vs. longer experimental eyes after wearing negative lenses in the recovering group). The equivalent comparison was made between a group experiencing a negative lens step-up and a group recovering from positive lens wear, when both groups were experiencing a similar amount of hyperopic defocus, with the main difference, again, being asymmetric eye lengths and/or recently modified eye growth. It was discovered that chick eyes in the positive lens step-up group reduced their rate of ocular elongation more than those in the group recovering from prior negative lens wear, implying that eye length or size had no effect when myopic defocus is present. On the other hand, eyes recovering from prior positive lens wear developed a greater myopic shift compared with negative lens-wearing eyes after the step-up, confirming the influence of a recently reduced eye length on increasing the eye's response to hyperopic defocus.

The results of this chapter are consistent with the idea that there may be an intrinsic mechanism, possibly non-visual, that prevents the eye from falling short of its normal, age-matched length or strives to match the length between the two eyes.

8.1.2.2 Study limitations from Chapter 4 and future experiments

There are five limitations of the experimental design for this chapter:

Firstly, the lens treatment started on various days of age (from 1 to 7 days of age, Table 4.1). Since the experiments in this chapter lasted up to two weeks, it was necessary to start lens treatment at an early age so the experiment could end before chicks turned three weeks old, when it became quite difficult to keep the Velcro rings on chick eyes. It is unlikely that the different starting age had a significant impact on the results, since chick eyes rapidly

emmetropize[74]. In addition, most of the experiments (10 out of 12) started when chicks were 6 or 7 days old.

Secondly, the lens treatment durations were different for positive and negative lenses. It was necessary for chick eyes to wear negative lenses longer than positive lenses to ensure full compensation for positive lenses (3 to 7 days of positive lens wear vs. 4 to 7 days of negative lens wear), since it takes longer to completely compensate for negative lenses. To rule out the possibility that different lens wearing durations might cause different responses after lens step-up (e.g., group 6 vs. group 10 in Chapter 4), the experiment was repeated multiple times with different measuring intervals and a longer observation period to ensure the validity of the results. Therefore, it is unlikely that different lens treatment durations for positive and negative lens wear caused different responses after lens step-up.

Thirdly, the experiments were conducted under light, so it is not possible to determine exclusively whether this intrinsic mechanism uses visual or non-visual cues. Even though the existence of an intrinsic, homeostatic mechanism was established in Chapter 3 where the experiments were conducted in darkness, suggesting there is a mechanism that is non-visual in nature, experiments in this chapter (and in the following chapters) were conducted under light. Therefore, there is a possibility that the intrinsic mechanism could be visual. However, it is unlikely to be a local visual signal within one eye: If it were, this mechanism would be influenced by the experimental eye's exposure to defocus. The fact that the experimental eyes responded differently, depending on the visual experience of their fellow eyes (results from Chapters 4 and 7), even though the experimental eyes were experiencing the same visual exposure argues against the possibility that this intrinsic mechanism is of a local visual nature. It would be interesting to conduct experiments under infra-red light to investigate whether this interaction between the eyes is visual or non-visual. In addition, even though this intrinsic mechanism is referred to as a "size-factor" throughout the thesis, it is not clear what parameter(s) this mechanism is sensitive to. Since this mechanism was uncovered through manipulating eye length or size, it would be reasonable to assume that it is either directly or indirectly sensitive to recent changes in eye length or size.

Fourthly, only two magnitudes of defocus were attempted using lens step-up in this chapter (5 and 8 D). While results from this chapter provide sufficient evidence showing the effect of the proposed intrinsic mechanism, for the completion of the study, it would be interesting to further study the compensation after the positive and negative lens step-up superimposing the eyes with a large variety of defocus, e.g., from 2 to 15 D. Future studies could superimpose the eyes with a larger variety in the degree of hyperopic defocus to study the threshold of the intrinsic mechanism: It is plausible that while a larger hyperopic defocus fails to induce further compensation for the strong negative lens, as shown in this chapter, a smaller hyperopic defocus might be able to induce further compensation at some level, if this small hyperopic defocus is below the threshold for the intrinsic mechanism(s) to detect. Furthermore, it is also possible that while chick eyes could further compensate for 8 D of functional myopic defocus after the lens step-up by reducing their rate of ocular elongation, they might not be able compensate for a much larger degree of myopic defocus.

Fifthly, results from this thesis cannot exclusively demonstrate whether it was an intrinsic, hemostatic mechanism or the refractive asymmetries between paired eyes that refrained the negative lens-wearing eyes from further elongating. By fitting chicks with positive or negative lenses over one eye, aiming to manipulate different eye lengths or sizes in the experimental eyes to potentiate the hypothesized intrinsic, possibly non-visual, mechanism(s), asymmetries in both the visual input (the experimental eyes were superimposed with hyperopic defocus after wearing negative lenses, whereas the fellow eyes were nearly emmetropic) and in eye length (the experimental eyes became significantly longer than the fellow eyes after compensating for the negative lenses) between the paired eyes were also created. It is possible that these asymmetries between paired eyes, either in isolation or in combination with the intrinsic mechanism, prevented the eyes from further elongating.

8.1.3 Chapter 5

8.1.3.1 Conclusions from Chapter 5

After discovering the dominance of myopic defocus in guiding eye growth against any intrinsic mechanism in the last chapter, in Chapter 5, the potency of myopic defocus was further studied by conducting an analysis to see if chick eyes could axially shorten to facilitate compensation for myopic defocus. Data from previous experiments in which chicks wore a positive lens over one eye for 3 days, either continuously or intermittently, were compared with data of normal, untreated chicks. The analyses showed that chick eyes wearing positive lenses could axially shrink to facilitate compensation for myopic defocus, against the intrinsic, homeostatic mechanism. The amount of reduction on average in axial length after wearing positive lenses for 3 days was three-fourths (mean change in axial length between 7 to 10 days of age, positive lens-wearing eyes vs. untreated, normal eyes, +40 μm vs. +188 μm). Most strikingly, 38.5% of positive lens-wearing eyes developed axial lengths that actually became shorter than before the lens-treatments, compared with 2% found in the untreated, normal eyes. The axial shortening was caused mostly by the reduction in vitreous chamber depth.

8.1.4 Chapter 6

8.1.4.1 Conclusions from Chapter 6

On possible candidate for the previously reported effects of the lens treatment in the experimental eyes that are non-visual in nature and can affect eye growth in the fellow eyes is a possible interaction between the treated eyes and the untreated fellow eyes. Therefore, in this chapter, we sought to determine how symmetrical the growth is between the two eyes, and studied the effects of eye growth in chicks wearing a lens on one eye, on the other untreated eye. We defined yoking as a phenomenon when wearing a lens over one eye changes the refractive state and eye growth in the fellow eye in the same direction as seen in the experimental eye, and the change in the untreated fellow eyes is different from what is observed in age-matched normals; and anti-yoking as a phenomenon when wearing a lens

over one eye changes the refractive state and eye growth in the fellow eye in the opposite direction as seen in the experimental eye, and the change in the untreated fellow eyes is different from what is observed in age-matched normals.

Paired eyes in untreated chicks were well correlated in their axial lengths from as early as 24 hours and on various days afterwards, demonstrating symmetrical size and/or symmetrical growth. Symmetrical growth between paired eyes tends to be interrupted in the beginning of lens treatment and regained later in the treatment. A correlation between the change in axial length in the untreated eye and lens wearing duration shows that monocular lens treatment tended to reduce eye growth in the fellow eyes after short lens wearing durations (1-2 days, anti-yoking for positive lens treatment and yoking for negative lens treatment) and to increase eye growth after longer lens wearing durations (up to 7 days, yoking for positive lens treatment and anti-yoking for negative lens treatment), and had minimal effect on the fellow eyes if the treatment duration was around 3-4 days. However, the size of these effects was relatively small: The amount of yoking and anti-yoking can cause an average of 39.2% overestimation of the effects of positive lens wear and 33.4% underestimation of the effects of negative lens wear. Finally, it should be noted that yoking and anti-yoking were only discovered in approximately half of the experiments conducted in this chapter, and that for intermediate lens wearing durations there were no detectable effects on the untreated fellow eye.

8.1.4.2 Study Limitations from Chapter 6 and Future Experiments

Two limitations of this study are that lens treatment started on various days of age (from 1 to 11 days of age), and the lens treatment durations were limited (1 to 4 and 7 days). It would be interesting to see if age would play a role in yoking and anti-yoking by starting lens treatment on the same day for all groups. It would also strengthen the conclusion of the current study if the animals could wear a strong lens for more durations, e.g., from 1 to 10 days.

8.1.5 Chapter 7

8.1.5.1 Conclusions from Chapter 7

To further investigate whether it was the proposed intrinsic, possibly non-visual mechanism or the asymmetry in visual input between the paired eyes that refrained the eyes from further elongating, chicks first wore a weak positive or negative lens over one eye to induce unequal eye length or size between paired eyes, then the lens powers were stepped in the experimental eyes as in Chapter 4, while the fellow eyes started to wear a weak lens of the same sign, superimposing both eyes with the same sign and amount of defocus. If it is the proposed intrinsic mechanism that controls eye growth, there should be no further compensation for the strong negative lens after the step-up, since these eyes were longer than the fellow eyes. On the other hand, if it is the asymmetry in visual input that controls eye growth, these eyes should further compensate for the strong negative lenses since now both eyes have the same visual input.

It was discovered that, in contrast to the findings in Chapter 4, chick eyes further elongated to fully compensate for the strong negative lenses after the step-up, against the proposed intrinsic mechanism, when both eyes experienced defocus of the same sign and magnitude. The results suggest that direction and magnitude of eye growth is governed by the sign of defocus when the two eyes both experience the same defocus.

One important notion that was touched on but never studied explicitly in this thesis is the effect of asymmetry in defocus and axial length between paired eyes on lens compensation. Monocular optical treatment causes changes in refractive error and ocular dimensions mostly in the lens-wearing eyes, thus creating an asymmetry between the paired eyes. It is not exactly clear how this asymmetry affects the sequential lens compensation. The fact that chick eyes did not further compensate for monocular hyperopic defocus after the negative lens step-up (an asymmetry in visual stimuli in paired eyes), and did further compensate for binocular hyperopic defocus after the step up (equal visual stimuli in paired eyes) indicates that the discrepancy in response could be due to the asymmetry in visual stimuli between the two eyes, not due to the hypothesized intrinsic, possibly non-visual factor *per se*. It is interestingly that chick eyes further compensated for the hyperopic defocus

after the step-up only when the visual input was equal in both eyes, suggesting that while the intrinsic mechanism is one of factors controlling eye growth, the coordination between the eyes may also contribute to eye growth regulation. It is curious how one eye could detect the visual input in the other eye. It could be done through visual coordination, or accommodation. Alternatively, it could be a combination of the intrinsic non-visual mechanism and the asymmetry in visual input between the paired eyes that prevents the eye from further elongating to compensate for hyperopic defocus after the step-up.

The results from this thesis do not explicitly support one possibility vs. the other. It would be interesting to further investigate this question by fitting a positive and a negative lens over paired eyes that underwent optic nerve section to remove one possible source of coordination between the eyes, as well as manipulate accommodation in monocular lens conditions.

The effect of a proposed intrinsic, possibly non-visual mechanism on eye growth when both eyes experienced the same visual input in terms of the sign and magnitude of defocus was also be assessed by comparing the change in refractive error and ocular dimension two days after recovery from positive lens wear to that two days after wearing a negative lens (both eyes were experiencing a similar amount of hyperopic defocus, with the main difference being the experimental eye shorter after wearing the positive lens). The equivalent comparison was made between paired eyes experiencing recovery from negative lens wear (experimental eye) and positive lens wear (fellow eye), when both eyes were experiencing a similar amount of myopic defocus, with the main difference, again, being asymmetric eye lengths and/or recently modified eye growth. It was discovered that the experimental and fellow eyes elongated by the same amount during the first 2 days recovering from prior positive lens wear or wearing negative lenses, suggesting that the proposed intrinsic mechanism did not significantly affect eye growth when both eyes experienced defocus of the same sign. On the other hand, eyes recovering from prior negative lens wear elongated significantly more compared with fellow eyes wearing positive lenses, against the effect of the proposed intrinsic mechanism.

8.2 The proposed intrinsic factor is only observed when the refractive states of the two eyes are asymmetrical

The effect of the proposed intrinsic, possibly non-visual mechanism on eye growth when both eyes in the same chick experienced the same visual input in terms of the sign and magnitude of defocus was also explored. By comparing the change in refractive error and ocular dimensions two days after recovery from positive lens wear (the experimental eye) to that two days after wearing a negative lens (the fellow eye, group 66 in Chapter 7), both eyes experienced a similar amount of hyperopic defocus, but the experimental eye that was recovering from prior positive lens wear was shorter than normal at recovery after compensating for the positive lens, whereas the fellow eye that just started to wear a negative lens had normal eye length. The equivalent comparison was made between paired eyes experiencing recovery from negative lens wear (the experimental eye) and positive lens wear (the fellow eye, group 67 in Chapter 7), when both eyes were experiencing a similar amount of myopic defocus, with the main difference, again, being asymmetric eye lengths and/or recently modified eye growth. If the intrinsic mechanism had an effect, it would act to induce more change in the axial length in the experimental eye (more elongation in the experimental eye recovering from prior positive lens wear in group 66, and less elongation in the experimental eyes recovering from prior negative lens wear in group 67), compared with their fellow eyes. It was discovered that the experimental and fellow eyes elongated by the same amount during the first 2 days recovering from prior positive lens wear (the experimental eye) or wearing negative lenses (the fellow eye), suggesting that the proposed intrinsic mechanism did not significantly affect eye growth when both eyes experienced defocus of the same sign. Similarly, eyes recovering from prior negative lens wear elongated significantly more compared with fellow eyes wearing positive lenses, also arguing against the effect of the proposed intrinsic mechanism when both eyes experienced defocus of the same sign.

8.2.1 Monocular vs. binocular lens step-up

8.2.1.1 Positive Lens Step-Up

To further elucidate the effects of the asymmetry in visual input between paired eyes, the change in refractive error and axial length in the experimental eyes 2 days after either lens step-up or recovery in monocularly treated groups (Chapter 4) and binocularly treated groups (Chapter 7) were directly compared.

When changes in experimental eyes in the monocularly treated group (group 8 from Chapter 4, fellow eyes untreated) were compared with changes in the experimental eyes in the binocularly treated group (group 64 from Chapter 7, fellow eyes wearing +5 D lenses) 2 days after +5 D stepping to +10 D lenses (from 11 to 13 days of age for group 8, from 14 to 16 days of age for group 64), experimental eyes in both groups showed a similar amount of change in refractive error (mean change, group 8 vs. group 64, +4.06 D vs. +4.15 D, $p > 0.05$, Fig. 8.1A, see Tables 4.1, A2.1, 7.1 and 7.2 for details), axial length (+0.14 mm vs. 0.13 mm, $p > 0.05$, Fig. 8.1B), vitreous chamber depth (+0.01 mm vs. –0.09 mm, $p > 0.05$, Fig. 8.1C), and choroidal thickness (+0.07 mm vs. +0.14 mm, $p > 0.05$, Fig. 8.1D). These results confirm the potency of myopic defocus against the size-factor, regardless of the asymmetry in visual input between the paired eyes.

8.2.1.2 Negative Lens Step-Up

When changes in experimental eyes in the monocularly treated group (group 14 from Chapter 4, fellow eyes untreated) were compared with changes in the experimental eyes in the binocularly treated group (group 65 from Chapter 7, fellow eyes wearing –5 D lenses), experimental eyes in the latter showed more compensation for –10 D lenses than in the former: Two days after –5 D lenses stepping to –10 D lenses (from 14 to 16 days of age), monocularly treated experimental eyes developed a 1.35 D hyperopic shift, whereas binocularly treated experimental eyes developed a 1.21 D myopic shift ($p < 0.05$, Fig. 8.1A, see Tables 4.1, A2.1, 7.1 and 7.2 for details). Interestingly, there was no difference in experimental eyes in axial length (mean change between 14 and 16 days of age, group 14

vs. group 2, +0.31 mm vs. +0.27 mm, $p > 0.05$, Fig. 8.1B), in vitreous chamber depth (+0.19 mm vs. +0.18 mm, $p > 0.05$, Fig. 8.1C), or choroidal thickness (+0.01 mm vs. –0.02 mm, $p > 0.05$, Fig. 8.1D) between the two groups. No consistent change between these two groups was discovered in either anterior chamber or lens thickness (refer to Tables A2.1 and 7.1). It is possible that the difference in change in refractive error between these two groups was caused by change in corneal curvature, which was not measured in this thesis.

To summarize, inhibition in the further ocular elongation to compensate for a sudden increase in hyperopic defocus (induced by stepping up the power of a negative lens) only occurred when the lens treatment was monocular. When there was equal visual input between the paired eyes, appropriate compensation occurred for the strong negative lenses after the step-up, indicating a dominance of the visual signal in guiding ocular growth under these conditions.

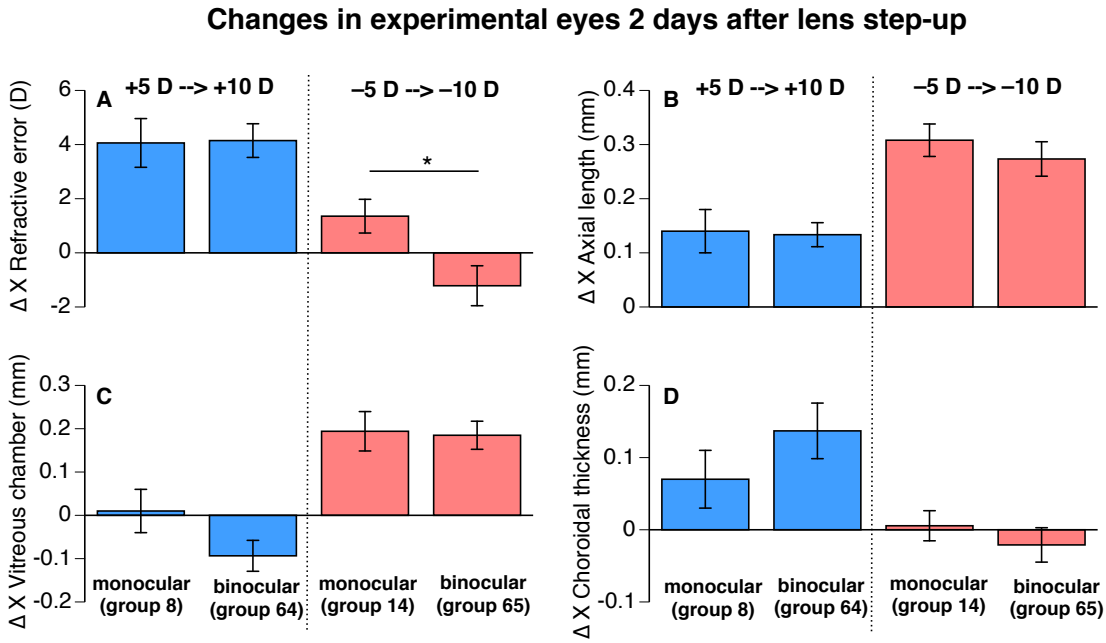

Figure 8.1. Comparison of change in experimental eyes between monocular and binocular lens step-up.
Data is shown as Mean ± SEM for (A) refractive error, (B) axial length, (C) vitreous chamber depth, and (D) choroidal thickness for positive (blue) and negative (red) lens treatment. * $p < 0.05$ (2-tailed, unpaired, *Student*'s t-test).

8.2.2 Monocular vs. binocular recovery

8.2.2.1 Recovery from Positive Lens Wear

When the change during recovery (change in the experimental eye 2 days after recovery) in the experimental eyes of the group of monocular recovery from +5 D lens wear (group 16 from Chapter 4) was compared to that in experimental eyes recovering from +5 D lens wear while the fellow eyes also experienced 5 D of hyperopic defocus (group 65 in Chapter 7), monocularly and binocularly treated eyes showed similar changes in refractive error (mean change between 14 and 16 days of age, group 16 vs. group 65, –5.63 D vs. –4.58 D, $p > 0.05$, Fig. 8.2A), axial length (+0.17 mm vs. +0.15 mm, $p > 0.05$, Fig. 8.2B), vitreous chamber depth (+0.25 mm vs. +0.26 mm, $p > 0.05$, Fig. 8.2C), and choroidal thickness (–0.21 mm vs. –0.21 mm, $p > 0.05$, Fig. 8.2D). These results suggest that binocular hyperopic defocus (group 65 in Chapter 7) did not cause the experimental eyes to recover faster than the experimental eyes that experienced monocular hyperopic defocus (group 16 in Chapter 4).

8.2.2.2 Recovery from Negative Lens Wear

When the change during recovery (change in the experimental eye 2 days after recovery) in the experimental eyes of the group of monocular recovery from –5 D lens wear (group 15 from Chapter 4) was compared with the rate of recovery in experimental eyes from binocular –5 D lens wear (group 67 in Chapter 7), monocularly and binocularly treated eyes showed similar changes in refractive error (mean change between 14 and 16 days of age, group 15 vs. group 67, +5.00 D vs. +4.93 D, $p > 0.05$, Fig. 8.2A) and axial length (+0.97 mm vs. +0.20 mm, $p > 0.05$, Fig. 8.2B). Interestingly, the monocularly treated experimental eyes showed significantly more reduction in vitreous chamber depth, possibly caused by more choroidal thickening in these eyes: Within the first 2 days of recovery (between 14 and 16 days of age), monocularly treated eyes (group 15 from Chapter 4) showed significantly more choroidal thickening and corresponding more vitreous shortening, compared with binocularly treated eyes (group 67 from Chapter 7, mean change, group 15 vs. group 67,

+0.15 mm vs. +0.07 mm for choroidal thickness, Fig. 8.2C; –0.11 mm vs. +0.02 mm for vitreous chamber depth, Fig. 8.2C; $p < 0.05$ for both). These results suggest that binocular myopic defocus (group 67 in Chapter 7) did not cause the experimental eyes to develop a larger hyperopic shift or to grow faster than the experimental eyes that experienced monocular myopic defocus (group 15 in Chapter 4), again, confirming the potency of myopic defocus in guiding eye growth.

