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.

Chapter 7: The effect of eye size on binocular lens compensation in chicks. I designed the experiments under the guidance of McFadden, collected the data assisted by

Sidhu A., and analyzed the data under the guidance of McFadden. I wrote the manuscript.

Some of the work in this thesis or arising from the paradigms developed in this thesis has been published in either a paper or abstract form:

1. Zhu X., Sidhu A., Cernota N. R, and Wallman J. The Effect of Eye Size on Lens-Compensation in Chicks. *Invest. Ophthal. Vis. Sci.* (2012) E-Abstract 3441.
2. Zhu X, McBrien NA, Smith EL, 3rd, Troilo D, Wallman J. Eyes in various species can shorten to compensate for myopic defocus. *Invest Ophthal Vis Sci* 2013;54:2634-2644.
3. Zhu X. Temporal integration of visual signals in lens compensation (a review). *Exp Eye Res* 2013;114:69-76.
4. Zhu X, Wallman J, and McFadden SA. Non-visual factors influencing emmetropization in chicks. *Invest. Ophthal. Vis. Sci.* (2016) E-Abstract 3791.

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Table of Contents

List of Figures

Figures in Appendices are listed below:

List of Tables

Tables in Appendices are listed below:

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.