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Plasticity in the growth of the chick eye: Emmetropization achieved by alternate morphologies

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ABSTRACT

Both refractive properties of the eyes and ambient light conditions affect emmetropization during growth. Exposure to constant light flattens the cornea making chicks hyperopic. To discover whether and how growing chick eyes restore emmetropia after exposure to constant light (CL) for 3, 7, or 11 weeks, we returned chicks to normal (N) conditions with 12 h. of light alternating with 12 h. of darkness (designated the "R", or recovery, condition) for total periods of 4, 7, 11, or 17 weeks. The two control groups were raised in CL conditions or raised in N conditions for the same length of time. We measured anterior chamber depths and lens thicknesses with an A-scan ultrasound machine. We measured corneal curvatures with an eight-axis keratometer, and refractions with conventional retinoscopy. We estimated differences in optical powers of CL, R and N chicks of identical age by constructing ray-tracing models using the above measurements and age-adjusted normal lens curvatures. We also computed the sensitivity of focus for small perturbations of the above optical parameters. Full refractive recovery from CL effects always occurred. Hyperopic refractive errors were absent when R chicks were returned to N for as little as 1 week after 3 weeks CL treatment. In R chicks exposed to CL for 11 weeks and returned to N, axial lengths, vitreous chamber depths and radii of corneal curvatures did not return to normal, although their refractions did. While R chicks can usually recover emmetropia, after long periods of exposure to CL, they cannot recover normal ocular morphology. Emmetropization following CL exposure is achieved primarily by adjusting the relationship between corneal curvature and axial length, resulting in normal refractions.

1. Introduction

It is well known that the growth of the eye is influenced by the state of focus on the retina, as well as by the magnitude and timing of illumination. In the early stages of growth, the eyes of chicks appear to be very malleable, responding with reversible changes in rate of vitreous chamber growth due to defocus (Wallman & Adams, 1987) and flattening of the cornea in constant light (Padmanabhan, Shih, & Wildsoet, 2007). It is the length of the malleability period following exposure to constant light (CL) that this paper addresses. The eye's response to CL and recovery from it is particularly interesting in that it involves simultaneous alterations of almost all the important optical parameters of the eye: the shape of the cornea, the depth of the anterior chamber, the shape of the lens, as well as the depth of the vitreous chamber (Li et al., 1995). It is generally assumed that only the refractive indices of the various media are unaffected. In this study we measured directly and estimated the changes in the optical parameters of the eye in response to CL and removal to normal (N) conditions with 12 h of light alternating with 12 h of darkness.

Raising chicks (*Gallus domesticus*) in CL alters proportional growth of the eye, producing the physiological change known as hyperopia, or "far-sightedness" (Harrison & McGinnis, 1967; Lauber, Schutze, & McGinnis, 1961; Li et al., 1992). CL chicks have small, flat, thick corneas with high stromal cell densities, shallow anterior chambers, and deeper vitreous chambers compared to N chicks (Li et al., 1995; Wahl et al., 2009).

Disproportionate growth resulting in CL-induced hyperopia is due to a damping effect of CL on the melatonin rhythm (Li & Howland, 2000). A reduction in average melatonin concentration occurs in the retina, pineal gland and blood circulation of CL chicks. When CL chicks are treated with melatonin eye drops during the subjective night, the eye is protected and grows normally. Conversely, when chicks in normal day/night cycles are treated with the melatonin receptor antagonist luzindole, they develop hyperopia (Li & Howland, 2002; Wahl et al., 2011).

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It is likely that shape changes of the eye are effected by connective tissues, because the higher stromal cell densities observed in CL chicks occur in corneas that are smaller than normal (Wahl et al., 2009), suggesting that the production of matrix is affected. There is a circadian rhythm in proteoglycan synthesis associated with the rhythm in ocular elongation (Nickla, Rada, & Wallman, 1999). The normal process of extracellular matrix accumulation may be slower in the mammalian CL sclera because collagen (hydroxyproline) and glycosaminoglycan production is decreased in CL (Norton & Rada, 1995). The intraocular pressure (IOP) of normal chick eyes is high during the day and low in the night (Li, Wahl, & Howland, 2002; Nickla, Wildsoet, & Wallman, 1998). The growth rhythm of the eye, as well as the IOP rhythm, are absent in CL conditions (Papastergiou et al., 1998). While these rhythms have not been established as important factors in normal ocular growth, their absence, correlated with abnormalities in ocular growth, is suggestive. Moreover, when melatonin rhythms are blocked in chicks raised in N conditions, they develop hyperopia (Li & Howland, 2002; Wahl et al., 2011).