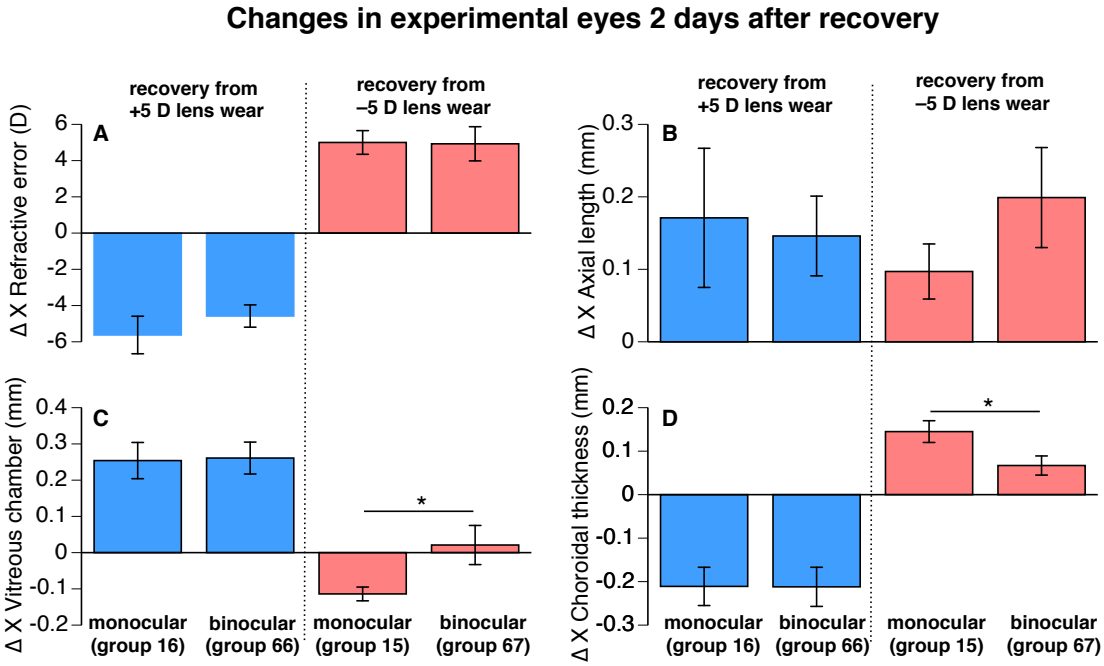

Figure. 8.2. Comparison of change in experimental eyes between monocular and binocular recovery.
Data is shown as Mean ± SEM for (A) refractive error, (B) axial length, (C) vitreous chamber depth, and (D) choroidal thickness (D) for +5 D (blue) and –5 D (red) lens treatment. * $p <$ 0.05 (2-tailed, unpaired, *Student*'s t-test).

To summarize, it seems that: (1) The "size-factor" is only effective in refraining the eye from becoming too long in the case of asymmetric defocus states between the two eyes, and in the face of a sudden increase in hyperopic defocus (see Fig. 8.3 below for a diagram), (2) symmetry in visual input in terms of the sign and magnitude of defocus between paired eyes can override the size-factor in the case of binocular negative lens wear, (3) myopic defocus dominated both the size-factor and asymmetry between paired eyes.

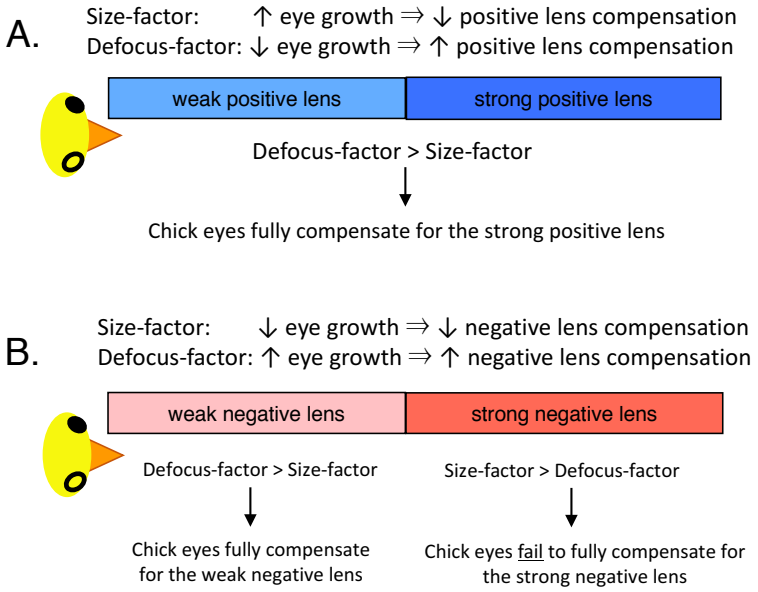

Figure 8.3. Diagrams for the interactions between the size- and defocus-factors during positive (A) and negative (B) lens step up.

8.3 Possible molecular pathway responsible for the non-visual cue(s)

The Hippo pathway has been shown to be a master regulator for size-determining purposes[246], and it is possible that it is involved in eye grow regulation. Several studies have shown that the Hippo pathway plays an important role in the eye in various species: (1) The Hippo pathway effector YAP controls frog retinal stem cell DNA replication time and genomic stability[282], (2) the Hippo pathway effector Yki downregulates Wg signaling to promote retinal differentiation in the *Drosophila* eye[186], (3) the Hippo pathway controls a switch between retinal progenitor cell proliferation and photoreceptor cell differentiation in Zebrafish[283]. The Hippo pathway has also been shown to regulate the Retinal Pigment Epithelium proliferation and differentiation. Mutation of the Hippo pathway, on the other hand, has been shown to cause abnormal eye growth. Overexpression of Yki phenocopies increases proliferation, defective apoptosis, and tissue overgrowth in *Drosophila*[284]. Further experiments are needed to further study the effect of the Hippo pathway in eye growth and its potential in reducing myopia progression:

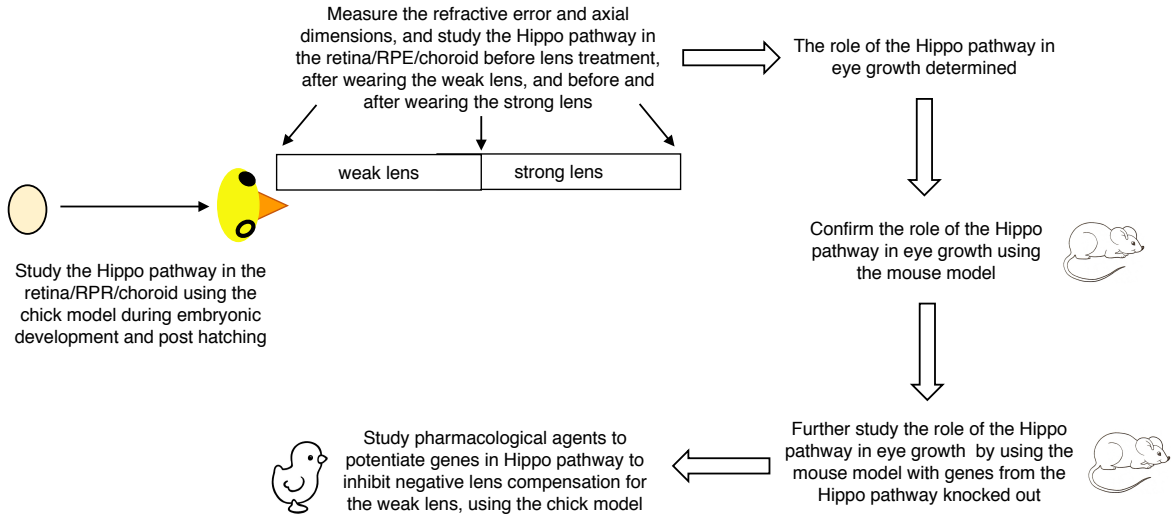

Figure 8.4. A diagram showing future experiments to further study the effect of the Hippo pathway.

8.4 Implications for human anisometropia

Anisometropia is defined as a difference in sphero-cylindrical refractive error of greater than 0.75 D between the right and left eyes, usually due to an interocular asymmetry in axial lengths[285]. If there is an intrinsic factor that is sensitive to the asymmetry in eye length or size between paired eyes and acts to prevent the eyes from deviating from their pre-programed size, it could potentially function to reduce human anisometropia.

Anisometropia is a unique refractive condition in that the paired eyes of the same individual grow to two different end points. Data from a large clinical population collected by Qin *et al.* showed that there is first a decrease in the prevalence of anisometropia during infancy and then an increase throughout childhood and in older age groups[286, 287]. Several longitudinal studies that examined the development of anisometropia during childhood all show an increase in the magnitude of anisometropia with age[287]. Studies investigating the influence of genetics in anisometropic development have yielded inconclusive results: Goldschmidt investigated the immediate families of 36 teenagers with high anisometropic myopia, and discovered that no siblings of the anisometropic probands displayed significant asymmetric refractive errors[288]. Three other studies examined the pedigree of myopia anisometropes, and found conflicting results: Ohguro *et al.* discovered an

autosomal-dominant inheritance pattern in a young male with 20 D of myopic anisometropia[289], whereas Feng *et al.* reported an autosomal-recessive inheritance pattern in a Chinese family with 5 D of myopic anisometropia[290], and Weiss suggested an x-linked recessive inheritance pattern in 3 female patients with a strong family history of myopic anisometropia[291].

The failure of the eyes to eliminate any asymmetry in both refractive error and axial length could be caused by the loss of regulation, from a wide range of causes. It could be caused by the disruption between processes promoting regulated growth (e.g., genetically programed organogenesis and growth, optically regulated growth and homeostasis, and non-optically regulated homeostasis) and processes promoting dysregulated growth (e.g., disruptive environmental intrauterine effects, and optically dysregulated growth)[292]. It would be important to further investigate the potential role of the hypothesized size-factor in anisometropic patients.

8.5 Implications for human myopia control

This thesis suggests that an intrinsic factor sensitive to recent asymmetric changes in eye length or size can prevent the eyes from further elongating to compensate for a sudden increase in hyperopic defocus. If the basis of this mechanism is non-visual, as discussed in Section 1.7.2, and the same mechanism exists in humans, it could have the potential to reduce myopic development and/or prevention in school-aged children.

The main stream of current myopia control is to utilize optical devices (e.g., bifocals, Progressive Additional Lenses, multi-zone contact lenses, and orthokeratology) to superimpose myopic defocus onto the (primarily peripheral) retina to slow myopia progression. None of these treatment options completely stops myopic progression. For those that do slow down myopic progression in the first a couple of years, the therapeutic effect slowly diminishes in the following years (a more detailed review on myopia control can be seen in Huang *et al.* (2016)[150]). One of the reasons that these optical treatments are not 100% effective might be that they do not address possible non-visual mechanisms that might also be involved in eye growth regulation.

On the other hand, if the intrinsic homeostatic mechanism does work, why do school-aged children still develop myopia? There are three possibilities: First, it is possible that this intrinsic mechanism normally remains dormant in the eye and can only be activated after a sudden change in the visual input in one eye that causes an asymmetry in visual input between the paired eyes, as shown by the monocular negative lens step-up. School-aged children develop myopia more gradually, and it is more common for myopic shifts to occur in both eyes. Therefore, the intrinsic mechanism may never be activated to exert its protective effect against myopia. Second, school-aged children might be outside of the age range during which the intrinsic mechanism can be effective. Only chicks one or two weeks after hatching were used in this thesis, which would be similar to the early emmetropization period in children during the first one of two years of life, years before they start school. Therefore, it would be important to study the potential effect of the intrinsic mechanism in older chicks whose age might be more comparable to school-aged children. Third, since usually paired eyes have similar refractive errors, the intrinsic mechanism does not exert its effect when paired eyes experience defocus of the same sign and magnitude.

Either way, if the exact genes or molecular cascades that are involved in the proposed intrinsic, homeostatic mechanisms could be identified, perhaps non-optical therapies could be developed to enhance the actions of these genes or molecular cascades to prevent myopic progression. Furthermore, one might also expect that myopia control could be optimized if the proposed potential non-optical therapy could be combined with optical treatment(s).

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Appendix 1. Supplemental Table and Figures for Chapter 3

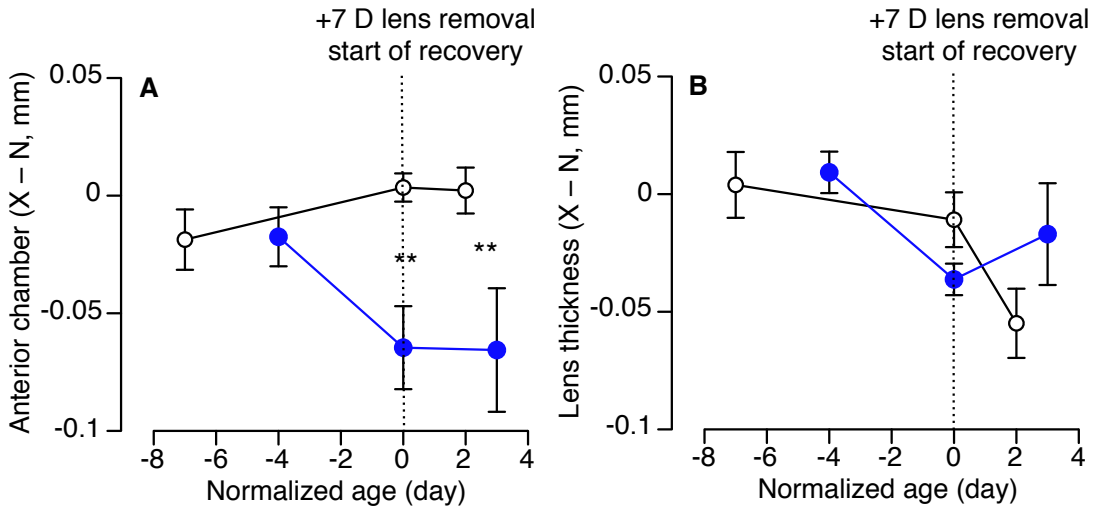

Figure. A1.1. Comparison for recovery in either light or darkness after positive lens wear.

(A) Anterior chamber depth and (B) and lens thickness (B). Data are shown as the inter-ocular difference between the experimental and fellow eyes (X – N, Mean ± SEM). Note that ages have been normalized so the day of lens removal (the start of recovery) is represented by zero on the X-axes, so days –7, 0, and 2 for group 1 in this figure correspond to days 7, 14, and 16 in Table 3.2, respectively; days –4, 0, and 3 for group 2 in this figure correspond to days 7, 11, and 14 in Table 3.2, respectively. Asterisks indicate statistical significant difference for inter-ocular difference between groups 1 and 2 on various days (** $p < 0.01$, Two-Way Mixed Measures ANOVA).

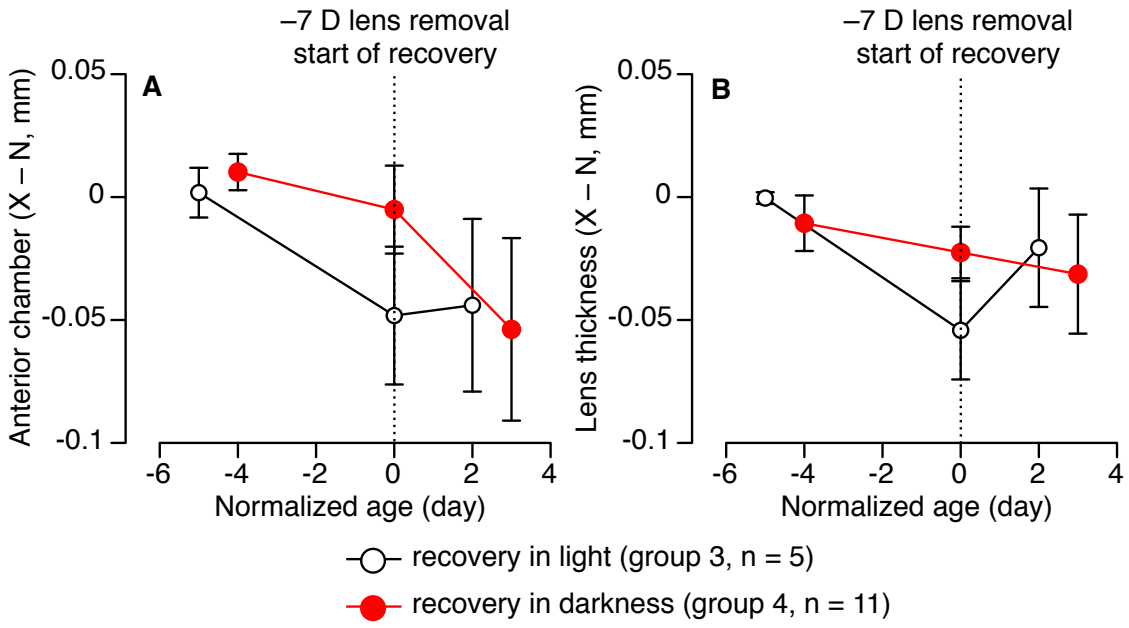

Figure. A1.2. Comparison for recovery in either light or darkness after negative lens wear.

(A) Anterior chamber depth and (B) lens thickness. Data are shown as the inter-ocular difference between the experimental and fellow eyes (X – N) at various ages. Note that ages have been normalized so the day of lens removal (the start of recovery) is represented by zero on the X-axes, so days –5, –2, 0, and 2 for group 3 in this figure correspond to days 7, 14, and 16 in Table 3.2, respectively; days –4, 0, and 3 for group 4 in this figure correspond to days 7, 11, and 14 in Table 3.2, respectively.

Table A1.1. Summary of actual values for ocular dimensions, refractive error, and sample size (n) for Chapter 3 (Mean ± SEM)

Group	n	Age	Eye	Anterior chamber depth (mm)	Lens thickness (mm)	Vitreous chamber depth (mm)	Choroidal thickness (mm)	Axial length (mm)	Refractive error (D)
1	8	7	X	1.20 ± 0.01	1.85 ± 0.03	5.15 ± 0.08	0.19 ± 0.02	8.72 ± 0.09	-0.69 ± 0.33
			N	1.22 ± 0.00	1.85 ± 0.03	5.15 ± 0.07	0.16 ± 0.01	8.69 ± 0.08	-0.23 ± 0.45
	8	14	X	1.37 ± 0.02	2.16 ± 0.02	5.02 ± 0.08	0.28 ± 0.02	9.18 ± 0.10	6.57 ± 0.41
			N	1.36 ± 0.02	2.17 ± 0.02	5.31 ± 0.07	0.20 ± 0.01	9.37 ± 0.07	0.23 ± 0.45
	8	16	X	1.42 ± 0.01	2.24 ± 0.02	5.38 ± 0.05	0.21 ± 0.01	9.59 ± 0.07	0.52 ± 0.41
			N	1.42 ± 0.01	2.30 ± 0.01	5.43 ± 0.05	0.20 ± 0.00	9.68 ± 0.06	0.25 ± 0.36
2	8	7	X	1.28 ± 0.02	1.88 ± 0.02	5.05 ± 0.03	0.20 ± 0.02	8.74 ± 0.05	-0.52 ± 0.69
			N	1.30 ± 0.02	1.87 ± 0.01	5.05 ± 0.04	0.18 ± 0.02	8.73 ± 0.04	-1.41 ± 0.32
	8	11	X	1.25 ± 0.03	1.94 ± 0.02	4.91 ± 0.04	0.29 ± 0.01	8.72 ± 0.07	5.75 ± 0.85
			N	1.32 ± 0.02	1.98 ± 0.02	5.14 ± 0.05	0.16 ± 0.01	8.92 ± 0.06	-1.33 ± 0.33
	8	14	X	1.37 ± 0.04	2.08 ± 0.02	5.21 ± 0.04	0.18 ± 0.00	9.17 ± 0.08	1.22 ± 1.02
			N	1.43 ± 0.02	2.10 ± 0.02	5.29 ± 0.06	0.13 ± 0.00	9.29 ± 0.06	-2.68 ± 0.53
3	5	1	X	1.22 ± 0.03	1.60 ± 0.01	5.18 ± 0.10	0.23 ± 0.03	8.55 ± 0.11	-2.06 ± 0.89
			N	1.22 ± 0.03	1.60 ± 0.01	5.10 ± 0.07	0.25 ± 0.03	8.49 ± 0.08	-2.20 ± 1.01
	5	6	X	1.20 ± 0.04	1.86 ± 0.02	5.43 ± 0.06	0.14 ± 0.01	8.95 ± 0.10	-5.78 ± 0.79
			N	1.24 ± 0.02	1.91 ± 0.02	5.01 ± 0.05	0.20 ± 0.01	8.69 ± 0.06	-1.16 ± 0.47
	5	8	X	1.27 ± 0.05	1.94 ± 0.02	5.20 ± 0.07	0.35 ± 0.02	9.08 ± 0.12	-0.06 ± 0.28
			N	1.31 ± 0.02	1.96 ± 0.01	5.04 ± 0.06	0.23 ± 0.04	8.87 ± 0.09	-1.37 ± 0.48
4	11	7	X	1.32 ± 0.02	1.92 ± 0.02	5.01 ± 0.06	0.22 ± 0.03	8.82 ± 0.04	-0.69 ± 0.42
			N	1.31 ± 0.01	1.93 ± 0.02	5.03 ± 0.05	0.21 ± 0.02	8.82 ± 0.04	-0.19 ± 0.23
	11	11	X	1.34 ± 0.02	2.02 ± 0.02	5.28 ± 0.07	0.17 ± 0.02	9.17 ± 0.07	-5.22 ± 0.46
			N	1.35 ± 0.01	2.04 ± 0.02	5.09 ± 0.07	0.23 ± 0.03	9.05 ± 0.06	-0.52 ± 0.33
	11	14	X	1.40 ± 0.03	2.13 ± 0.02	5.36 ± 0.07	0.18 ± 0.00	9.42 ± 0.07	-2.92 ± 0.53
			N	1.46 ± 0.02	2.17 ± 0.02	5.23 ± 0.06	0.19 ± 0.02	9.40 ± 0.05	-1.11 ± 0.46

Appendix 2. Supplemental Table and Figures for Chapter 4

Table A2.1. Summary of actual values for ocular dimensions, refractive error, and sample size (n) for Chapter 4 (Mean ± SEM)

Group	n	Age	Eye	Anterior chamber depth (mm)	Lens thickness (mm)	Vitreous chamber depth (mm)	Choroidal thickness (mm)	Axial length (mm)	Refractive error (D)
5	9	6	X	1.29 ± 0.02	1.88 ± 0.02	5.05 ± 0.04	0.16 ± 0.01	8.72 ± 0.06	0.64 ± 0.43
			N	1.28 ± 0.02	1.89 ± 0.02	5.05 ± 0.04	0.16 ± 0.01	8.71 ± 0.06	0.14 ± 0.39
	9	7	X	1.30 ± 0.03	1.91 ± 0.02	4.86 ± 0.04	0.27 ± 0.04	8.68 ± 0.07	5.83 ± 0.92
			N	1.28 ± 0.01	1.95 ± 0.01	5.02 ± 0.04	0.13 ± 0.01	8.71 ± 0.05	0.42 ± 0.44
	9	9	X	1.30 ± 0.02	1.95 ± 0.02	4.59 ± 0.04	0.44 ± 0.05	8.63 ± 0.07	14.19 ± 1.36
			N	1.31 ± 0.02	2.04 ± 0.02	5.05 ± 0.05	0.19 ± 0.01	8.92 ± 0.07	0.34 ± 0.37
	9	11	X	1.30 ± 0.03	2.02 ± 0.02	4.61 ± 0.06	0.43 ± 0.03	8.70 ± 0.08	16.92 ± 1.07
			N	1.34 ± 0.02	2.11 ± 0.02	5.11 ± 0.07	0.22 ± 0.01	9.11 ± 0.09	0.03 ± 0.15
6	4	1	X	1.26 ± 0.02	1.58 ± 0.02	5.24 ± 0.07	0.20 ± 0.04	8.60 ± 0.05	-1.74 ± 0.83
			N	1.26 ± 0.01	1.58 ± 0.02	5.25 ± 0.05	0.18 ± 0.05	8.60 ± 0.02	-1.24 ± 1.00
	4	4	X	1.21 ± 0.01	1.76 ± 0.02	4.97 ± 0.07	0.21 ± 0.02	8.48 ± 0.05	6.76 ± 0.56
			N	1.26 ± 0.01	1.78 ± 0.01	5.20 ± 0.05	0.16 ± 0.01	8.74 ± 0.06	-1.34 ± 1.14
	4	6	X	1.22 ± 0.02	1.84 ± 0.02	4.96 ± 0.06	0.17 ± 0.01	8.51 ± 0.06	7.62 ± 0.41
			N	1.29 ± 0.01	1.88 ± 0.02	5.15 ± 0.07	0.18 ± 0.02	8.83 ± 0.07	-1.23 ± 0.53
	4	8	X	1.22 ± 0.01	1.90 ± 0.02	4.76 ± 0.07	0.29 ± 0.03	8.50 ± 0.06	15.85 ± 1.25
			N	1.33 ± 0.01	1.97 ± 0.03	5.19 ± 0.05	0.18 ± 0.01	8.99 ± 0.08	-1.00 ± 0.14
	4	11	X	1.23 ± 0.02	2.04 ± 0.02	4.80 ± 0.05	0.27 ± 0.04	8.68 ± 0.08	14.71 ± 0.99
			N	1.39 ± 0.00	2.07 ± 0.02	5.23 ± 0.06	0.21 ± 0.01	9.23 ± 0.05	-1.45 ± 0.53
	4	13	X	1.23 ± 0.03	2.06 ± 0.01	4.97 ± 0.07	0.24 ± 0.01	8.84 ± 0.07	15.77 ± 0.55
			N	1.41 ± 0.01	2.09 ± 0.02	5.38 ± 0.07	0.19 ± 0.01	9.41 ± 0.08	-0.37 ± 0.36
	4	17	X	1.15 ± 0.03	2.19 ± 0.02	5.13 ± 0.04	0.20 ± 0.02	9.02 ± 0.05	18.38 ± 0.60
			N	1.37 ± 0.01	2.23 ± 0.03	5.68 ± 0.07	0.20 ± 0.00	9.83 ± 0.07	-0.45 ± 0.18
7	6	7	X	1.28 ± 0.02	1.91 ± 0.02	5.09 ± 0.07	0.17 ± 0.02	8.79 ± 0.10	0.19 ± 0.71
			N	1.28 ± 0.02	1.92 ± 0.03	5.08 ± 0.08	0.19 ± 0.01	8.79 ± 0.10	-0.97 ± 0.61
	6	11	X	1.31 ± 0.02	2.00 ± 0.01	4.81 ± 0.09	0.36 ± 0.03	8.82 ± 0.12	9.37 ± 1.34
			N	1.34 ± 0.02	2.04 ± 0.01	5.18 ± 0.09	0.18 ± 0.01	9.07 ± 0.12	0.32 ± 0.71
	6	13	X	1.33 ± 0.01	2.08 ± 0.01	4.95 ± 0.09	0.38 ± 0.02	9.08 ± 0.10	10.27 ± 0.95
			N	1.37 ± 0.02	2.09 ± 0.02	5.33 ± 0.09	0.20 ± 0.01	9.32 ± 0.11	0.73 ± 0.41
	6	15	X	1.38 ± 0.02	2.13 ± 0.02	5.09 ± 0.08	0.37 ± 0.01	9.32 ± 0.09	10.68 ± 0.49
			N	1.40 ± 0.02	2.13 ± 0.03	5.47 ± 0.08	0.21 ± 0.01	9.57 ± 0.13	0.13 ± 0.23
	6	18	X	1.41 ± 0.03	2.19 ± 0.03	5.21 ± 0.06	0.33 ± 0.02	9.49 ± 0.08	11.15 ± 0.30
			N	1.44 ± 0.03	2.20 ± 0.04	5.59 ± 0.08	0.21 ± 0.00	9.79 ± 0.11	0.14 ± 0.27
8	6	7	X	1.27 ± 0.01	1.98 ± 0.02	4.98 ± 0.03	0.27 ± 0.02	8.84 ± 0.04	-0.01 ± 0.42
			N	1.27 ± 0.01	1.97 ± 0.02	5.01 ± 0.04	0.26 ± 0.03	8.85 ± 0.07	-0.32 ± 0.30
	6	11	X	1.37 ± 0.01	2.08 ± 0.01	4.90 ± 0.03	0.34 ± 0.02	9.02 ± 0.03	5.32 ± 0.69
			N	1.37 ± 0.01	2.11 ± 0.01	5.13 ± 0.06	0.26 ± 0.02	9.20 ± 0.06	-0.09 ± 0.23
	6	13	X	1.39 ± 0.01	2.12 ± 0.02	4.90 ± 0.05	0.41 ± 0.04	9.16 ± 0.04	9.37 ± 0.60
			N	1.40 ± 0.01	2.14 ± 0.01	5.28 ± 0.06	0.23 ± 0.01	9.40 ± 0.06	0.26 ± 0.18
	6	15	X	1.40 ± 0.01	2.14 ± 0.02	4.94 ± 0.06	0.49 ± 0.03	9.31 ± 0.07	8.65 ± 0.67
			N	1.40 ± 0.01	2.18 ± 0.01	5.41 ± 0.05	0.24 ± 0.01	9.57 ± 0.06	0.34 ± 0.34
	6	18	X	1.42 ± 0.01	2.20 ± 0.01	5.03 ± 0.08	0.44 ± 0.04	9.43 ± 0.08	10.47 ± 0.58
			N	1.42 ± 0.01	2.23 ± 0.01	5.51 ± 0.06	0.25 ± 0.00	9.75 ± 0.07	0.44 ± 0.32

Table A2.1 Cont.

Group	n	Age	Eye	Anterior chamber depth (mm)	Lens thickness (mm)	Vitreous chamber depth (mm)	Choroidal thickness (mm)	Axial length (mm)	Refractive error (D)
9	10	7	X	1.28 ± 0.01	1.88 ± 0.01	5.11 ± 0.04	0.21 ± 0.02	8.81 ± 0.05	-0.20 ± 0.39
			N	1.28 ± 0.01	1.88 ± 0.01	5.14 ± 0.04	0.20 ± 0.01	8.82 ± 0.04	-0.50 ± 0.33
	5	9	X	1.31 ± 0.02	1.94 ± 0.03	5.18 ± 0.09	0.12 ± 0.01	8.88 ± 0.10	-3.92 ± 1.10
			N	1.31 ± 0.01	1.99 ± 0.02	4.96 ± 0.07	0.22 ± 0.02	8.82 ± 0.07	0.35 ± 0.28
	10	11	X	1.32 ± 0.02	2.01 ± 0.01	5.42 ± 0.05	0.16 ± 0.01	9.25 ± 0.06	-5.72 ± 0.66
			N	1.36 ± 0.02	2.04 ± 0.01	5.08 ± 0.04	0.23 ± 0.01	9.05 ± 0.04	0.94 ± 0.17
	8	14	X	1.34 ± 0.02	2.13 ± 0.01	5.71 ± 0.05	0.12 ± 0.00	9.63 ± 0.05	-9.12 ± 0.47
			N	1.42 ± 0.01	2.13 ± 0.01	5.23 ± 0.04	0.21 ± 0.02	9.31 ± 0.05	0.61 ± 0.18
	8	16	X	1.40 ± 0.02	2.21 ± 0.01	5.93 ± 0.07	0.17 ± 0.01	10.03 ± 0.08	-12.09 ± 0.36
			N	1.45 ± 0.01	2.19 ± 0.02	5.28 ± 0.04	0.20 ± 0.01	9.46 ± 0.05	0.52 ± 0.08
	8	18	X	1.42 ± 0.03	2.25 ± 0.03	6.19 ± 0.08	0.15 ± 0.01	10.35 ± 0.09	-14.02 ± 0.85
			N	1.48 ± 0.01	2.25 ± 0.01	5.39 ± 0.05	0.24 ± 0.02	9.69 ± 0.05	0.38 ± 0.07
10	11	7	X	1.28 ± 0.01	1.88 ± 0.01	5.06 ± 0.04	0.19 ± 0.02	8.73 ± 0.05	-0.63 ± 0.30
			N	1.28 ± 0.01	1.88 ± 0.01	5.05 ± 0.04	0.21 ± 0.02	8.74 ± 0.05	-0.16 ± 0.39
	6	9	X	1.30 ± 0.02	1.93 ± 0.02	5.19 ± 0.06	0.12 ± 0.01	8.88 ± 0.06	-3.94 ± 0.83
			N	1.33 ± 0.01	1.98 ± 0.01	4.98 ± 0.06	0.18 ± 0.02	8.80 ± 0.07	0.07 ± 0.33
	11	11	X	1.30 ± 0.01	2.00 ± 0.01	5.36 ± 0.04	0.15 ± 0.01	9.14 ± 0.05	-4.58 ± 0.24
			N	1.35 ± 0.01	2.04 ± 0.01	5.03 ± 0.04	0.20 ± 0.02	8.95 ± 0.05	0.52 ± 0.32
	11	14	X	1.29 ± 0.02	2.13 ± 0.01	5.52 ± 0.05	0.15 ± 0.02	9.41 ± 0.06	-7.21 ± 0.61
			N	1.39 ± 0.01	2.14 ± 0.01	5.11 ± 0.03	0.19 ± 0.01	9.16 ± 0.04	0.48 ± 0.08
	11	16	X	1.31 ± 0.02	2.18 ± 0.01	5.61 ± 0.06	0.18 ± 0.02	9.61 ± 0.07	-4.66 ± 0.91
			N	1.41 ± 0.01	2.20 ± 0.01	5.21 ± 0.04	0.19 ± 0.01	9.35 ± 0.06	0.66 ± 0.06
	11	18	X	1.37 ± 0.03	2.25 ± 0.01	5.71 ± 0.08	0.27 ± 0.03	9.93 ± 0.08	-3.97 ± 0.80
			N	1.45 ± 0.02	2.26 ± 0.01	5.29 ± 0.03	0.22 ± 0.01	9.56 ± 0.05	0.51 ± 0.12
11	4	13	X	1.31 ± 0.06	2.17 ± 0.02	5.29 ± 0.11	0.20 ± 0.02	9.29 ± 0.11	-7.69 ± 1.32
			N	1.38 ± 0.02	2.17 ± 0.02	4.99 ± 0.06	0.22 ± 0.01	9.09 ± 0.05	-0.65 ± 0.53
	4	14	X	1.31 ± 0.07	2.18 ± 0.02	5.36 ± 0.10	0.21 ± 0.02	9.37 ± 0.11	-6.81 ± 0.28
			N	1.40 ± 0.02	2.20 ± 0.03	5.03 ± 0.08	0.20 ± 0.02	9.16 ± 0.06	-0.07 ± 0.22
	4	16	X	1.35 ± 0.06	2.23 ± 0.03	5.40 ± 0.16	0.24 ± 0.05	9.55 ± 0.12	-3.34 ± 1.46
			N	1.44 ± 0.01	2.25 ± 0.02	5.11 ± 0.09	0.20 ± 0.02	9.34 ± 0.08	-0.25 ± 0.36
	4	19	X	1.39 ± 0.07	2.31 ± 0.02	5.58 ± 0.14	0.22 ± 0.05	9.82 ± 0.16	-3.28 ± 1.76
			N	1.49 ± 0.01	2.34 ± 0.03	5.25 ± 0.14	0.21 ± 0.02	9.62 ± 0.14	0.17 ± 0.32
12	10	7	X	1.19 ± 0.02	1.81 ± 0.01	5.35 ± 0.05	0.15 ± 0.01	8.80 ± 0.06	-3.26 ± 1.19
			N	1.23 ± 0.02	1.83 ± 0.02	5.10 ± 0.05	0.18 ± 0.01	8.66 ± 0.06	0.89 ± 0.60
	10	9	X	1.17 ± 0.03	1.90 ± 0.01	5.46 ± 0.06	0.12 ± 0.01	8.97 ± 0.08	-5.74 ± 1.05
			N	1.22 ± 0.02	1.90 ± 0.02	5.10 ± 0.05	0.18 ± 0.01	8.73 ± 0.05	-0.02 ± 0.28
	10	11	X	1.13 ± 0.02	2.00 ± 0.01	5.47 ± 0.07	0.15 ± 0.01	9.07 ± 0.08	-4.90 ± 1.15
			N	1.20 ± 0.03	2.02 ± 0.02	5.10 ± 0.06	0.18 ± 0.01	8.82 ± 0.07	0.59 ± 0.42

Table A2.1 Cont.

Group	n	Age	Eye	Anterior chamber depth (mm)	Lens thickness (mm)	Vitreous chamber depth (mm)	Choroidal thickness (mm)	Axial length (mm)	Refractive error (D)
	10	7	X	1.28 ± 0.02	1.87 ± 0.02	5.02 ± 0.05	0.20 ± 0.02	8.68 ± 0.05	-0.12 ± 0.40
			N	1.29 ± 0.01	1.86 ± 0.02	5.02 ± 0.03	0.18 ± 0.01	8.65 ± 0.04	-0.65 ± 0.29
	10	11	X	1.30 ± 0.02	2.06 ± 0.02	5.21 ± 0.06	0.14 ± 0.01	9.03 ± 0.09	-4.13 ± 0.27
			N	1.34 ± 0.02	2.06 ± 0.01	4.99 ± 0.06	0.19 ± 0.01	8.90 ± 0.08	-0.06 ± 0.17
13	10	14	X	1.37 ± 0.03	2.14 ± 0.01	5.45 ± 0.08	0.17 ± 0.01	9.44 ± 0.10	-7.33 ± 0.34
			N	1.43 ± 0.01	2.13 ± 0.01	5.10 ± 0.05	0.22 ± 0.01	9.21 ± 0.07	0.17 ± 0.21
	10	16	X	1.39 ± 0.04	2.23 ± 0.02	5.59 ± 0.08	0.18 ± 0.02	9.70 ± 0.10	-9.44 ± 0.43
			N	1.45 ± 0.01	2.21 ± 0.02	5.17 ± 0.05	0.22 ± 0.02	9.36 ± 0.07	0.16 ± 0.14
	10	18	X	1.43 ± 0.04	2.27 ± 0.01	5.74 ± 0.09	0.20 ± 0.01	9.94 ± 0.10	-8.79 ± 0.40
			N	1.49 ± 0.01	2.24 ± 0.01	5.25 ± 0.06	0.22 ± 0.01	9.53 ± 0.08	0.42 ± 0.09
	9	7	X	1.24 ± 0.02	1.89 ± 0.02	5.04 ± 0.04	0.17 ± 0.01	8.66 ± 0.05	-0.39 ± 0.43
			N	1.24 ± 0.02	1.90 ± 0.02	5.05 ± 0.03	0.19 ± 0.02	8.70 ± 0.03	-0.26 ± 0.41
	9	11	X	1.24 ± 0.03	2.04 ± 0.02	5.12 ± 0.07	0.18 ± 0.01	8.90 ± 0.08	-4.22 ± 0.37
			N	1.28 ± 0.02	2.08 ± 0.02	4.98 ± 0.05	0.20 ± 0.02	8.86 ± 0.06	-0.10 ± 0.18
14	9	14	X	1.28 ± 0.04	2.15 ± 0.01	5.31 ± 0.07	0.17 ± 0.01	9.24 ± 0.10	-7.19 ± 0.35
			N	1.34 ± 0.02	2.17 ± 0.02	5.09 ± 0.07	0.25 ± 0.02	9.18 ± 0.06	0.13 ± 0.24
	9	16	X	1.31 ± 0.04	2.23 ± 0.02	5.50 ± 0.07	0.17 ± 0.01	9.55 ± 0.10	-5.84 ± 0.49
			N	1.41 ± 0.03	2.20 ± 0.02	5.18 ± 0.07	0.25 ± 0.02	9.37 ± 0.06	-0.18 ± 0.29
	9	18	X	1.32 ± 0.05	2.32 ± 0.02	5.66 ± 0.06	0.19 ± 0.01	9.81 ± 0.10	-4.85 ± 0.54
			N	1.44 ± 0.03	2.28 ± 0.03	5.27 ± 0.06	0.27 ± 0.01	9.59 ± 0.08	0.29 ± 0.30
	7	7	X	1.26 ± 0.01	1.88 ± 0.02	5.03 ± 0.04	0.18 ± 0.01	8.68 ± 0.04	-0.32 ± 0.29
			N	1.25 ± 0.01	1.89 ± 0.02	5.04 ± 0.04	0.19 ± 0.01	8.70 ± 0.04	-0.87 ± 0.37
	7	11	X	1.34 ± 0.02	2.12 ± 0.02	5.28 ± 0.05	0.14 ± 0.01	9.22 ± 0.06	-4.88 ± 0.50
			N	1.33 ± 0.02	2.13 ± 0.02	5.19 ± 0.02	0.24 ± 0.02	9.23 ± 0.05	-0.42 ± 0.31
15	7	13	X	1.38 ± 0.01	2.14 ± 0.03	5.17 ± 0.05	0.29 ± 0.02	9.32 ± 0.07	0.12 ± 0.21
			N	1.37 ± 0.01	2.14 ± 0.02	5.28 ± 0.02	0.23 ± 0.01	9.37 ± 0.04	0.39 ± 0.28
	7	15	X	1.41 ± 0.00	2.24 ± 0.03	5.29 ± 0.11	0.27 ± 0.01	9.55 ± 0.11	0.46 ± 0.22
			N	1.41 ± 0.00	2.18 ± 0.02	5.29 ± 0.03	0.22 ± 0.01	9.45 ± 0.04	-0.10 ± 0.19
	7	18	X	1.42 ± 0.00	2.24 ± 0.02	5.44 ± 0.10	0.24 ± 0.00	9.69 ± 0.11	0.16 ± 0.34
			N	1.42 ± 0.01	2.22 ± 0.02	5.40 ± 0.03	0.22 ± 0.00	9.61 ± 0.03	-0.16 ± 0.22
	6	7	X	1.28 ± 0.03	1.91 ± 0.02	5.05 ± 0.08	0.22 ± 0.04	8.78 ± 0.10	-0.06 ± 0.43
			N	1.28 ± 0.02	1.91 ± 0.02	5.04 ± 0.06	0.23 ± 0.02	8.77 ± 0.08	-0.75 ± 0.45
	6	14	X	1.40 ± 0.03	2.09 ± 0.03	4.97 ± 0.06	0.39 ± 0.05	9.19 ± 0.12	6.20 ± 0.43
			N	1.44 ± 0.02	2.13 ± 0.02	5.23 ± 0.04	0.21 ± 0.01	9.33 ± 0.07	-0.11 ± 0.25
16	6	16	X	1.40 ± 0.04	2.20 ± 0.03	5.23 ± 0.05	0.18 ± 0.02	9.36 ± 0.05	0.58 ± 0.61
			N	1.46 ± 0.03	2.17 ± 0.02	5.28 ± 0.05	0.18 ± 0.01	9.41 ± 0.07	0.70 ± 0.18
	6	18	X	1.45 ± 0.04	2.29 ± 0.02	5.47 ± 0.08	0.28 ± 0.02	9.80 ± 0.12	0.16 ± 0.09
			N	1.50 ± 0.05	2.24 ± 0.02	5.46 ± 0.06	0.20 ± 0.02	9.72 ± 0.11	-0.23 ± 0.31

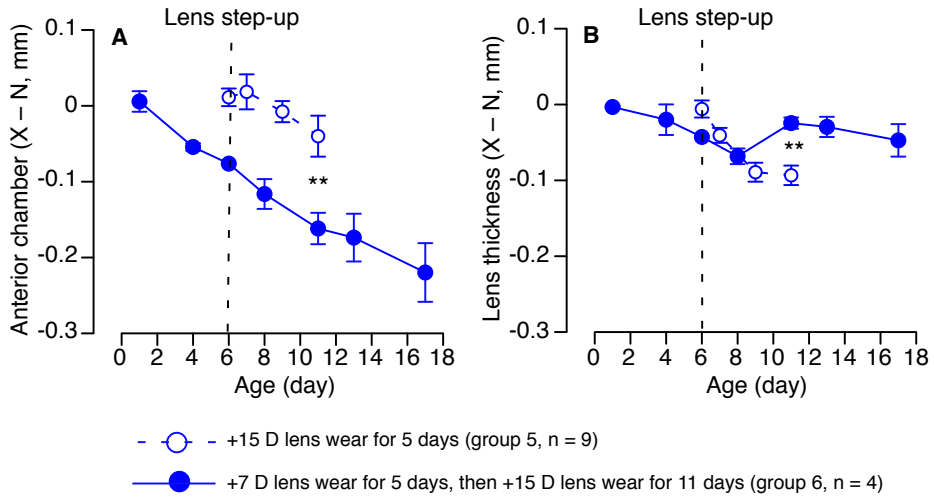

Figure. A2.1. Time course of compensation for +15 D lenses and for first +7 D then +15 D lenses.

Chicks in the control group wore +15 D lenses from the beginning. Data is shown as the inter-ocular difference (X-N, Mean ± SEM) for anterior chamber depth (A) and lens thickness (B). There was no difference between the inter-ocular difference between these two groups at the beginning of the treatment (day 6 for group 5 and day 1 for group 6, $p > 0.05$ for all these parameters, Two-Way Mixed Measures ANOVA). Comparison of the inter-ocular difference between these two groups yielded a significant difference for both anterior chamber and lens thickness on day 11 (**: $p < 0.01$).