The morphology and physiological optics of the chick eye are particularly sensitive to fluctuations in melatonin levels during the developmental period (Wahl et al., 2011). Since melatonin rhythms affect ocular growth (Wiechmann & Summers, 2008), and melatonin rhythms are damped by CL (Li & Howland, 2000, 2002) one can conclude that it is through this damping during development in CL that abnormal ocular growth occurs. We wished to determine whether, and for how long, these effects of CL-induced damping of the melatonin rhythm are reversible during the growth period of the chick. This has already been investigated in a study of lens-induced ametropia for recovery from a single age (Padmanabhan et al., 2007) which found that effects were reversible after hatchlings had been exposed to CL for 2 weeks.

Here we report on the extent of morphological and optical recovery possible in chicks following various durations of CL exposure. Because we found that refraction always returned to normal while morphology did not, we used a model eye to explain this outcome.

2. Methods

2.1. Animal husbandry and lighting regimes

Hatchling Cornell-K strain chicks (average weight 35.8 ± 2 g.) were used in this study, and they were 1 day old at the start of the experiment. The illumination level in the aviary was 700 lux during the light-on period. Illumination was supplied by fluorescent lamps (Sylvania 40 W, Cool White). Hatchling chicks were raised in temperature controlled brooders (30 °C). Food (Agway), crop gravel, and water were provided *ad libitum*. Two different control groups of chicks were raised either under N or CL for up to 17 weeks. The experimental group, R (“recovery”) was raised under CL for 3, 7, or 11 weeks (Table 1) and then placed in N for the remainder of the experiment. The number of chicks in each experimental group is given in Table 1.

All animals were handled in strict accordance with good animal practice as defined by the N.I.H. and the Cornell Institutional Animal Care and Use Committee (IACUC), and all animal work was approved by the Cornell IACUC under protocol number 89-101-01.

2.2. Corneal curvature and refraction measurements

All measurements were made on the right eyes of the chicks using techniques described in Li et al. 1995. We used an infrared keratometer and a conventional streak retinoscope to measure the corneal curvatures and refractions of the chicks. An “A” scan ultrasound (3M Biosound, Esoate, Indianapolis, IN) was used to measure axial length, fitted with a 10 MHz ultrasound probe extended with a 10 mm length of soft rubber tubing filled with ultrasound transmission gel (Aquasonic; Parker Laboratories, Fairfield NJ). Proparacaine HCl, (0.5%) was used as a corneal anesthetic. No other anesthetic agents were used in this study. The chick was hand-held and the open end of the tube was placed on the corneal surface near the optic axis. Prior work has shown that when both eyes of the chick receive the same treatment, the results in both eyes are virtually identical (Li & Howland, 2006) so only measurements from right eyes are reported. All measurements were made during the day between 10:00 am and 2:00 pm. We monitored those aspects of the eye known to be affected or possibly affected by CL. These included corneal radius of curvature, anterior chamber depth, refraction, lens thickness and vitreous chamber depth. Measurements were made at the end of the study for the two control groups of chicks (N or CL) exposed to 12/12 or constant light cycles. For the experimental chicks (R) measurements were made at the end of the experiment and at the times when chicks were changed from CL to a 12/12 cycle. Corneal curvature measurements were made by taking video images of reflections from an array of eight infra-red light emitting diodes arranged in a 30-cm circle around a video camera at a distance of 137 mm from the animal. Measurements were made in four orthogonal meridians and averaged. The distance between opposed LEDs is inversely proportional to the dioptric power of the cornea, and the apparatus was calibrated using ball bearings of known diameter (Glasser, Troilo, & Howland, 1994).

Table 1
Designations and numbers of control and experimental chicks in groups.

Type	Group	Number of chicks
Normal (control)	N4	6
“	N7	6
“	N11	8
“	N17	3
Constant light (control)	CL4	7
“	CL7	5
“	CL11	6
“	CL17	3
Recovery (experimental)	CL3/N1	6
“	CL3/N4	6
“	CL7/N4	2
“	CL11/N6	3

2.3. Bootstrap and Monte Carlo tests of significant differences between optical parameters of N, CL and R birds

Because some of our sample sizes were very small, we were reluctant to use conventional parametric statistics to compute significance differences between mean optical parameters of different treatments. Accordingly, we wrote a bootstrap computer program to compute the probabilities that the average numerical results of different treatments differ significantly from each other. We first entered the data for each treatment beginning with the number of data points, followed by the data. The number of data points in each treatment need not have been, and often were not, equal. The program then computed and stored the means of each treatment and their differences. For each comparison it gathered all of the data of the two treatments into one distribution. It then drew two samples (with replacement) from this distribution, each sample of the pair having the same size as one or the other of the treatments. This procedure was repeated 500,000 times. The ratio