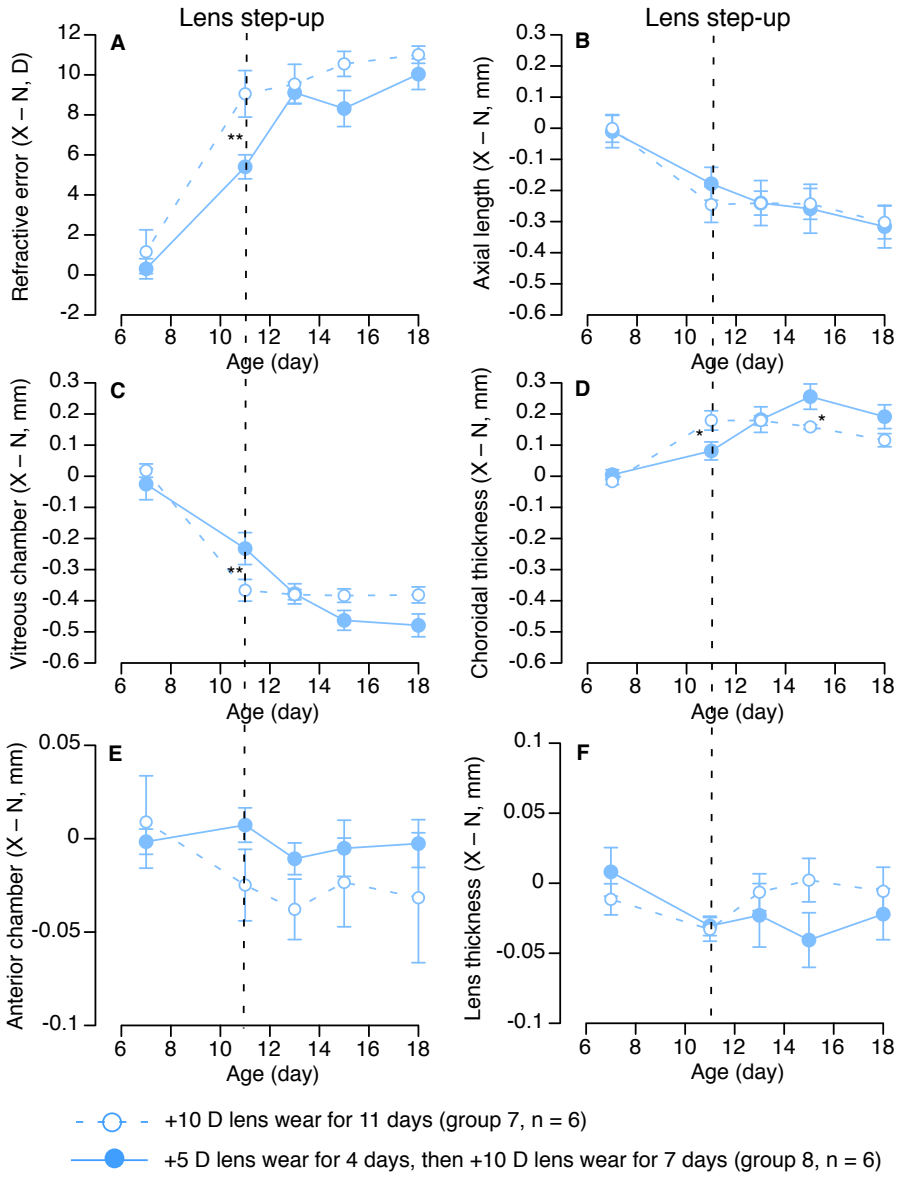

Figure A2.2. Time course of compensation for +10 D lenses and for first +5 D then +10 D lenses.

Data is shown as the inter-ocular difference (X-N, Mean ± SEM) for refractive error (A), axial length (B), vitreous chamber depth (C), choroidal thickness (D), anterior chamber depth (E), and lens thickness (F). There was no difference between the inter-ocular difference between these two groups at the beginning of the treatment. Asterisks indicate significant difference in the inter-ocular difference between these two groups on various days (*: $p < 0.05$, **: $p < 0.01$, Two-Way Mixed Measures ANOVA).

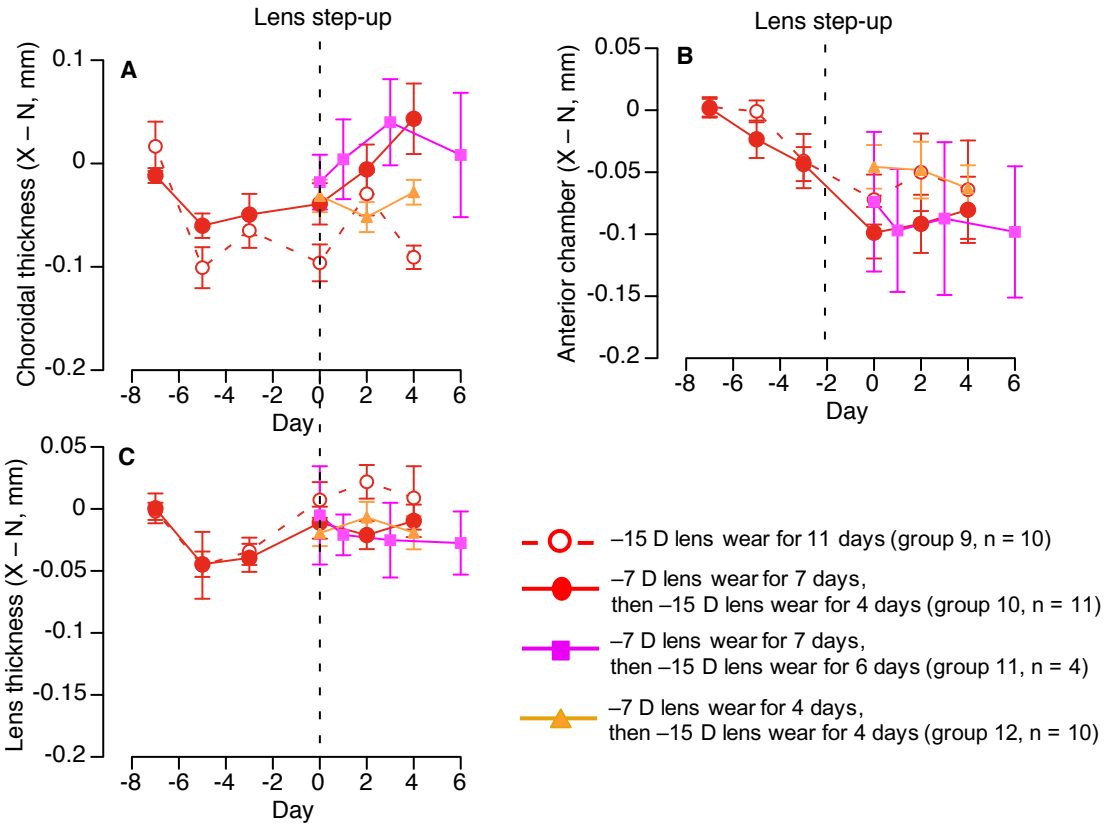

Figure A2.3. Time course of compensation for –15 D lenses and for first –7 D then –15 D lenses.

Data is shown as the inter-ocular difference (X-N, Mean ± SEM) for (A) choroidal thickness, (B) anterior chamber depth, and (C) lens thickness.

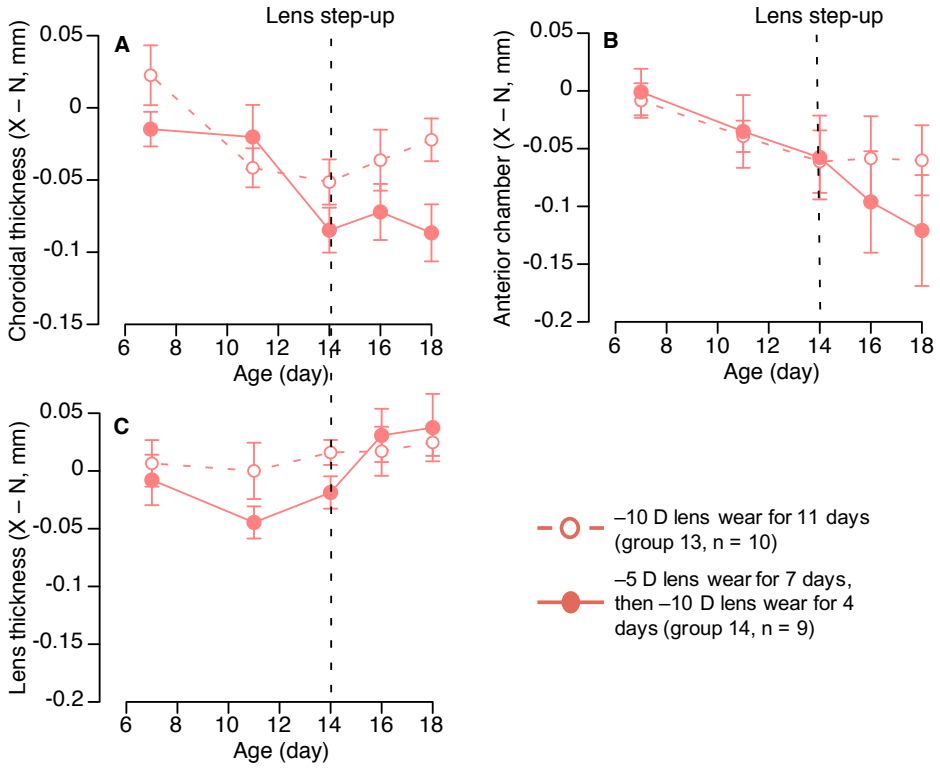

Figure A2.4. Time course of compensation for –10 D lenses and for first –5 D then –10 D lenses.

Data is shown as the inter-ocular difference (X-N, Mean ± SEM) for choroidal thickness (A), anterior chamber depth (B), and lens thickness (C).

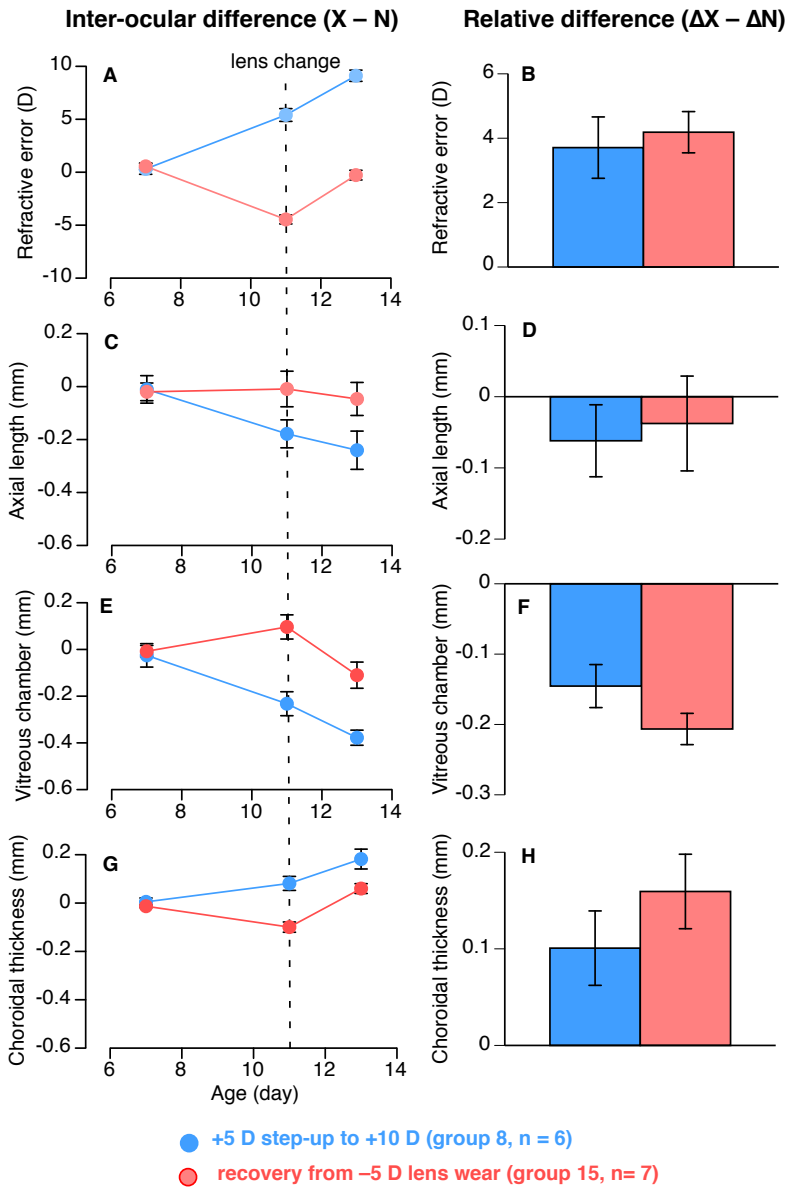

Figure A2.5. Comparisons between positive lens step-up and recovery from negative lens wear.

Line scatter plots show the inter-ocular difference (X – N) for the time course (up to 2 days after the step-up or recovery), and bar charts show the relative change (ΔX–ΔN) within the first 2 days after the step- up or recovery, in refractive error (Figs. A and B), axial length (Figs. C and D), vitreous chamber depth (Figs. E and F), and choroidal thickness (Figs. G and H). Asterisks show the level of statistical significance for comparisons between the step-up group and recovery group for the relative change (**: $p < 0.01$, 2-tailed, unpaired, *Student's* t-test).

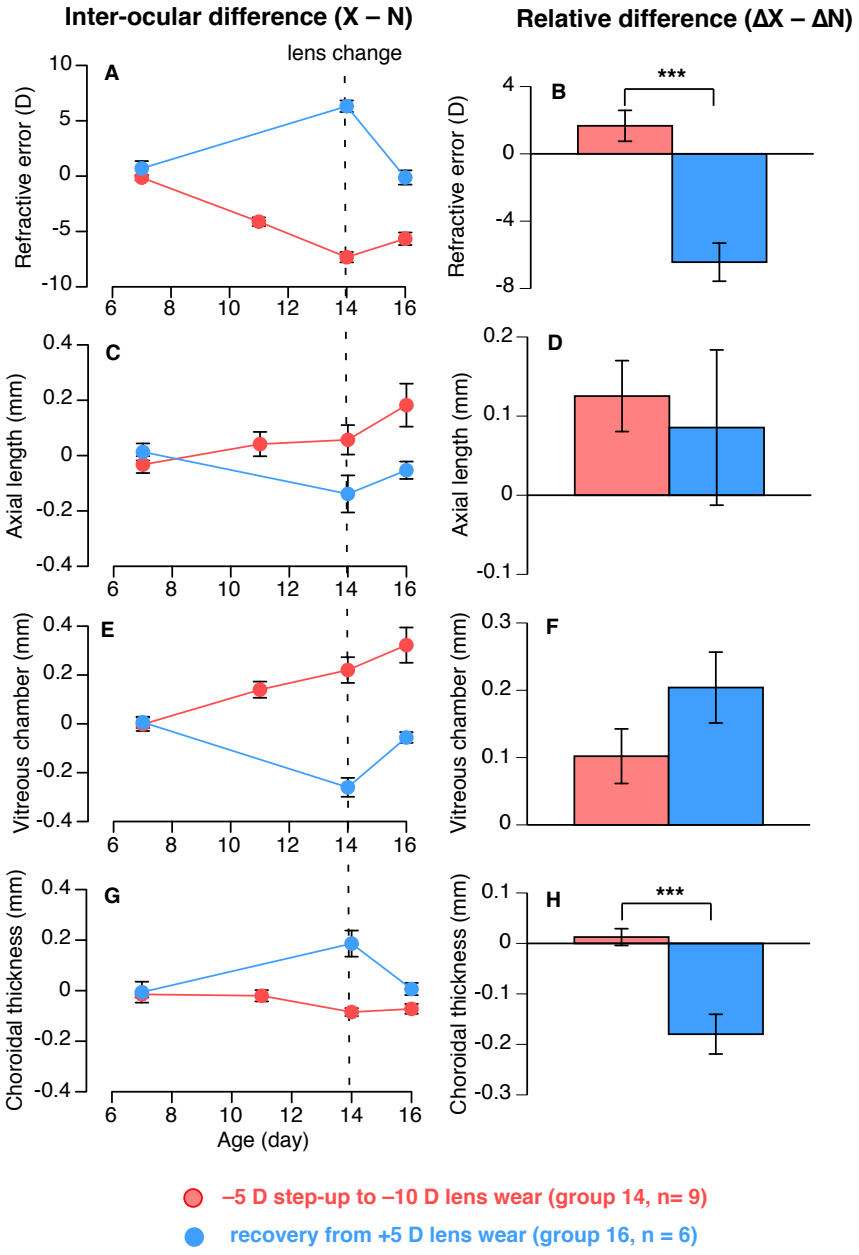

Figure A2.6. Comparisons between negative lens step-up and recovery from positive lens wear

Line scatter plots show the inter-ocular difference (X – N) for the time course (up to 2 days after the step-up or recovery), and bar charts show the relative change (ΔX–ΔN) within the first 2 days after the step- up or recovery, in refractive error (Figs. A and B), axial length (Figs. C and D), vitreous chamber depth (Figs. E and F), and choroidal thickness (Figs. G and H). Asterisks show the level of statistical significance for comparisons between the step-up group and recovery group for the relative change (***: $p < 0.001$, 2-tailed, unpaired, *Student's* t-test).

Appendix 3. Supplemental Figures for Chapter 7

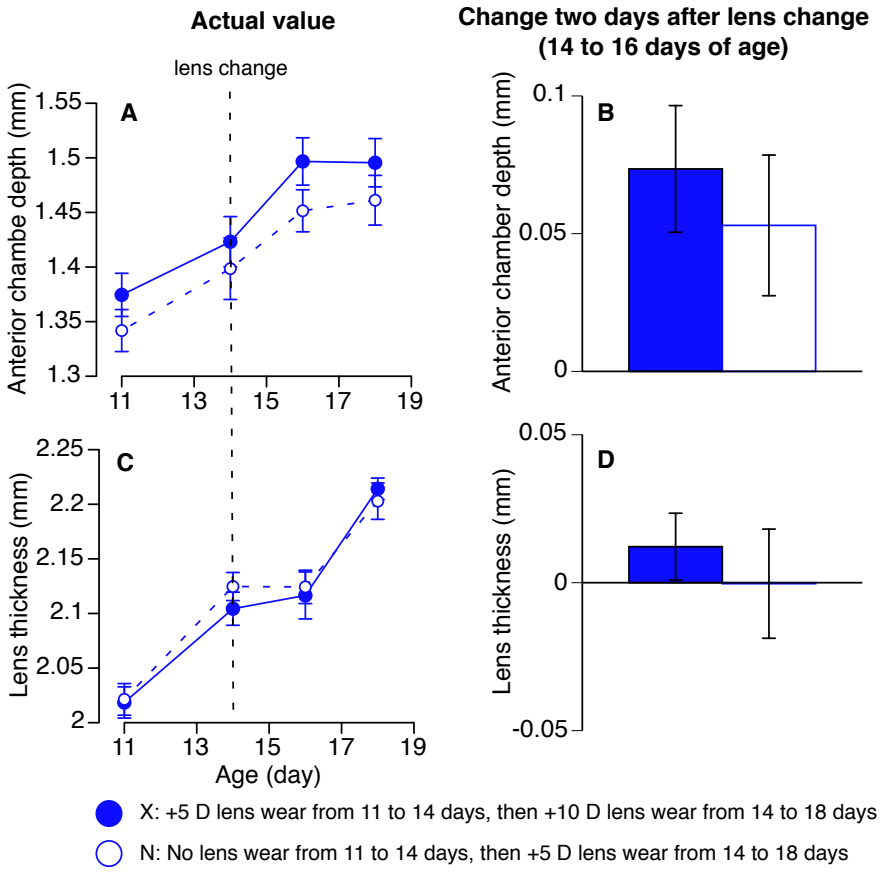

Figure A3.1. Time course of binocular positive lens treatment.
Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 2 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

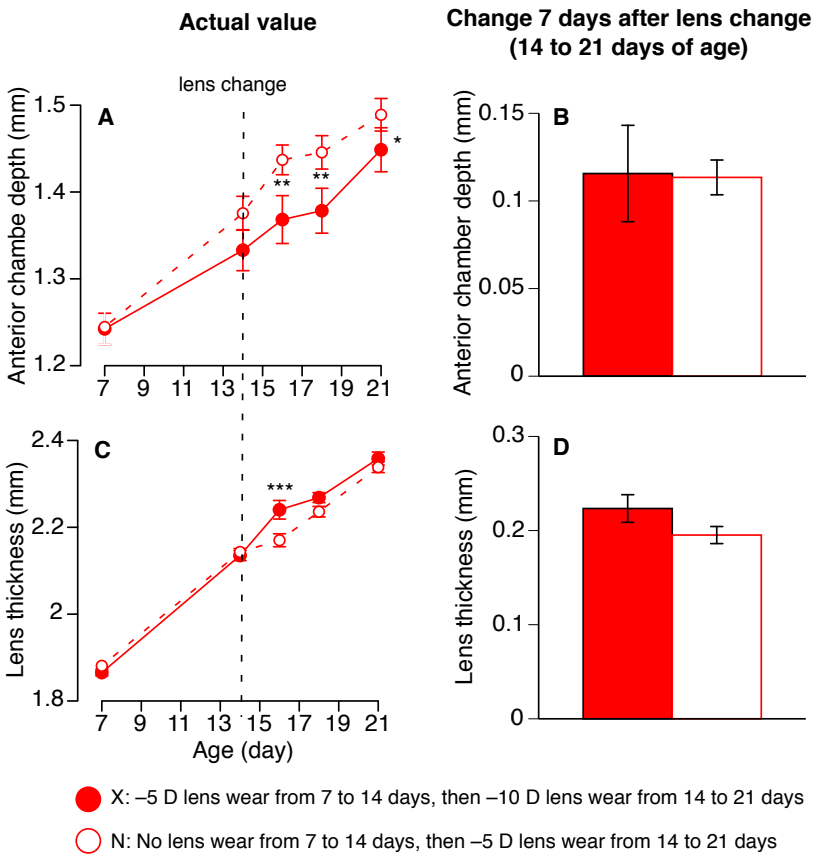

Figure A3.2. Time course of binocular negative lens treatment.
Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 7 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

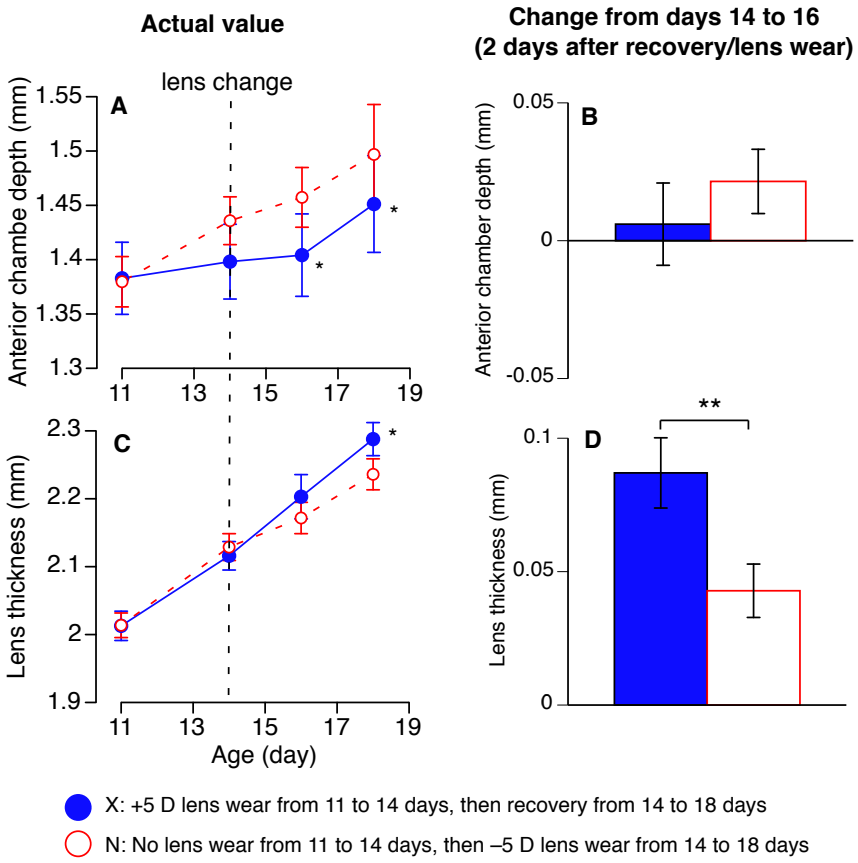

Figure A3.3. Comparison between positive lens recovery and negative lens wear. Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 2 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

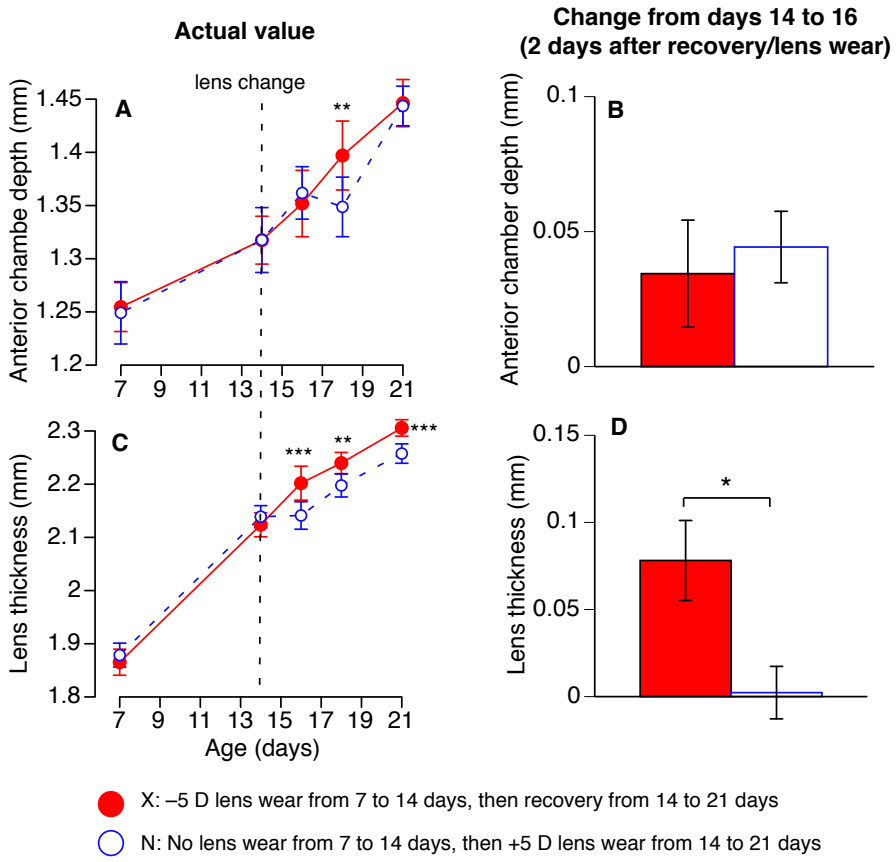

Figure A3.4. Comparison between negative lens recovery and positive lens wear.
Data is shown as Mean ± SEM, for both the actual values for the experimental and fellow eyes (left panel), and for the change 2 days after lens change (right panel). Asterisks on the left and right panels indicate statistical significance between actual values in the experimental and fellow eyes at various ages (Two-Way Mixed Measures ANOVA) and statistical significance between the change in the experimental and fellow eyes (paired, 2 tailed *Student*'s t-test), respectively. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Appendix 4. Previous Publications

Eyes in Various Species Can Shorten to Compensate for Myopic Defocus

Xiaoying Zhu,[1] Neville A. McBrien,[2] Earl L. Smith III,[3] David Troilo,[4] and Josh Wallman[1,5]

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[5]Deceased March 3, 2012.

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Purpose. We demonstrated that eyes of young animals of various species (chick, tree shrew, marmoset, and rhesus macaque) can shorten in the axial dimension in response to myopic defocus.

Methods. Chicks wore positive or negative lenses over one eye for 3 days. Tree shrews were measured during recovery from induced myopia after 5 days of monocular deprivation for 1 to 9 days. Marmosets were measured during recovery from induced myopia after monocular deprivation, or wearing negative lenses over one or both eyes, or from wearing positive lenses over one or both eyes. Rhesus macaques were measured after recovery from induced myopia after monocular deprivation, or wearing negative lenses over one or both eyes. Axial length was measured with ultrasound biometry in all species.

Results. Tree shrew eyes showed a strong trend to shorten axially to compensate for myopic defocus. Of 34 eyes that recovered from deprivation-induced myopia for various durations, 30 eyes (88%) shortened, whereas only 7 fellow eyes shortened. In chicks, eyes wearing positive lenses reduced their rate of ocular elongation by two-thirds, including 38.5% of eyes in which the axial length became shorter than before. Evidence of axial shortening in rhesus macaque (40%) and marmoset (6%) eyes also occurred when exposed to myopic defocus, although much less frequently than that in eyes of tree shrews. The axial shortening was caused mostly by the reduction in vitreous chamber depth.

Conclusions. Eyes of chick, tree shrew, marmoset, and rhesus macaque can shorten axially when presented with myopic defocus, whether the myopic defocus is created by wearing positive lenses, or is the result of axial elongation of the eye produced by prior negative lens wear or deprivation. This eye shortening facilitates compensation for the imposed myopia. Implications for human myopia control are significant.

Keywords: emmetropization, myopia, hyperopia, ocular length, chick, tree shrew, marmoset, rhesus macaque

Many animal studies have shown that eyes can compensate for imposed defocus by changing choroidal thickness and the rate of ocular elongation, above or below that found in normal untreated growing eyes. For instance, when wearing a positive lens that puts the focal plane in front of the photoreceptors, the eye decreases its rate of ocular elongation and increases choroidal thickness, thereby pushing the retina forward to meet the focal plane; the opposite happens in the case of wearing a negative lens. Among the various animal species used, chick eyes have been shown to be able to compensate for the widest range of defocus.[1]

It usually is assumed that, when eyes compensate for myopic defocus imposed by positive lenses, their rate of ocular elongation is reduced, so the eye either elongates at a slower rate than normal or, at the most, stops its growth. Even though it seems more natural that an eye in a growing animal should elongate rather than actually shorten (reduced length from the front of the cornea to the back of the sclera), there seems no obvious reason why an eye experiencing myopic defocus cannot axially shorten or shrink through a mechanism, such as extracellular matrix remodeling of the sclera, thereby further facilitating compensation. Given that tissues are remodeled continuously under homeostatic control, we ask why should axial shortening or shrinkage be more implausible than elongation or enlargement.

Previous studies have shown that organ size can fluctuate drastically under physiologic conditions. In Burmese pythons, which typically feed once every a couple of months, the heart, lungs, liver, intestinal mucosa, and kidneys all alternate between a large and a small size. After a large meal, the increase in mass of these organs ranges between 50% and 150% (as percentage of fasted mass).[2] In many seasonally breeding birds, the gonads can shrink by 87% when the day length decreases from 13 to 12 hours (e.g., spotted antbirds[3]). If other organs can fluctuate in size, perhaps eyes as well can shrink when needed. In this study, we demonstrate that eyes of chick, tree shrew, marmoset, and rhesus macaque also can shorten axially in response to myopic defocus when wearing positive lenses, or recovering from wearing negative lenses or form deprivation, by summarizing earlier data from four independent

laboratories of Josh Wallman, Neville A. McBrien, David Troilo, and Earl L. Smith III.

Some of the results from chicks have been presented previously either in a preliminary form (Zhu X and Wallman J. *IOVS* 2009;50:ARVO E-Abstract 3929) or in separate studies for different purposes.[4–6] For the tree shrew, data relating to scleral metabolism and induced myopia in the same animals have been reported in a separate study for different purposes.[7] For the marmoset data[8–10] and rhesus macaque data[11–15] some findings have been presented in separate reports for different purposes related to recovery from myopia.

Materials and Methods

Animals

White Leghorn chicks were obtained from either Cornell University (Cornell K-strain; Ithaca, NY) or Truslow Farms (Hyline-W98-strain; Chestertown, MD). Chicks were housed in a heated, sound-attenuated chamber (76 × 61 cm), with a 14:10 hour light–dark cycle in the Wallman laboratory. Maternally reared tree shrews (*Tupaia belangeri*) from the breeding colony of the McBrien laboratory were used. Animals were transferred from the breeding colony 15 days after natural eye opening, on the day experimental procedures commenced. Eye opening occurred at 20 ± 3 days (mean ± SD) after birth. Animals were housed individually in large stainless steel cages and kept on a 15:9 hour light–dark cycle. Maternally reared marmosets (*Callithrix jacchus*) from the breeding colony in the Troilo laboratory were used. Animals were kept in group enclosures on a 10:14 light–dark cycle. Rhesus macaques (*Macaca mulatta*) were obtained at 1 to 3 weeks of age and housed in the primate nursery in the Smith laboratory. They were maintained on a 12:12 hour light–dark cycle. Care and use of all animals adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the local animal ethics committees at the respective investigators' institutions.

Experimental Procedure

The Table includes the treatment details and sample sizes for each treatment for each species.

For chicks, PMMA plastic lenses (12 mm diameter with a back optic radius of 7 mm) or glass lenses (not conspicuously curved) of −7, +6, +7, or +10 diopters (D) were used. Each lens was glued between a rigid plastic ring and a Velcro ring, and attached to a mating Velcro ring glued to the feathers around the chicks' eyes. Lenses were cleaned at least twice a day. The majority of chicks wore a lens over one eye for 3 days. Some chicks wore negative lenses (−7 D) continuously, and the rest of them wore positive lenses (+6, +7, or +10 D) either continuously (with or without a weak diffuser) or for various durations (specifically, 20 seconds per 20 minutes, 5 seconds per 5 minutes, 2 minutes per 10 minutes or hour, 5 minutes per 4 hours, and 30 minutes per 2, 4, or 12 hours) with darkness between episodes. These chicks were measured by ultrasound biometry before and after 3 days of treatment. Another set of the chicks wore various lenses on one eye and had the fellow control eyes measured by ultrasound biometry repeatedly within 1 hour. Data from only the untreated fellow eyes were used to calculate the SD, an index of measurement error in chicks. The starting age of all chicks was one week old in all the experiments.

For tree shrews, one of the paired eyes was deprived of vision by a translucent occluder fitted to a head-mounted goggle 15 days after natural eye opening. The translucent occluder remained in place for 5 days, while the fellow eye was left untreated. After 5 days deprivation was discontinued, all tree shrews (n = 39) had ultrasound biometry (A-scan ultrasound) performed immediately on removal of the head-mounted goggle holding the occluder. A total of 34 animals were allowed to recover from the induced myopia for periods of 1, 3, 5, 7, or 9 days, with $n \geq 5$ in each recovery group.

For marmosets, the conditions examined that might result in reduced eye growth included rearing some with either positive contact lenses (+5 D) over one eye or spectacle lenses over both eyes (+3 D or +5 D) starting at the age of 4 months (mean age = 128 days) for various durations to impose myopic defocus. Details of the lenses used are reported in the original studies.[8–10] The rest of the animals used in this analysis had myopia induced by wearing either a translucent occluder or negative contact lens (−5 D) over one eye, or spectacle lenses over both eyes (−3 D or −5 D) starting at the age of 10 weeks, and these devices were removed when the marmosets were roughly 4 months old (mean age = 112 days), and the eyes were allowed to recover. Another group of untreated marmosets also were measured by ultrasonography periodically.

Rhesus macaques wore negative lenses (specifically, −3 D over one eye [OD −3 D], −3 D over both eyes [OU −3 D], −6 D over both eyes [OU −6 D], negative sequential lenses over both eyes [OU NS], or occluders over one eye [FD]) starting at the age of 3 or 4 weeks (mean age = 25 days) for roughly 4 months (mean duration = 135 days), after which the eyes were allowed to recover. Another group of untreated rhesus macaques also were measured by ultrasonography periodically.

Axial Biometry Measurements

Internal ocular dimensions were measured with A-scan ultrasonography from the anterior surface of the cornea for all four species, but to different tissues at the back of the eye with different measuring intervals, for different animals.

For chicks, A-scan ultrasonography was conducted with a 30 MHz transducer (Model 176599; Panametrics, Waltham, MA) and sampled at 100 MHz with a Sonix 8100 A/D board (Sonix, Inc., Springfield, VA) on a computer.[16] The internal ocular dimensions (from the anterior surface of the cornea to the outer surface of the sclera) were measured with chicks anesthetized with 1.5% of isoflurane.[17] Ocular length was defined as the sum of anterior chamber depth, lens thickness, vitreous chamber depth, and the thicknesses of the retina, choroid, and sclera. For chicks that wore various lenses for 3 days, the eyes were measured at the beginning and end of each experiment, and the rest of the chicks were measured repeatedly within an hour.

For tree shrews, the length of the eye (from the anterior surface of cornea to the inner surface of the sclera) was measured before and after recovery using A-Scan ultrasonography. In tree shrews, ultrasound measures were made using a 10 MHz, 6.35 mm diameter ultrasound transducer focused at 22 mm and driven by a Panametrics 5052 pulser/receiver that was coupled to a 15 mm Perspex (Lucite International, Southhampton, United Kingdom) standoff perfused continuously with 0.9% saline (flow rate 0.8 mL/min). The standoff was positioned by hand so the saline column contacted the anesthetized cornea (0.5% proxymetacaine HC1) without any applanation. Waveform echoes passed from the pulser/receiver into a LeCroy 9400 digital storage oscilloscope (sample rate 100 megasamples/s; LeCroy, Geneva, Switzerland). To enhance the signal-to-noise ratio, each stored waveform was the average of 20 single incoming waveforms. Six stored waveforms from independent positioning of the transducer were collected for each eye and transferred to PC for subsequent measurement.

TABLE. Details and Sample Sizes for Each Treatment for All Species

Species	Treatment	Ocularity	Sample Size
Chicks	−7 D lens, continuous	Monocular	24
	+6 or +7 D lens, continuous	Monocular	36
	+7 D lens with a weak diffuser, continuous	Monocular	13
	+6 D lens, 5 s every 5 min	Monocular	10
	+6 D lens, 20 s every 20 min	Monocular	9
	+7 D lens, 2 min every 10 min	Monocular	7
	+6 D lens, 2 min every h	Monocular	14
	+10 D lens, 5 min every 4 h	Monocular	6
	+6 D lens, 30 min every 2 h	Monocular	6
	+6 or +10 D lens, 30 min every 4 h	Monocular	76
	+6 D lens, 30 min every 12 h	Monocular	6
	+6 and −6 D lenses, each worn alternately for 15 min every 4 h	Monocular	12
Tree shrews	1 d of recovery after 5 d of form deprivation	Monocular	5
	3 d of recovery after 5 d of form deprivation	Monocular	8
	5 d of recovery after 5 d of form deprivation	Monocular	10
	7 d of recovery after 5 d of form deprivation	Monocular	5
	9 d of recovery after 5 d of form deprivation	Monocular	6
Marmosets	Recovery from −5 D contact lens wear	Monocular	15
	Recovery from −3 or −5 D spectacle lens wear	Binocular	24
	Recovery from form deprivation	Monocular	17
	+3 or +5 D spectacle lens wear	Binocular	18
	+5 D contact lens wear	Monocular	20
	Untreated, normal marmosets	N.A.	20
Rhesus macaques	Recovery from form deprivation	Monocular	9
	Recovery from −3 D lens wear on the right eye (plano lens on the left eye)	Monocular	9
	Recovery from −3 D lens wear on both eyes	Binocular	10
	Recovery from −6 D lens wear on both eyes	Binocular	3
	Recovery from wearing negative lenses sequentially	Binocular	4
	Untreated, normal rhesus macaques	N.A.	40

Details and sample sizes for each treatment for all species (except for chicks whose untreated fellow eyes were measured repeatedly within an hour and form-deprived tree shrews without recovery; these values are given in the text).

Conversion of time to distance used previously reported values for the tree shrew eye.[18] At the end of the recovery period equatorial dimensions (superior-inferior and nasal-temporal) of the enucleated tree shrew eyes were measured with a digital caliper.

For marmosets, the length of the eye (from the anterior surface of the cornea to the inner surface of the sclera) was measured repeatedly using A-scan ultrasonography during periods of positive lens wear, or recovery from deprivation or from negative lens-induced myopia. A 33 MHz piezoelectric immersion transducer (model PZ25-0.25-SU-R1.00; Panametrics) driven by an ultrasound pulser/receiver (model 5072 PR-15U; Panametrics) was used. The transducer was coupled to the eye with a 16-mm water-filled plexiglass stand-off that positioned the focal zone of the sound wave inside the vitreous chamber of marmoset eyes for all ages. The ultrasound signal was digitized for analysis using a 100 MHz analogue-to-digital conversion board (model STR-8100; Sonix, Inc.; or model NI-PCI-5922; National Instruments, Austin, TX).

Similarly, for rhesus macaques, the length of the eye also was measured repeatedly by A-scan ultrasonography during recovery from either form-deprivation or wearing negative lenses. However, the length of the eye (or "axial length") was from the anterior surface of the cornea to the anterior surface of the retina. Thus, axial length can be affected by changes in choroidal thickness. The great majority (67%) of data ($n = 4$, 3, and 26 in the groups of FD, OD −3 D, and untreated, respectively, and all of the binocularly-treated animals) were obtained with an instrument (Mentor Image 2000, 7 MHz transducer; Mentor, Norwell, MA) that provided information on individual ocular components, in particular vitreous chamber depth. This instrument (Mentor Image 2000, 7 MHz transducer; Mentor) provided the average of 10 separate measures. The instrument (Mentor Image 2000, 7 MHz transducer; Mentor) used a weighted average velocity of sound in the ocular media of 1550 m/s to calculate intraocular distances. The rest of the data ($n = 5$, 6, and 14 in the groups of FD, OD −3 D, and untreated, respectively) were obtained with the OTI A-scan (OTI scan 1000, 12 MHz transducer).

Statistics

Data are presented as mean ± SD. Two different statistical methods were used to compare the number of eyes that shortened axially versus the number of eyes that did not in the treated eyes and control eyes, respectively.

For chicks and tree shrews, the number of treated eyes that shortened while wearing positive lenses (chicks) or recovering from deprivation (tree shrews) versus those that did not was compared to their fellow control eyes with χ^2 tests.

For marmosets and rhesus macaques, the hypothesis that more eyes that wore positive lenses, or recovered from deprivation or wearing negative lenses shortened compared to eyes from normal, untreated animals was tested using a bootstrapping method[19] (Matlab, version R2010b; Mathworks, Natick, MA). Analysis consisted of the following steps: Firstly, the change in axial length in treated eyes between either the initiation of positive lens wear, or recovery from deprivation or negative lenses and the next time the animals were measured (mean = 31 and 14 days for marmosets and rhesus macaques, respectively) was calculated. For animals that wore devices over both eyes, change in axial length from both treated eyes

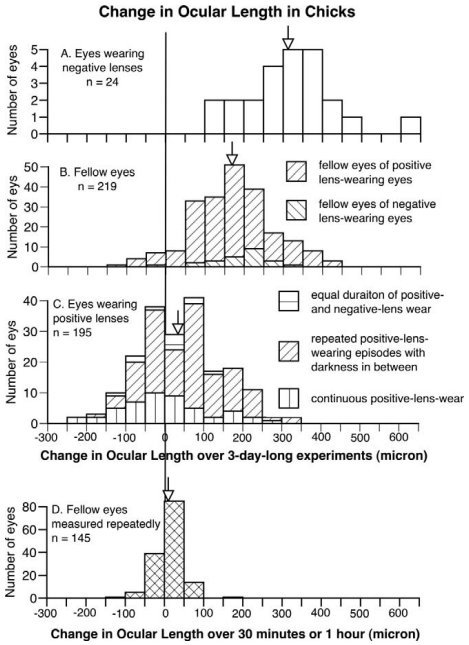

Figure 1. The frequency distributions of change in ocular length (front of cornea to back of sclera) in negative lens–wearing eyes (**A**), all the fellow eyes (**B**), positive lens–wearing eyes (**C**), all measured 3 days apart, and untreated eyes measured repeatedly within an hour from another group (**D**), in chicks. *Arrows* indicate the average of each group. It is clear that, while negative lenses increased the rate of ocular elongation, positive lenses decreased it, with 38.5% of the positive lens–wearing eyes shortening during the course of the experiment (on the *left side* of zero). Furthermore, data from eyes measured repeatedly (**D**) show the error of biometry measurements in ocular length. When very little eye growth was expected within an hour, most of the points are close to zero.

was used for analysis. Data from monocularly- versus binocularly-treated animals were analyzed separately.

Secondly, data from eyes of untreated animals were used for comparison. Since the eyes of the treated and untreated animals were measured on different days, the hypothetical axial length of the eyes of the untreated animals on the same day that the treated animals were measured was interpolated based on the axial length data from that particular normal animal (Igor Pro, version 5.02; Wavemetrics, Lake Oswego, OR), provided that this normal animal was measured frequently enough. Therefore, for x treated animals, x sets of axial length can be calculated for each normal, untreated animal. Furthermore, if there were x treated animals and y untreated animals, a total of xy sets of axial length can be calculated. Specifically, for marmosets, there were a total of 94 treated animals and 20 untreated animals (note that data from some treated and untreated animals were not included for calculation due to either lack of ultrasound data or infrequent measurements), thus leaving a total of 1334 sets of axial length that were calculated (see Fig. 6). For rhesus macaques, there were 35 treated animals and 40 normal animals. Hence, a total of 1400 ($35 \times 40 = 1400$) sets of axial length was calculated. Since these data are not independent from each other, bootstrapping methods, instead of χ^2 tests, were used to compare the numbers of eyes that shortened versus those that did not for treated and untreated animals.

Thirdly, the number of treated eyes that shortened and those that did not was compared to the number of eyes from untreated animals that shortened during the same duration (calculated with interpolation) and those that did not, using the bootstrapping procedure. Specifically, having observed that, for a given treatment, a certain number, n, out of m treated eyes shortened, we analyzed whether such an event could have occurred by chance. To estimate the probability of observing n out of m treated eyes shortening axially, we used the bootstrapping procedure to build a distribution representing the probability of observing an arbitrary number of shortening eyes in a group of m. To build that distribution, we pooled measurements from untreated eyes, and drew random samples of m measurements 50,000 times. For each randomly selected sample, we counted the number of measurements less than 0 (eyes that shortened). The resulting distribution allowed us to calculate the probability of observing at least n shortening eyes in a sample of size m by counting the number of times out of 50,000 that we had drawn random samples of size m that also contained at least n measurements less than 0. If we observed fewer than 2500 such occurrences (less than 5% of the bootstrapped samples), we concluded that the observed number of eyes that shortened axially could not be attributed to chance.

Furthermore, the 95% confidence intervals (CIs) for A-scan ultrasonography measures of axial length for the different species was used to assess whether the observed axial shortening in response to myopic defocus could be accounted for by measurement error. For chicks, the 95% CIs were calculated from control eyes ($n = 145$) that were measured twice within an interval of one hour, during which time their fellow eyes wore various lenses. For rhesus macaques the repeatability of measures from 20 eyes was measured to establish 95% CIs. For tree shrews[18] and marmosets[8] the 95% CIs were used from previously reported data on normal animals from the two laboratories.

Results

For chicks, as expected, negative and positive spectacle lenses increased and decreased the rate of ocular elongation, respectively (Fig. 1). Eyes wearing negative lenses for 3 days ($n = 24$) elongated twice as much as fellow eyes of positive and negative lens–wearing eyes ($n = 219$, mean change in ocular length, 314 vs. 171 μm, $P < 0.001$, unpaired 1-tailed Student's *t*-test, Figs. 1A, 1B), whereas eyes wearing positive lenses for 3 days ($n = 195$) elongated less than a quarter as much as these fellow eyes (mean 40 vs. 171 μm, $P < 0.001$, Figs. 1B, 1C). In chicks wearing positive lenses, 75 out of 195 (38.5%) positive lens–wearing eyes became shorter than at the start of the experiment (mean shortening ± SD −63 ± 49 μm, Fig. 1C), whereas only 10 fellow eyes shortened (−58 ± 34 μm, Fig. 1B). The frequency of eye shortening in the positive lens–wearing eyes and their fellow eyes was significantly different ($P < 0.001$, χ^2 test).

The 95% CI for ocular length measures was estimated from repeated measures on 145 fellow eyes, each measure separated by 1 hour. This provided a SD of 26 μm, resulting in 95% CIs of ±51 μm. As a matter of fact, the SD of these measurements (SD = 26 μm) overestimated the measurement error because it was based on a heterogeneous sample of experimental animals measured at different times of day. Using this SD and supposing

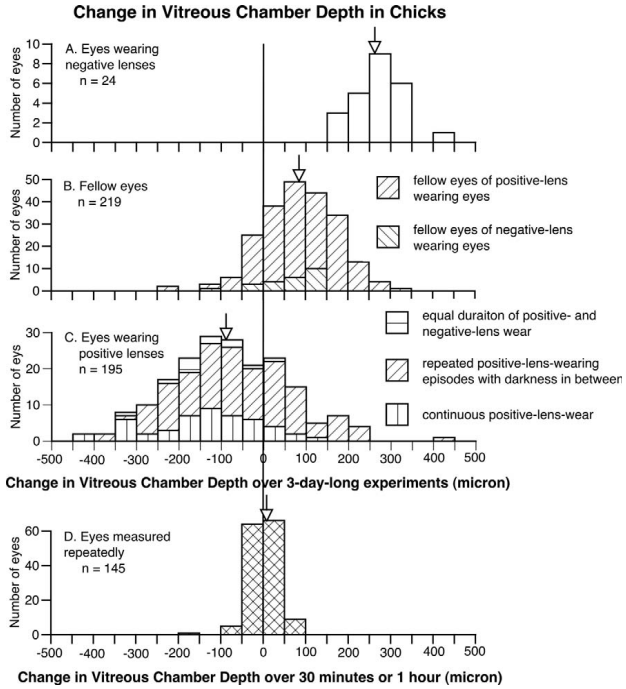

Figure 2. The frequency distributions of change in vitreous chamber depth in negative lens–wearing eyes (**A**), all the fellow eyes (**B**), positive lens–wearing eyes (**C**), all measured 3 days apart, and untreated eyes measured repeatedly within an hour from another group (**D**), in chicks. *Arrows* indicate the average of each group. Similar to Figure 1, it is clear that, while negative lenses increased the vitreous chamber depth, positive lenses decreased it (wearing positive lenses caused the vitreous chamber to shorten in approximately two thirds of the eyes). Again, data from eyes measured repeatedly (**D**) show the accuracy and validity of biometric measurements of vitreous chamber depth.

the changes in the length of individual positive lens–wearing eyes approximated a normal distribution (Fig. 1D), zero change in ocular length in eyes wearing positive lenses would be 1.54 SDs below the mean (40 μm). Therefore, if measurement error were the only cause, we would expect 6.2% of these 195 eyes (12 eyes) to have shortened, rather than 38.5% (75 eyes) that were encountered ($P < 0.0001$, Fisher's exact test).

Not surprisingly, the shortening in ocular length also resulted in a decrease in the depth of the vitreous chamber (Fig. 2). The vitreous chamber depth in positive lens–wearing eyes decreased by −84 μm over 3-day–long experiments (Fig. 2C), whereas the vitreous chamber depth in the fellow eyes of positive and negative lens–wearing eyes elongated by the same amount (Fig. 2B, $P < 0.001$, unpaired 1-tailed Student's *t*-test). Furthermore, significantly reduced axial enlargement in anterior chamber depth and lens thickness also was found in positive lens–wearing eyes, although to a smaller degree (anterior chamber depth, positive lens–wearing eyes versus all fellow eyes 1 vs. 15 μm, $P < 0.01$; lens thickness 89 vs. 102 μm, $P < 0.01$). The choroids in positive lens–wearing eyes thickened significantly more than those in the fellow eyes (32 vs. −26 μm, $P < 0.001$). This change, however, only caused a reduction in vitreous chamber depth without changing ocular length. Indeed, the shortening of the vitreous chamber in positive lens–wearing eyes cannot be explained fully by choroidal thickening in these same eyes (vitreous chamber shortening versus choroidal thickening 84 vs. 32 μm), but is a consequence of the reduced ocular length. No significant change was found in retinal or scleral thickness during the course of experiments.

To rule out the possibility of abnormal growth in the chicks whose positive lens–wearing eyes shortened, the ocular growth of the fellow eyes in these chicks was compared to the rest of the fellow eyes, since a systemic pathologic condition could have reduced eye growth not only in the lens-wearing eye, but also in the fellow eye. Among 195 positive lens–wearing chicks, while 75 treated eyes shortened (38.5%), only 10 fellow eyes shortened (5.1%). This percentage was not significantly different from the percentage of eyes that shortened in all the fellow eyes (11 of 219 fellow eyes or 5.0%, $P = 0.26$, Fisher's exact test). Although the fellow eyes of those that had shortened lengthened slightly less on average than untreated fellow eyes in general (mean 131 vs. 171 μm), this may be related to the known yoking between eyes.[20–22] This difference cannot explain the shortening of the lens-wearing eyes. In addition, wearing positive lenses caused the eyes to shorten axially over a wide range of paradigms, suggesting that axial shortening of positive lens–wearing eyes in chicks was not the result of pathology, but was the product of an active compensatory mechanism for superimposed myopic defocus.

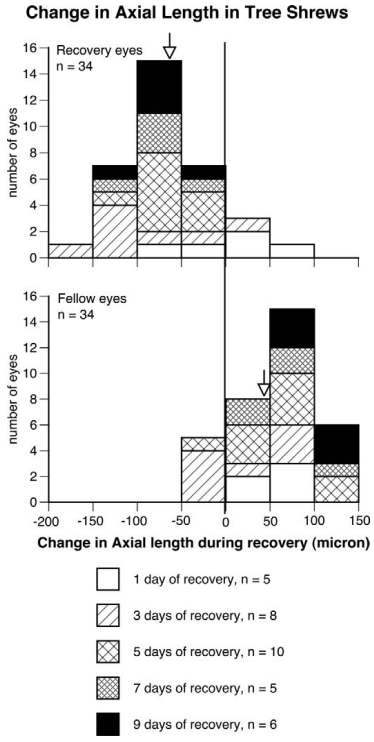

FIGURE 3. The frequency distributions of change in axial length (from the anterior surface of cornea to the inner surface of sclera) in the treated eyes that recovered from deprivation-induced myopia for various durations (*top*) and in their untreated, fellow eyes within the same duration (*below*) in tree shrews. A total of 30 treated eyes shortened during recovery, whereas 5 fellow eyes shortened within the same duration ($P < 0.0001$, χ^2 test).

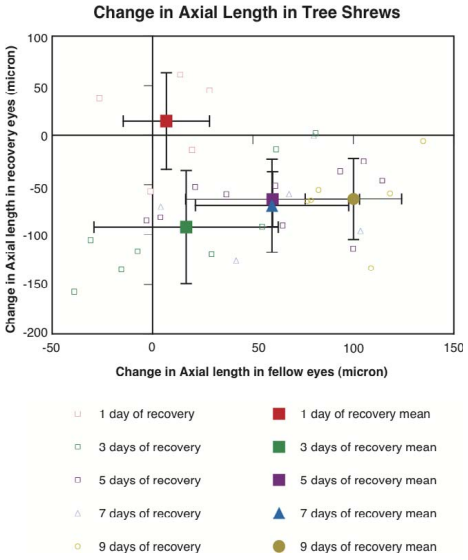

FIGURE 4. Change in axial length (from the anterior surface of cornea to the inner surface of sclera) in the recovery eyes (*y*-axis) plotted against that in the fellow eyes (*x*-axis) in tree shrews. *Small* and *open symbols* represent individual eyes, and *large* and *solid symbols* represent averages for each group (mean ±1 SD). It is clear that most of the recovery eyes shortened (*below zero* on the *y*-axis), whereas their fellow eyes grew (*above zero* on the *x*-axis). The figure also shows that the largest mean difference between treated and fellow control eyes occurred in the 9 day recovery group (≈150 μm), while the largest mean degree of axial shortening in treated eyes (92 μm) occurred in the 3 days recovery group.

For tree shrews, similarly, eyes recovering from deprivation-induced myopia shortened axially to compensate for myopic defocus (Fig. 3): After 1, 3, 5, 7, or 9 days of recovery following 5 days of monocular deprivation, 30 of 34 (88%) treated eyes shortened (mean change in axial length from cornea to the inner surface of the sclera during recovery −60 ± 53 μm, mean ± SD), whereas only 5 fellow eyes shortened axially (mean change 49 ± 48 μm, $P < 0.0001$, χ^2 test, Fig. 3). The shortening in axial length also resulted in a decrease in the depth of vitreous chamber (data not shown): The vitreous chamber depth in recovering eyes decreased by 89 μm, whereas the vitreous chamber depth in the fellow eyes elongated by 16 μm ($P < 0.0001$, paired 1-tailed Student's *t*-test). Furthermore, small but significantly reduced growth in anterior chamber depth also was found (recovery eyes versus fellow eyes −3 vs. 16 μm, $P < 0.05$, paired 2-tailed Student's *t*-test). The 95% CIs for axial length measures in tree shrew have been reported previously to be ±40 μm,[18] which is markedly less than the mean axial shortening of 60 μm observed in the tree shrew eyes recovering from experimentally-induced myopia in our study.

Another striking finding in tree shrews is that the percentage of eyes that shortened axially was correlated positively with the recovery duration (Figs. 3, 4). After 1 day of recovery ($n = 5$), 2 treated and 2 fellow eyes (from different animals) shortened, respectively. After 3 ($n = 8$) and 5 ($n = 10$) days of recovery, 7 and 10 treated eyes shortened, respectively, versus 4 and 1 fellow eye that shortened ($P < 0.0001$ for the 5-day recovery group, χ^2 test). After 7 and 9 days of recovery ($n = 5$ and 6, respectively), all of the treated eyes shortened and all of the fellow eyes grew ($P < 0.0001$). Furthermore, it is clear from Figure 4 that more than 68% of treated eyes (mean ± 1 SD) in groups that had more than 3 days of recovery (5–9 days of recovery) shortened, reinforcing the finding that the majority of eyes of tree shrews shorten axially to compensate for myopic defocus. Figure 4 also shows that the greatest relative difference between recovering and fellow control eyes occurred in the 9-day recovery group (≈ 150 μm), although the largest mean shortening in treated eyes (mean = 92 μm) occurred in the 3-day recovery group.

As eyes did shorten axially in absolute terms during compensation for myopic defocus, it is possible that, during recovery, eye growth was reduced in the axial direction, but could have increased in the equatorial direction. However, measurements, using digital caliper, of equatorial dimensions (superior/inferior and medial/lateral) from these enucleated tree shrews' eyes demonstrated that the equatorial enlargement observed after 5 days of induced myopia (mean

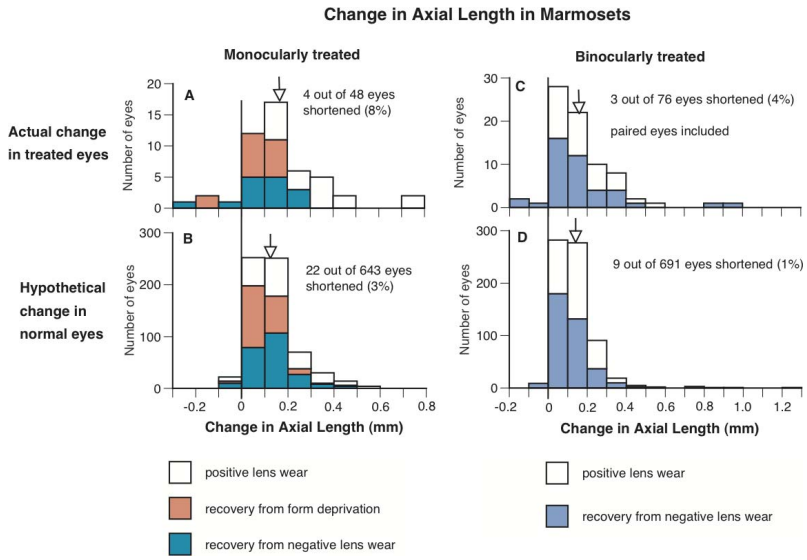

Figure 5. The frequency distributions of change in axial length (from the anterior surface of cornea to the posterior surface of sclera) in the treated eyes of marmosets that either wore positive lenses, or recovered from form deprivation or wearing negative lenses (**A**, **C**), and in untreated eyes in normal animals grouped to match the same duration of visual exposure as the treated eyes through interpolation (**B**, **D**). *Left* and *right* represent the change in axial length in the eyes of monocularly- and binocularly-treated marmosets (and the corresponding interpolated untreated eyes), respectively. It is clear that a higher percentage of the treated eyes shortened (axial change *below zero*) than the calculated data from age-matched normal eyes. The *vertical lines* indicate where zero is on the *x*-axes, and the *arrows* indicate the mean change in axial length for each group of eyes.

difference of superior/inferior + medial/lateral between treated versus control was 90 ± 24 μm) incrementally reduced the longer the recovery period (group mean differences between treated and fellow control eyes for 1-, 3-, 5-, 7-, and 9-day recovery groups were 91 ± 18, 77 ± 15, 20 ± 13, 16 ± 14, and 17 ± 21 μm, respectively). Thus, equatorial enlargement from induced myopia reduced gradually during recovery from myopia, but did not show absolute shortening in the equatorial dimension, suggesting that the change in eye growth is predominantly in the axial direction.

For marmosets, eyes that either wore positive lenses to impose myopia, or recovered from induced myopia from deprivation or from wearing negative lenses also could shorten axially to compensate for myopic defocus, although this was seen with much less frequency compared to eyes of tree shrews, chicks, or rhesus macaque (Fig. 5). For monocularly treated marmosets that either wore +5 D contact lenses, or recovered from myopia induced by wearing −5 D contact lenses or occluders ($n = 15$ to 18 in each group), 8% of the treated eyes shortened axially (4 treated eyes of 48 eyes consisting of 2 eyes recovering from −5 D contact lenses and 2 eyes recovering from form-deprivation; mean change in axial length ± SD 165 ± 185 μm, Fig. 5A), whereas only 3% of the calculated, age-matched (interpolated) normal eyes shortened (22 of 643 calculated eyes, Fig. 5B; $P < 0.05$ for pooled data, bootstrapping; when data from each group were analyzed separately, only recovery from wearing occluders reached statistical significance, $P < 0.05$). For binocularly-treated marmosets that either wore +3 D or +5 D spectacle lenses, or recovered from myopia induced by wearing −3 D or −5 D spectacles lenses ($n = 18$ and 24, respectively), 4% of treated eyes shortened axially (3 treated eyes of 76 eyes, all 3 eyes from animals recovering from wearing −3 D or −5 D lenses; mean change in axial length 173 ± 180 μm, Fig. 5C), whereas only 1% of calculated, age-matched (interpolated) normal eyes shortened (9 of 691 eyes, Fig. 5D, $P > 0.05$ for pooled data; when data from each group were analyzed separately, $P < 0.001$ for the binocular negative lens–wearing group). The 95% CIs for axial length measures in marmoset have been reported previously to be ±33 μm.[8]

Axial length data from eyes of rhesus macaques (from the anterior corneal surface to the anterior surface of retina) were analyzed in a similar fashion as the marmoset eyes, and it showed that rhesus macaque eyes also can shorten axially to compensate for myopic defocus (Fig. 6). For monocularly treated macaques that recovered from deprivation-induced myopia (FD, $n = 9$) or wearing −3 D lenses (OD −3D, $n = 9$), a total of 33% of eyes shortened axially (6 of 18 eyes, mean change in axial length ± SD 57 ± 179 μm, Fig. 6A), whereas only 15% (111 of 720 eyes) of calculated, age-matched (interpolated) normal eyes shortened (Fig. 6B, $P > 0.05$ for pooled data, bootstrapping; when data from each group was analyzed separately, the FD group showed a significant difference, $P < 0.05$).

The frequency of eye shortening in binocularly-treated macaques was stronger than that observed in monocularly-treated macaques, although it still was just under half the treated eyes (47%). These rhesus macaques recovered from wearing either −3 D (OU −3 D, $n = 10$) or −6 D lenses (OU −6 D, $n = 3$), or negative sequential lenses (OU NS, $n = 4$) over both eyes. A total of 47% of the binocularly-treated recovering eyes shortened axially (16 of 34 eyes; 12, 1, and 3 eyes from

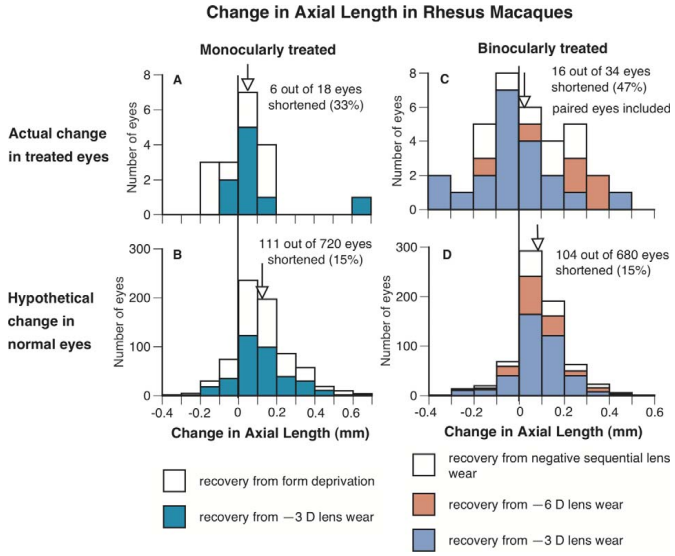

Figure 6. The frequency distributions of change in axial length (from the anterior surface of the cornea to the anterior surface of the retina) in the treated eyes of rhesus macaques that recovered from form deprivation, or wearing negative lenses (**A**, **C**) and in untreated eyes in normal animals grouped to match the same duration of visual exposure as the treated eyes through interpolation (**B**, **D**). *Left* and *right* represent the change in axial length in the eyes of monocularly- and binocularly-treated eyes in rhesus macaques (and the corresponding interpolated untreated eyes), respectively. It is clear that a higher percentage of the treated eyes shortened (axial change *below zero*) than the calculated data from age-matched normal eyes. The *vertical lines* indicate where zero is *x*-axes, and the *arrows* indicate the mean change in axial length for each group of eyes.

the groups of OU –3 D, OU –6 D, and OU NS, respectively; mean ± SD 22 ± 181 μm, change in axial length, Fig. 6C), whereas only 15% (104 of 680) of calculated, age-matched (interpolated) normal eyes shortened (Fig. 6D, $P < 0.001$ for pooled data, bootstrapping; when data from each group were analyzed separately, the groups of OU –3 D and OU –6 D also showed a significant difference of $P < 0.001$ and $P < 0.05$, respectively). The 95% CIs for axial length measures for rhesus macaque were calculated from repeated measures on 20 rhesus macaque eyes and found to be ±47 μm.

Since the axial length in rhesus macaques was measured to the anterior surface of the retina, there is the possibility that the axial shortening was caused by choroidal thickening. If that were the case, one would expect that the amount of choroidal thickening would equal the amount of eye shortening. However, previous findings suggest that the axial shortening discovered in these rhesus macaque eyes was not caused exclusively by choroidal thickening. Choroidal thickening in rhesus macaques has been suggested to be on average 50 μm, with the largest amount of thickening noted to be 102 μm.[23] Among the rhesus macaque eyes that shortened axially, in more than half of the eyes the shortening was more than the maximal choroidal thickening reported previously.[23] Of the 22 macaque eyes that shortened axially, 11 shortened by more than 100 μm, ranging from 120 to 310 μm (mean shortening in these 22 eyes −122 ± 85 μm), amounts substantially larger than could be accounted for by choroidal thickening.

Therefore, although it is likely that choroidal thickening in rhesus macaque eyes contributed to the observed axial shortening measured in these eyes, roughly in half of the eyes that shortened, the magnitude of shortening could not be accounted for by choroidal thickening alone, indicating that rhesus macaque eyes also can shorten axially to compensate for myopic defocus.

Discussion

Our data provided evidence that avian and mammalian eyes can shorten axially to compensate for myopic defocus. That eye shortening was found in a variety of species, regardless of the structural differences in the sclera and eye sizes, suggests that the same mechanism modulating eye growth is conserved evolutionarily.

In tree shrews, where the majority of treated eyes (88%) exposed to myopic defocus shortened axially, the finding that the enlarged equatorial diameters observed after 5 days of induced myopia reduced incrementally during recovery from myopia, such that there was no statistical difference between treated and fellow eyes across all recovery groups, supports the likelihood that the treated eyes compensating for myopic defocus actually shrank (reduction in surface area). For chicks, marmosets, and rhesus macaques we can only state that eyes of these species can shorten axially in response to myopic defocus as no equatorial measures were available. However, it would seem likely a similar process occurs across all species.

It might be considered that it would be more difficult for chick eyes to shorten because the chick sclera has a more rigid cartilaginous layer composed of chondrocytes, whereas the outer layer of mammalian eyes has only fibrous sclera composed of fibroblasts and myofibroblasts,[24] which theoretically should make it easier to remodel. However, an earlier study by Kusakari et al. found evidence of scleral remodeling in

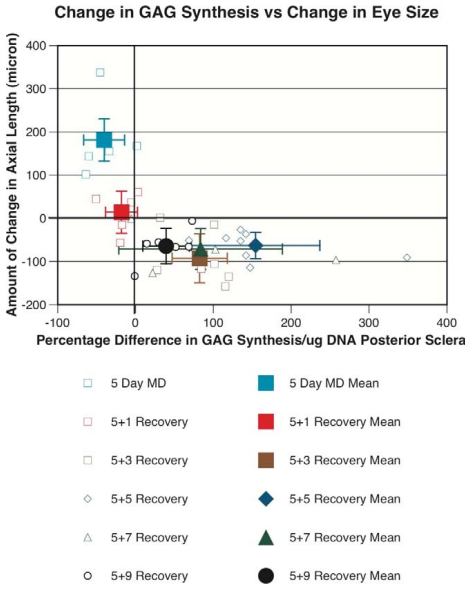

Figure 7. Change in axial length in the treated eyes of tree shrew (*y*-axis) plotted against the percentage difference in glycosaminoglycan synthesis in the posterior sclera between treated and control eyes (*x*-axis) in tree shrews. *Small* and *open symbols* represent individual eyes, and *large* and *solid symbols* represent averages for each group (mean ± SD). It is clear that most of the recovery eyes that shortened axially (27 of 30—*below zero* on the *y*-axis) had an increase in glycosaminoglycan synthesis in the posterior sclera compared to their fellow eyes, whereas tree shrew eyes that elongated due to deprivation-induced myopia (*above zero* on the *y*-axis) underwent a decrease in glycosaminoglycan synthesis in the posterior sclera, when compared to their fellow eyes.

the posterior sclera during induced myopia in chicks.[25] Specifically, after deprivation-induced myopia, the boundary between the cartilaginous and fibrous sclera became indistinct, and some spindle-shaped transitional mesenchymal cells that showed morphologic features of fibroblasts and chondrocytes were discovered between the two layers, suggesting possible transformation of the two cell types during altered eye growth. These findings support the possibility of remodeling during compensation that could lead to actual eye shortening in chicks.

In tree shrews we have direct evidence that the eyes that shortened axially also underwent active sclera remodeling, which was in the opposite direction to that found for eyes that were enlarging due to developing myopia. On the morning of the day when final biometric measures were collected on each tree shrew, animals were given an intraperitoneal injection of radiolabeled sulfate (^{35}S) to label glycosaminoglycans (GAGs) in the sclera of tree shrews. Six hours after injection of radiolabeled sulfate (when sulfate incorporation in the sclera is at its peak[7]), collection of in vivo biometric measures was completed, then animals were given a terminal dose of anesthesia, and scleral tissue was collected and processed for measurement of GAG synthesis using procedures described previously.[7] Of the 30 tree shrew eyes that actually underwent axial shortening during recovery from induced myopia, 27 eyes (90%) underwent an increase in GAG synthesis in the posterior sclera (central 5 mm). Of the 4 eyes recovering from myopia that did not show shortening, 3 eyes had been recovering for only 24 hours and only 1 of these eyes had an increase in GAG synthesis (Fig. 7). On the contrary, for the tree shrews that had been deprived of pattern vision monocularly for 5 days, but not allowed any recovery from myopia, all treated eyes underwent enlargement over the 5 days of MD with an average elongation of the axial length of 181 ± 90 μm, with 4 of the 5 myopic eyes undergoing a significant reduction in GAG synthesis (−40.3 ± 26%, $n = 5$, $P < 0.01$). Interestingly, it also was found that the same 27 recovering eyes that showed an increase in GAG synthesis in the posterior sclera also showed an increase in GAG synthesis in the equatorial sclera, although to a smaller degree, giving further evidence that tree shrew eyes recovering from myopic defocus underwent eye shrinkage and not just axial shortening. This relationship between changes in eye size and GAG synthesis, such that eyes that shortened underwent increased GAG synthesis in the sclera and eyes that elongated underwent reduced GAG synthesis in the sclera, provides strong evidence for active regulation of scleral metabolism to facilitate eye size changes in both directions.

The frequency of eyes that shortened axially to compensate for myopic defocus shows marked differences across the different animal species evaluated. Using the not unreasonable criterion for axial shortening as any reduction in ocular length (as in fellow eyes and normal eyes the norm is for axial elongation) then 88% of treated eyes in tree shrews recovering from myopia shortened axially, 38.5% of chick eyes treated with positive lenses for 3 days shortened, 47% of binocularly-treated and 33% of monocularly-treated rhesus macaque eyes shortened axially, and 8% of monocularly-treated and 4% of binocularly-treated marmoset eyes shortened axially. While the percentage values for axial shortening in response to myopic defocus of treated eyes of tree shrews and chicks are very different from the response of their fellow eye data, with only 5 fellow eyes (6.8%) of tree shrews shortened axially and 10 fellow eyes (5.1%) of chicks shortened axially, it is pertinent also to review the frequency of axial shortening in relation to 95% CIs for A-scan ultrasonography of axial length on the four species. Using the 95% CI values reported for each species in the results section, it is found that 71% of treated tree shrew eyes, 19.5% of treated chick eyes, 35% of binocularly- and 33% of monocularly-treated rhesus macaque eyes, and 6.2% of monocularly- and 4% of binocularly-treated marmoset eyes shortened axially more than the 95% CI value for axial length measures. Thus, under the specific experimental paradigms used with each species, the vast majority of eyes shortened axially in response to myopic defocus in tree shrew, while in chicks and rhesus macaques 20% to 35% of eyes shortened axially in response to myopic defocus and around 5% of marmoset eyes shortened axially.

We consider the above differences in the frequency of axial shortening between species were likely due to the experimental paradigms used, in particular the relatively older age of the primates when recovery or positive lens wear began and in the case of chicks the very short period of positive lens wear of only 3 days. For chicks and tree shrews, either positive lens wear or recovery from myopia was initiated during the most susceptible period for influencing postnatal refractive development and eye size,[26,27] and at considerably younger ages (especially in relative developmental terms) than marmosets or rhesus macaques, whereas for marmosets and rhesus macaques recovery from monocular deprivation or negative lens wear only started after 4 or 5 months, respectively, at which time the rate of postnatal eye size changes has past their most susceptible period for influencing refractive development.[8]

Although positive lens wear in chicks was initiated at the most susceptible period, the duration of lens wear of only 3 days is likely to have limited the degree and frequency of eyes that shortened axially compared to tree shrew eyes that had recovery periods up to 9 days. Compared to the monocularly-treated macaques, the binocularly-treated rhesus macaques showed a stronger trend of axial eye shortening. This may have been caused by the yoking effect, an interaction between paired eyes that drives both eyes to change in the same direction.

Eye growth is controlled by local retinal mechanisms, as demonstrated by the fact that after the eye and brain are disconnected, either by optic nerve section[28] or blocking the action potentials of retinal cells by tetrodotoxin,[29,30] chick and tree shrew eyes still develop deprivation-induced myopia. Chick eyes also have been shown to maintain the ability to compensate for positive or negative spectacle lenses after optic nerve section.[21,31] These results all suggest that the retina can modulate eye growth in response to altered visual stimuli without input from the brain. In addition, this local mechanism can alter eye growth selectively within a limited region or quadrant of the eye when diffusers[32] or lenses[33] degrade the retinal image in that part of the eye, while leaving the rest of the retinal image relatively intact. It seems highly likely that active shortening of eyes, as reported in our study, also is controlled by local ocular mechanisms.

In summary, we have presented an analysis of data from various established animal models of refractive error development showing the capacity of eyes in young, rapidly growing animals to shorten axially to facilitate compensation for myopic defocus. It would be interesting to determine if this phenomenon also exists in children or adolescents, since older adult human eyes have been shown to shorten axially, possibly in response to the increased refractive power of the cornea and the lens.[34] These results suggest the possibility that combining distance correction with some myopic defocus in the correct amount and duration might cause the developing human eye to shorten axially or shrink. If this were the case, then strategies for preventing or reducing the axial elongation of the eye that results in high myopia in human, and the associated ocular pathologies, might be treated feasibly.

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Temporal integration of visual signals in lens compensation (a review)

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ABSTRACT

Postnatal eye growth is controlled by visual signals. When wearing a positive lens that causes images to be focused in front of the retina (myopic defocus), the eye reduces its rate of ocular elongation and increases choroidal thickness to move the retina forward to meet the focal plane of the eye. When wearing a negative lens that causes images to be focused behind the retina (hyperopic defocus), the opposite happens. This review summarizes how the retina integrates the constantly changing visual signals in a non-linear fashion to guide eye growth in chicks: (1a) When myopic or hyperopic defocus is interrupted by a daily episode of normal vision, normal vision is more effective in reducing myopia caused by hyperopic defocus than in reducing hyperopia caused by myopic defocus; (1b) when the eye experiences alternating myopic and hyperopic defocus, the eye is more sensitive to myopic defocus than to hyperopic defocus and tends to develop hyperopia, even if the duration of hyperopic defocus is much longer than the duration of myopic defocus; (2) when the eye experiences brief, repeated episodes of defocus by wearing either positive or negative lenses, lens compensation depends on the frequency and duration of individual episodes of lens wear, not just the total daily duration of lens wear; and (3) further analysis of the time constants for the hypothesized internal emmetropization signals show that, while it takes approximately the same amount of time for the signals to rise and saturate during lens-wearing episodes, the decline of the signals between episodes depends strongly on the sign of defocus and the ocular component. Although most extensively studied in chicks, the nonlinear temporal integration of visual signals has been found in other animal models. These findings may help explain the complex etiology of myopia in school-aged children and suggest ways to slow down myopia progression.

After decades of studies on myopia conducted on various animals, including tree shrews (Sherman et al., 1977; Norton and Rada, 1995), rhesus monkeys (Wiesel and Raviola, 1977; von Noorden and Crawford, 1978; Hung et al., 1995), chicks (Schaeffel et al., 1988; Irving et al., 1992), marmosets (Troilo and Judge, 1993; Whatham and Judge, 2001), guinea pigs (McFadden et al., 2004), and mice (Tejedor and de la Villa, 2003; Schaeffel et al., 2004), it has become clear that the growth of the eye, like the growth of other organs in our body, is under homeostatic control, and that the homeostatic control mechanism depends, at least in part, on visual signals that exert strong control over the axial length of the eye (Wallman and Winawer, 2004).

To see far objects clearly, the focal length of the eye needs to match its physical length, so the images will be focused on the photoreceptors in the retina, a state known as emmetropia. When presented with defocus (i.e., when an image is not focused on the photoreceptors), the eye has a short term focusing mechanism (accommodation) and a long-term focusing mechanism (emmetropization). Emmetropization is the capacity to compensate for defocus by changing both the rate of ocular elongation and the thickness of the choroid (a vascular layer lying between the retinal pigment epithelium and sclera) to bring the retina closer to the focal plane. When the image is focused in front of the retina (so called "myopic defocus", since the eye is now functionally myopic) by wearing a positive lens, the eye reduces its rate of ocular elongation and increases choroidal thickness to move the retina forward to meet the focal plane (Fig. 1). Given enough time, the eye will restore emmetropia with the positive lens in place, and will therefore appear hyperopic without the lens. The opposite happens when wearing a negative lens that focuses images behind the retina ("hyperopic defocus", Fig. 1).

Among the species used in myopia research, chicks are the most commonly used, mostly because, compared with other species, chicks have been shown to be able to compensate for the widest range of defocus within a relatively short period of time (Irving et al., 1992). Indeed, young chick eyes have two distinguishing traits facilitating compensation: Their eyes (which grow at a relatively steady rate when measured until at least 42 days old

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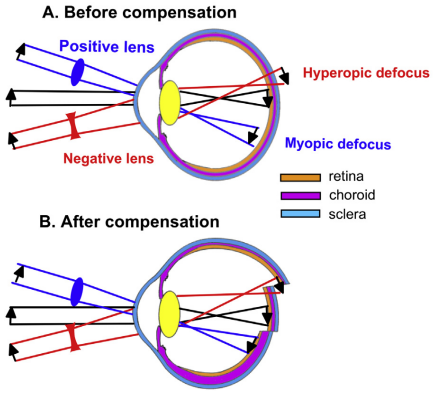

Fig. 1. Schematics of ocular compensation for defocus of opposite signs. (A) shows an emmetropic eye with a schematic representation of the myopic and hyperopic defocus produced by wearing a positive and negative spectacle lens, respectively. (B) shows ocular compensation: The eye reduces axial length and increases choroidal thickness to compensate for the positive lens, and increases axial length and reduces choroidal thickness to compensate for the negative lens. In either case, the eye becomes emmetropic again with the spectacle lens in place, since the image is now again focused on the retina. Adapted form Wallman and Winawer (Neuron, 2004; 43:447–68).

(Gottlieb et al., 1987)) change their rate of growth within a day or two to compensate for both myopic and hyperopic defocus, and their choroids show large changes in thickness to compensate for both myopic and hyperopic defocus (Wallman et al., 1995). Indeed, compensatory changes in choroidal thickness have been found in tree shrews (Siegwart and Norton, 1998), marmosets (Troilo et al., 2000), rhesus macaques (Hung et al., 2000), guinea pigs (Howlett and McFadden, 2006, 2009), and even in humans (Chakraborty et al., 2012; Woodman et al., 2012). However, the magnitude of change in choroidal thickness found in primates is much smaller than that found in chicks. These two compensatory components (axial length and choroidal thickness) have different temporal dynamics in chicks: Choroidal compensation happens more rapidly (within a few hours), whereas axial compensation takes a day or two to occur (Zhu et al., 2005).

In real life, every region of the retina experiences a dynamic mixture of myopic and hyperopic defocus, changing constantly depending on one's fixation point, accommodative state, and the surrounding environment. Because the pattern of defocus in the retina changes rapidly over space and time, but the compensatory growth mechanism is relatively slow, the eye faces a significant challenge: The eye must integrate visual information over space and time to infer whether it needs to increase its length (or accelerate growth), reduce its length (or slow its growth), or to maintain its current size (or growth rate). To better understand the emmetropization mechanism, it is essential to study the eye's response not only to the average level of defocus but also to the variations in magnitude and type of defocus that occur naturally at each region of the retina during normal emmetropization, i.e., to study the temporal integration of visual signals.

This review summarizes studies on the temporal integration of visual signals. Significantly, experimental results have led to a greater appreciation of the fact that the temporal integration of different types of retinal defocus is decidedly non-linear. This review also asks the question if or how a strategy of lens wear in children might be able to exploit these nonlinearities to slow down or even arrest myopic progression.

1. The linear model of temporal integration of visual signals

It is now clear that the retina can use visual signals it receives to guide eye growth toward emmetropization through a local mechanism (Wallman et al., 1987; Diether and Schaeffel, 1997), even after the connection to the brain has been severed by optic nerve section (Troilo and Wallman, 1991; Wildsoet and Wallman, 1995). Given the massive number of visual signals the retina receives during every waking moment, how does the retina process these visual signals to guide eye growth? Flitcroft (1998) proposed that the retina simply averages visual signals over a period of time to guide eye growth toward emmetropization. Wallman and Winawer (2004) then described a simple linear model of emmetropization in which internal emmetropization signals rise and fall in a linear fashion (Fig. 2A). Imagine an emmetropic eye experiencing several brief episodes of defocus with darkness between these episodes: If the eye experiences two episodes of myopic defocus (of the same magnitude and duration), the hypothesized internal emmetropization signal would rise in the direction guiding compensation for myopic defocus in a linear fashion during exposure, incrementing like a counter, and remain stable between exposures (during darkness). If the eye then experiences an equally long period of hyperopic defocus, the signal would start going to the opposite direction guiding compensation for hyperopic defocus, decrementing again like a counter. If such a linear model accurately describes the retina's response to defocus (assuming that the retina weighs myopic and hyperopic defocus equally), then one can infer that: (1) Equal duration of myopic and hyperopic defocus would cancel each other out, leaving no accumulated signal, and thus normal eye growth; and (2) a more stringent test of linearity would examine the specific temporal dynamics with the prediction that the final magnitude of the signal (and the final compensation) depends on the total exposure to defocus that the retina experiences over time each day.

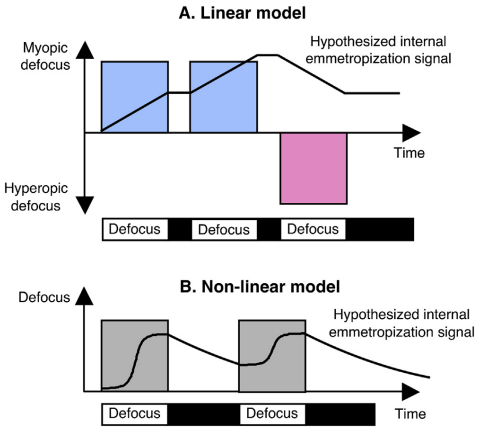

Fig. 2. Schematics for the linear model (A) and one type of the non-linear model (B) for the hypothesized internal emmetropization signal. The linear model (A) proposes that the signal is linearly related to the duration of defocus, and that signals produced by defocus of opposite signs cancel out each other. The non-linear model (B) proposes that the signal rises slowly and eventually saturates (provided the episode is long enough) and decays toward zero during periods of darkness between episodes. Adapted from Zhu and Wallman (Invest Ophthalmol Vis Sci, 2009b; 50:37–46), with permission from the Association for Research in Vision and Ophthalmology ©ARVO.

2. The non-linear model of temporal integration of visual signals

Results from previous studies, however, argue against this linear model. Indeed, there is strong evidence indicating three non-linearities in the temporal integration of defocus:

(1a) ***The retina does not weigh myopic and hyperopic defocus equally: Short periods of normal vision reduce experimental myopia caused by wearing negative lenses or occluders but have minimal effects on experimental hyperopia caused by wearing positive lenses***

It has been shown that a short period of "normal vision" (viewing without any lens or occluder on the eye) each day cancels myopia from wearing negative lenses or occluders during the rest of the day (Napper et al., 1995; Schmid and Wildsoet, 1996); by contrast, it takes a much longer period of normal vision to cancel out hyperopia from wearing positive lenses during the rest of the day (Schmid and Wildsoet, 1996). Specifically, in chicks, a daily episode of normal vision of as little as 2–3 h (17%–25% of the light phase) reduced myopia caused by wearing occluders (Napper et al., 1995) or negative lenses (Schmid and Wildsoet, 1996) the remainder of the day (9–10 h) by more than 95%. This phenomenon has also been found in tree shrews (Shaikh et al., 1999) and monkeys (Smith et al., 2002; Wensveen et al., 2006; Kee et al., 2007). Hyperopia caused by myopic defocus, on the other hand, is much more resilient in chicks: A daily episode of 3 h of normal vision (25% of the light phase) reduced hyperopia caused by wearing positive lenses the remainder of the day (9 h, 75% of the light phase) by only 10% (Schmid and Wildsoet, 1996). Furthermore, chicks that wore a positive lens for only 3 h per day and no lens for the remaining 9 h per day, still developed a significant hyperopic shift (Schmid and Wildsoet, 1996). When the percentage of myopia from each treatment paradigm (the final refractive error after wearing either negative lenses or occluders interrupted by normal vision as a percentage of the final refractive error after wearing these devices uninterrupted) in chicks, tree shrews, and rhesus monkeys is plotted against the duration of daily normal vision, a strikingly consistent trend is found across different species (Fig. 3): It is clear that as little as 2 h of daily normal vision reduced myopia progression by approximately 80% in all these different species (Smith et al., 2002). This suggests that the "diluted" response to wearing negative lenses or occluders might reflect an evolutionarily preserved strategy to prevent the eye from elongating too much in response to episodes of hyperopic defocus (Smith et al., 2002).

(1b) ***When positive and negative lenses are worn alternately, the eye is more responsive to myopic defocus and develops hyperopia***

In chicks, when positive and negative lenses are worn alternately, the eye is more responsive to myopic defocus and develops hyperopia, rather than averaging out the defocus of the opposite signs (Winawer and Wallman, 2002; Zhu et al., 2003; Winawer et al., 2005). Specifically, when Winawer and Wallman (2002) put positive and negative lenses alternately on chick eyes for equal duration per episode (with darkness between episodes), the eyes developed hyperopia, despite wearing negative lenses for the same duration (Fig. 4). When negative lenses were worn 5 times longer than positive lenses per episode, eyes still became hyperopic (Winawer and Wallman, 2002). In an extreme case, only four 2-min long daily episodes of positive lens wear canceled out the effect of wearing negative lenses the reminder of the day, and caused hyperopia (Zhu et al., 2003) (Fig. 4). Similar protective effects of

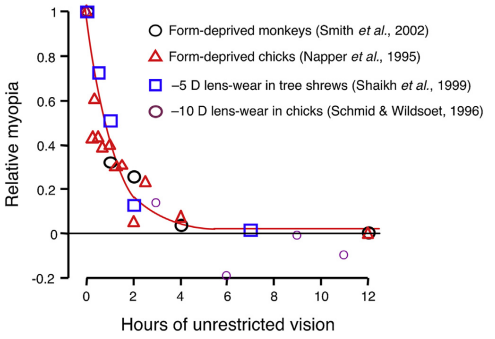

Fig. 3. The relationship between myopia and the amount of normal vision per day across different species. A similar amount of relative myopia (the final refractive error after wearing either negative lenses or occluders interrupted by normal vision, as a percentage of the final refractive error after wearing these devices uninterrupted) was found in various species wearing either negative lenses or diffusers interrupted by different amounts of normal vision. It is clear that 2 h of daily normal vision reduced myopia by approximately 80% in different species. Adapted from Smith et al. (Invest Ophthalmol Vis Sci, 2002; 43:291–9), with permission from the Association for Research in Vision and Ophthalmology ©ARVO.

myopic defocus were also found in tree shrews when young tree shrews wore positive lenses binocularly (McBrien et al., 2012), but were absent when older tree shrews wore positive lenses monocularly (Norton et al., 2006). On the other hand, myopic defocus was found to be less protective than normal vision in rhesus monkeys, possibly because the degree of imposed myopic defocus was too large (Kee et al., 2007).

In the real world, of course, the eyes do not experience any type of defocus for a sustained period of time, because of temporal fluctuation of defocus, depending on one's fixation point, accommodative state, and the surrounding environment. Therefore, to better mimic the visual experience in the real world, Winawer et al. (2005) designed a two-drum system that could project myopic and

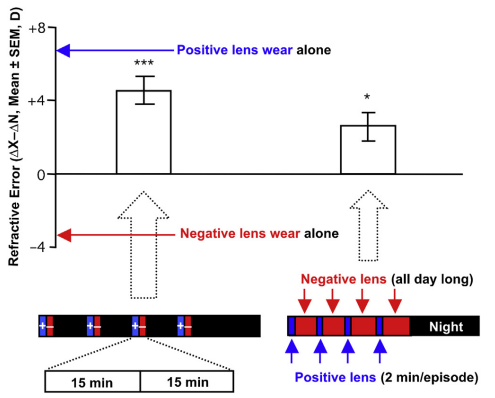

Fig. 4. The effect of brief, repeated episodes of positive lens wear on refractive error in chicks. Wearing positive lenses for brief, repeated episodes caused hyperopia, even though the eyes wore negative lenses for either the same duration (the bar on the left) or the rest of the day (the bar on the right). The blue and red arrows indicate the change in refraction (relative to control eyes) produced when the chicks wore only positive and negative lenses, respectively.

hyperopic defocus in chicks' eyes in rapid succession in a fraction of a second (Fig. 5). The chick was placed at the center of the inner drum made of a metal scrim. When the light source inside the inner drum was turned on and the light source outside the inner drum (between the inner drum and the outer drum) was turned off, the metal scrim appeared bright and opaque but the outer drum was dark and invisible from the point of view of the chick inside the inner drum. By contrast, when the light source inside the inner drum was turned off and the light source outside the inner drum was turned on, the metal scrim became dark and invisible to the chick while the outer drum appeared bright. When the chick was wearing a positive lens while sitting at the center of the inner drum (under cycloplegia), the inner and outer drums would project hyperopic defocus and myopic defocus, respectively (Fig. 5A). Therefore, by switching rapidly between the light sources inside and outside the inner drum, the authors were able to project myopic and hyperopic defocus in rapid succession to the lens-wearing eye (the fellow control eye had normal vision in a chamber) (Fig. 5).

Similar to previous findings, the authors found that myopic defocus had a dominating effect on lens compensation. Equal duration of brief myopic and hyperopic defocus (from 0.5 s to 15 min each) in rapid succession still caused hyperopia, and bigger hyperopic shifts were found with slower alternations. Unequal duration of brief myopic and hyperopic defocus (5 min of myopic defocus and 25 min of hyperopic defocus) also caused hyperopia. But, when the duration of myopic defocus was too short (0.5 s of myopic defocus and 2.5 s of hyperopic defocus), there was no obvious compensation, possibly because both myopic and hyperopic defocus canceled each other out.

Taken together, these findings support the hypothesis that, instead of using the "average" defocus signal over a period of time to guide emmetropization, the controller in the retina is much more sensitive to myopic defocus than to hyperopic defocus in the guidance of eye growth.

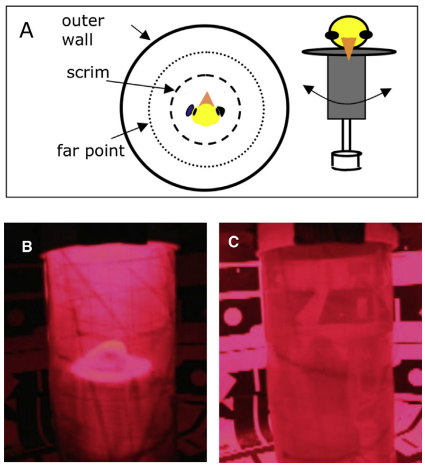

Fig. 5. Two-drum system. (A) The schematics of the two-drum system (top view). The inner and outer drums project hyperopic and myopic defocus to chick's lens-wearing eye, respectively. (B) and (C): Photographs of the inner scrim with the outer drum in the background. To the chick inside, the inner surface of the metal scrim appears opaque when illuminated from the inside (B), and clear when illuminated from the outside (C). Note that these photographs depict the effect of lighting on the appearance of the two drums, as viewed from outside the inner drum (not from the chick's perspective): When the outside surface of the inner drum appears transparent (B) or opaque (C) from the photographer's point of view, the inside surface of the inner drum will appear opaque (B) or invisible (C) from the chick's viewpoint inside the drum. Adapted from Winawer et al. (Vision Res, 2005; 45:1667–77).

(2) ***Lens compensation depends on the frequency and duration of individual episodes of lens-wear, not just the total exposure of lens wear per day***

In chicks, lens compensation has been found to depend on both the frequency of exposure to defocus and on the duration of each exposure, not just on the total duration of lens wear.

Schwahn and Schaeffel (1997) studied the effects of brief, repeated flickering lights on lens compensation and form deprivation myopia (FDM) in chicks. Chicks, wearing either positive or negative lenses, were raised in flickering lights of different frequencies (6 and 12 Hz) and duty cycles (an index of the relative durations of the light period and the subsequent dark period of a flicker cycle, expressed as the percentage of the flicker cycle that is occupied by the light period, 4%–75%). The duration of each light pulse ranged from a minimum of 3 ms (produced by a 4% duty cycle at 12 Hz) to a maximum of 83 ms (produced by a 50% duty cycle at 6 Hz). Despite the extremely short duration of each light pulse, chick eyes still partially compensated for defocus, although the magnitude of compensation was less compared that found in the normal lighting condition (no flicker). Similar results have been found in other studies (Crewther and Crewther, 2002, 2003; Crewther et al., 2006).

Winawer and Wallman (2002) gave chicks brief, repeated episodes of either positive or negative lens wear, with dark intervals between lens-wearing episodes. The chicks in different groups had the same total duration of lens wear per day, but with different durations of lens-wearing episodes at different frequencies, from 2 s every 2 min (the highest frequency, and the shortest lens-wearing episodes and the shortest dark intervals between lens wear) to 28 min every day (the lowest frequency, and the longest lens-wearing episodes and the longest dark intervals between lens wear). More frequent lens-wearing episodes (shorter duration per episode and shorter dark interval between episodes) caused more robust lens compensation, compared with less frequent lens-wearing episodes (longer duration per episode and longer dark interval). When the duration of each lens-wearing episode was less than a minute, however, very little compensation ensued.

Taken together, these findings also suggest that the temporal integration of visual signals is not simply a linear function and that the magnitude of lens compensation is not necessarily simply proportional to the total duration of lens wear. Ohngemach et al. (2001) discovered that the sensitivity to deprivation myopia varies over the day and that intermittent periods of normal vision inhibit deprivation myopia more if they occur in the evening than in the morning. It is possible that the emmetropization signal rises and saturates quickly during each lens-wearing episode (within a matter of minutes), and declines slowly between episodes, i.e., the nonlinear model of the hypothesized internal emmetropization signal (Fig. 2B) (Flitcroft, 1999; Wallman and Winawer, 2004; Zhu and Wallman, 2009b). Not only do these separate signals rise and decay in a non-linear fashion, but also the effects of the two signals add together in a non-linear manner.

(3) ***Lens compensation strongly depends on the sign of defocus and the ocular component***

In light of previous findings, it would be important to further analyze the temporal properties in lens compensation. Since it is still unclear which signaling molecules or biochemical pathways are responsible for the hypothesized internal emmetropization

signal, Zhu and Wallman (2009b) conducted two experiments to study the effects of the signal on axial length and choroidal thickness, and tried to infer the temporal properties of the rise and fall of the signal from the experimental results. The authors tried to find: (1) How brief they could make brief, repeated episodes of lens-wear (with darkness in between successive periods of lens wear) and still generate a robust response to lens wear; and (2) how long they could make the period of darkness between successive episodes of lens wear and still generate a robust response to lens wear. Consequently, in these experiments, the total duration of lens wear was different between different lens-wearing paradigms.

For several days, chicks were exposed to brief, repeated periods of either positive or negative lens wear, with a period of darkness between successive periods of lens wear in both experiments. In the first experiment, the authors varied the lens-wearing duration, and kept the frequency of lens wear constant (from 10 s to 10 min per hour). The axial and choroidal compensation resulting from each particular treatment paradigm were compared to those resulting from wearing lenses without any interruptions. The authors specifically calculated the "rise time" for both positive and negative lens wear: How long each brief repeated episode of lens wear should be to generate 50% of the maximum response to uninterrupted lens wear. In the second experiment, the authors held the duration of each repeated episode of lens wear constant (0.5 h per episode to ensure signal saturation), and varied the dark interval between lens wear (from 0.5 to 47.5 h). The authors studied the "fall time": How long each dark interval between lens wear should be for the emmetropization signal to decline sufficiently during the dark interval so that it can produce only 50% of the maximum response to uninterrupted lens wear. The authors showed that the rise times were very similar for both axial length and choroidal thickness for both positive and negative lenses: It takes a couple of minutes (1–4 min) for the emmetropization signal to rise to 50% maximum potency. The fall times for axial length, however, was drastically different for positive and negative lenses: It took 24 h for the emmetropization signal to decay by 50% for positive lenses, but only 0.4 h for negative lenses (Fig. 6). The fall times for choroidal thickness were also different for positive (6.7 h) and negative (3.2 h) lenses, even though the difference between the two was not as big as that found for axial length. Schwahn and Schaeffel (1997) found that the suppression of negative lens-induced myopia was correlated with the dark phase duration, but the suppression of positive lens-induced hyperopia was not, further supporting different fall-times for positive and negative lens compensation.

In summary, it seems that the controller in the retina does not just simply average out the defocus it experiences over a period of time and use the "mean defocus" to guide eye growth. Rather, there is a more complicated homeostatic controlling mechanism that generates a signal that rises and eventually saturates during defocus and decays slowly between episodes of defocus. The time constants for this signal are drastically different for axial length and choroidal thickness for positive and negative lens wear.

Furthermore, rather than the single emmetropization signal postulated in the linear model, it is likely that separate biochemical signals mediate the response to myopic defocus (growth inhibition) and hyperopic defocus (growth stimulation). In general, it is very hard to inhibit positive lens compensation compared with negative lens compensation, either visually or pharmacologically. First, as mentioned before, compensation for positive and negative lens shows distinctively different temporal dynamics (Schmid and Wildsoet, 1996; Schwahn and Schaeffel, 1997; Winawer and Wallman, 2002; Zhu et al., 2003, 2005; Zhu and Wallman, 2009b) and circadian rhythms (Nickla et al., 1998). Second, except for insulin, various pharmacological agents, such as muscarinic antagonists, dopamine agonists, and neurotoxins against catecholamines,

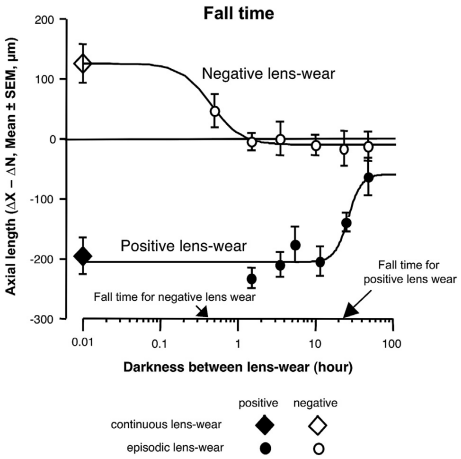

Fig. 6. The fall-times of the hypothesized internal emmetropization signals for ocular length for both positive and negative lens wear in chicks. The large diamonds represent the relative change in ocular length (change in ocular length in the lens-wearing eye over the course of the experiment minus the change in the follow, untreated eye) following uninterrupted lens wear (hence, zero hour of darkness between episodes), and the small circles represent the relative change following particular treatment paradigms of various dark intervals. It is clear that the paradigm of having the dark interval of 24 h between positive lens-wearing episodes still caused 50% of maximal ocular inhibition seen in continuous positive lens wear, and that only 0.4 h of dark interval between negative lens-wearing episodes already caused the maximal ocular elongation seen in continuous negative lens wear to decline by 50%. Therefore, the fall-times (arrows) are approximately 24 and 0.4 h for positive and negative lens wear, respectively. Adapted from Zhu and Wallman (Invest Ophthalmol Vis Sci, 2009b; 50:37–46), with permission from the Association for Research in Vision and Ophthalmology ©ARVO.

all block only myopia, not hyperopia (as recently reviewed by Ganesan and Wildsoet (2010)).

All in all, there are three nonlinearities in the temporal integration of visual signals in lens compensation: (1) The retina is more sensitive to myopic defocus than to hyperopic defocus, suggested by the findings that when myopic or hyperopic defocus is interrupted by a daily episode of normal vision, normal vision is more effective in reducing myopia caused by hyperopic defocus than in reducing hyperopia caused by myopic defocus, and that when the eye experiences alternating myopic and hyperopic defocus, the eye is more responsive to myopic defocus than to hyperopic defocus and tends to develop hyperopia, even if the duration of hyperopic defocus is much longer than the duration of myopic defocus. (2) When the eye experiences brief, repeated episodes of defocus by wearing either positive or negative lenses, lens compensation depends on the frequency and duration of the lens wear, not just the total duration of lens wear per day. (3) Further analysis of the time constants for the hypothesized internal emmetropization signals show that, while it takes approximately the same amount of time for the signals to rise and saturate during lens-wearing episodes, the decline of the signals between episodes depends strongly on the sign of defocus and the ocular component.

3. Significance of temporal properties of the non-linear model of the hypothesized internal emmetropization signal

The temporal properties of the non-linear model of the hypothesized internal emmetropization signal help explain why short

but frequent episodes of lens wear is more effective in causing lens compensation, compared with long but infrequent episodes of lens wear, while the total duration of daily lens exposure was kept the same. Compared with long but infrequent lens-wearing episodes, short but frequent exposures to defocus are more effective in generating the emmetropization signal because the short episodes can also cause the signal to rise and saturate, while the short dark intervals between frequent exposures prevent the signal from declining between lens-wearing episodes. Extremely short lens-wearing episodes (each less than a minute in duration), by contrast, do not give the emmetropization signal enough time to rise during episodes. Thus, no compensation takes place.

The long fall time of axial length for positive lenses found in chicks also helps explain why hyperopia is the "favored" result of compensation, even when the duration of positive lens wear is much shorter than that of negative lens wear.

The different fall times for axial length and choroidal thickness for positive and negative lens wear help explain the dissociation between axial and choroidal compensation previously discovered by Winawer and Wallman (2002). Specifically, when chicks wore spectacle lenses without interruption, axial and choroidal compensation occurred simultaneously, at least during the first few days of lens treatment. This was not necessarily the case during intermittent lens wear (with periods of darkness between lens-wearing episodes): If episodes of intermittent positive or negative lens wear were brief and frequent, both increased axial elongation and choroidal thinning (after wearing negative lenses) and axial inhibition and choroidal thickening (after wearing positive lenses) were found, similar to results with uninterrupted lens wear. But, if lens treatment was not frequent enough (when the duration of dark intervals between lens-wearing episodes were too long), only axial inhibition (after wearing positive lenses) and choroidal thinning (after wearing negative lenses) could still be found after several days of lens treatment (Winawer and Wallman, 2002; Zhu and Wallman, 2009b). A similar dissociation of axial and choroidal compensation was also found either when chicks' daily rhythms were interrupted by repeated light exposure during the night (Kee et al., 2001), or when chicks wore a weak diffuser over a positive lens (Park et al., 2003). It has been proposed that a signal cascade, which originates in the retina and passes through the retinal pigment epithelium and the choroid and eventually reaches the sclera, controls choroidal thickness and the scleral extracellular matrix biosynthesis, thus controlling ocular growth, as reviewed by Wallman and Winawer (2004). Given the different fall times for choroidal thickness and axial length, it is possible that there are two independent signals originated in the retina that control choroidal and axial compensation via independent mechanisms. On the other hand, Zhu et al. (2005) has shown that a transient choroidal thickening does take place right after each episode of positive lens wear (which could be missed if measured several hours later), and Nickla (2006, 2007, 2010) has shown that this transient choroidal thickening is necessary in reducing the rate of ocular elongation.

Understanding the temporal integration of visual signals can help us understand the etiology of myopia in school-aged children, and develop effective therapies to reduce myopic progression. If the findings on the temporal integration of visual signals found in chicks generalize to human myopia, they suggest that the temporal pattern of defocus, for example from near work such as reading, may play a larger role in determining one's myopic progression than the total amount of time one spends doing near work.

It is widely observed that myopia is highly associated with the level of education (Rosenfield and Gilmartin, 1998), and it has often been suggested that the aspect of education associated with myopia might be prolonged hyperopic defocus during reading. However, the correlation between the degree of myopic development and the total amount of near work (during which the eye is subjected to hyperopic defocus) per day is weak (Saw et al., 1996, 2001, 2002; Tan et al., 2000; Mutti et al., 2002), indicating that the total duration of reading per day may not capture the most important factor in determining myopic development. Indeed, the findings on the temporal integration of visual signals suggest that the temporal dynamics of near work (e.g., the duration of study and, especially, the length of breaks in between) should be further analyzed to predict whether a child will develop myopia, and may even help predict the speed of myopia progression.

4. Integration of simultaneous myopic and hyperopic defocus

In addition to the temporal integration of alternating myopic and hyperopic defocus, it is important to study the effect of simultaneous myopic and hyperopic defocus. It has been shown that presenting chick eyes with simultaneously competing myopic and hyperopic defocus, using either mixed astigmatic (toric) lenses with opposite lens powers on the two perpendicular meridians (McLean and Wallman, 2003), lens-cone devices with two target planes (Diether and Wildsoet, 2005) or dual-power lenses that had different combinations of lens powers (Tse et al., 2007), caused hyperopia, confirming the dominating effect of myopic defocus in chicks. Moreover, Benavente-Perez et al. (2012) reported similar results in marmosets by using multi-zone contact lenses with alternating powers. Therefore, it seems that when the retina experiences myopic and hyperopic defocus simultaneously, myopic defocus still dominates compensation, although to a smaller degree than when the eye experiences myopic and hyperopic defocus in succession. In the future studies of temporal and spatial integration of visual signal, one should analyze defocus from four dimensions: The sign of the defocus, the magnitude of the defocus, the spatial location of the defocus, and the duration of the defocus, to better control its effects on emmetropization (Tse et al., 2007).

5. Remaining questions

There are still many questions remaining to be answered regarding the temporal integration of visual signals. For example, what signaling pathways are responsible for the integration of visual signals? A number of signaling molecules have been suggested to be involved in emmetropization, e.g., glucagon (Fischer et al., 1999; Feldkaemper and Schaeffel, 2002; Vessey et al., 2005a, 2005b; Fischer et al., 2008; Zhu and Wallman, 2009a) and retinoic acid (Seko et al., 1996; Mertz and Wallman, 2000; McFadden et al., 2004), but it is not clear how these molecules work together to accurately control eye growth toward emmetropization.

Another question to ask would be: Does the non-linearity in the temporal integration of visual signals found in chicks also exist in primates or humans? If it does, to what extent? Also, is the signaling pathway in humans, assuming its existence, the same as that in chicks? In fact, the results found in animal studies lead to a puzzle about the development of human myopia: If myopic defocus protected against the development of myopia in humans, it would be very hard for school-aged children to develop myopia, as long as they had frequent brief breaks from near work, since very brief periods of myopic defocus would be sufficient to prevent myopia. So, why does myopic defocus seem less potent in arresting axial elongation in children than in chicks? One possibility is that school-aged children are beyond the age when myopic defocus can cancel out the effect of hyperopic defocus. A more optimistic possibility is that the pattern of temporal integration in children is different from the pattern found in chicks. Because the chick eye is growing so fast, the chick's emmetropization mechanism may have evolved to be sensitive to myopic defocus and to shut down axial elongation for

several hours after only a few minutes of exposure to myopic defocus. Because the child's eye is growing relatively slowly, the human emmetropization mechanism may require relatively long periods of exposure to more extreme myopic defocus before it will shut down axial elongation for hours or days.

Although alternating periods of myopic defocus and hyperopic defocus tend to produce slight hyperopia in chicks, this effect has not been found in children. However, projecting myopic defocus onto the peripheral retina (while allowing the central retina to receive clear images) may produce somewhat similar effects in chicks and in children. Placing dual-zone spectacle lenses on chicks, Liu and Wildsoet (2011) have shown that projecting myopic defocus onto the peripheral retina reduced axial elongation and caused hyperopia. In fact, myopic defocus slowed down axial elongation more effectively when that myopic defocus was confined to the peripheral retina rather than when the same amount of myopic defocus was projected onto the entire retina. Interestingly, recent studies have also shown that both dual-focus soft contact lenses (Anstice and Phillips, 2011) and contact lenses with progressive positive power in the periphery (Sankaridurg et al., 2011) can reduce the rate of axial elongation in childhood myopia. Both the dual-zone contact lens and a contact lens with progressive positive power in the periphery projected myopic defocus onto the peripheral retina, allowing the child to retain clear foveal vision and still receive a sufficiently strong dose of myopic defocus on the peripheral retina to slow down axial elongation. Furthermore, Phillips (2005) has shown that, when dominant eyes of school-aged children were corrected for distance and fellow eyes were undercorrected, myopia progression in the fellow eyes was significantly slower than in the distance-corrected dominant eyes, suggesting that the myopic defocus the fellow undercorrected eyes experienced at all levels of accommodation (driven by the dominant eye) reduced myopia progression. By confining myopic defocus to the peripheral retina of both eyes or to the entire retina of only one eye, clinicians may be able to slow down axial elongation in both eyes or in one eye respectively, while still enabling a child to have high acuity distance vision in the central visual field. Slowing down the progression of myopia and even preventing the development of myopia in school-aged children, perhaps, may not be a dream after all.

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