Diurnal Fluctuations and Developmental Changes in Ocular Dimensions and Optical Aberrations in Young Chicks

Yibin Tian and Christine F. Wildsoet

Purpose. To investigate further the emmetropization process in young chicks by studying the diurnal fluctuations and developmental changes in the ocular dimensions and optical aberrations, including refractive errors, of normal eyes and eyes that had the ciliary nerve sectioned (CNX).

Methods. The ocular dimensions and aberrations in both eyes of eight CNX (surgery on right eyes only) and eight normal chicks were measured with high-frequency A-scan ultrasonography and aberrometry, respectively, four times a day on five different days from posthatching day 13 to 35. A fixed pupil size of 2 mm was used to analyze aberration data. Repeated-measures ANOVA was applied to examine the effects of age, time of day, and surgery.

Results. Refractive errors and most higher-order aberrations decreased with development in both normal and CNX eyes. However, although normal eyes showed a positive shift in spherical aberration with age, changing from negative spherical aberration initially, CNX eyes consistently exhibited positive spherical aberration. Anterior chamber depth, lens thickness, vitreous chamber depth, and thus optical axial length all increased with development. Many of these ocular parameters also underwent diurnal changes, and mostly these dynamic characteristics showed no age dependency and no effect of CNX. Anterior chamber depth, vitreous chamber depth, and optical axial length were all greater in the evening than in the morning, whereas the choroids were thinner in the evening. Paradoxically, eyes were more hyperopic in the evening, when they were longest. Although CNX eyes, having enlarged pupils, were exposed to larger higher-order aberrations, their growth pattern was similar to that of normal eyes.

Conclusions. Young chicks that are still emmetropizing, show significant diurnal fluctuations in ocular dimensions and some optical aberrations, superimposed on overall increases in the former and developmental decreases in the latter, even when accommodation is prevented. The possibility that these diurnal fluctuations are used to decode the eye's refractive error status for emmetropization warrants investigation. That eyes undergoing ciliary nerve section have more higher-order aberrations but do not become myopic implies a threshold for retinal image degradation below which the emmetropization process is not affected.

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It has become increasingly apparent that the eye cannot be viewed as a static system, optically, anatomically, or physiologically. A number of ocular parameters, including refractive errors, ocular dimensions and intraocular pressure (IOP), undergo dynamic changes on both short (seconds) and longer time scales. One interesting manifestation of these dynamics is the various diurnal rhythms that have been reported in conjunction with ocular function. For example, rhythms in melatonin production, IOP, pupil size, and corneal epithelial thickness have been reported.[1–6]

Of relevance to the current work are the more recent reports of diurnal fluctuations in ocular dimensions known to influence the refractive state of the eye. Chicks, rabbits, monkeys, and humans all show such fluctuations.[7–12] The fact that diurnal rhythms in ocular dimensions are a feature preserved across these various species suggests that these rhythms have functional significance, and it has been speculated that they subserve emmetropization, the process by which the growth of various ocular components of young eyes is regulated to achieve a developmental end point of emmetropia.[8,9,12] Such diurnal dimensional fluctuations will generate fluctuations in the type and amount of retinal image blur via their effect on the refractive state of the eye. That they may have significance for developmental eye growth regulation is the prediction from animal studies demonstrating that retinal image quality is important for normal eye growth. When retinal image quality is degraded (e.g., by putting a diffuser or defocusing lens in front of an eye), the eye shows altered growth responses. In the case of imposed defocus, the retina appears capable of distinguishing between the blur induced by plus and minus lenses to elicit compensatory growth responses (i.e., decreased and increased elongation, respectively).[13,14]

The cues used to decode the sign of defocus during emmetropization are not known. Plausibly, the eye could use odd-error cues from astigmatism and higher-order aberrations to decode the sign of defocus (Hunter J, et al. *IOVS* 2003;44: ARVO E-Abstract 4341).[15] Drawing on an analogy with accommodation in humans where accommodative microfluctuations play a role in decoding the sign of defocus,[16] diurnal fluctuations in refractive errors and/or higher order optical aberrations could play a similar role in emmetropization. Short-term fluctuations in higher-order aberrations[17] as well as changes on the scale of days, weeks, and months have been reported in young adult humans.[18,19] Because the power spectra of short-term aberrational changes and accommodative microfluctuations are very different from each other, it is likely that they have different origins. Although developmental decreases in both astigmatism and higher-order aberrations have been reported in very young chicks (Kisilak M, et al. *IOVS* 2002;43: ARVO E-Abstract 2924; Hunter J, et al. *IOVS* 2004;45:ARVO E-Abstract 4299; Kisilak M, et al. *IOVS* 2005;46:ARVO E-Abstract 1971),[20] no study to date has addressed the question of whether still-growing eyes show diurnal fluctuations in aberrations, as shown for IOP and ocular dimensions.

The purpose of the present study was to quantify the diurnal fluctuations in optical aberrations and ocular dimensions in the growing eyes of young chicks. Specifically, we were interested in whether there were diurnal fluctuations in refractive errors and/or optical aberrations that also were consistent and large enough to be used by the emmetropization mechanism to decode the sign of defocus. We also were interested in how such patterns evolve in growing eyes and whether optical and ocular dimensional changes are correlated. We made measurements in both normal chicks and those that had undergone unilateral ciliary nerve section that both eliminated all refractive fluctuations associated with accommodation and achieved maximum dilation of the pupil, thereby removing a primary limiting factor to aberrational influences on the retinal image. Some of these results have been reported in abstract form (Tian Y et al. *IOVS* 2005;46:E-Abstract 2283).[21]

METHODS

Animals

In this study, we used 16 White-Leghorn chicks (*Gallus gallus domesticus*) obtained from a commercial hatchery (Privett Hatcheries, Portales, NM) and housed in a University of California animal research facility. Illumination during rearing was provided by daylight (full-spectrum) fluorescent lighting, set to a 12-hour light (from 9 AM to 9 PM), 12-hour dark diurnal cycle. Food and water were provided ad libitum. Care and use of animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Lesioning Surgery

Half of the 16 chicks underwent ciliary nerve section (CNX) surgery on their right eyes at 2 days of age under isoflurane anesthesia (1.5% in oxygen).[22,23] This lesioning surgery served the primary purpose of eliminating accommodation and had the additional advantage of providing a maximally dilated pupil, so allowing the optical aberrations of the chick eye to be fully characterized. By preventing accommodation, which is known to influence optical aberrations in humans,[24] we were able to isolate growth-related changes. We chose surgery over using topical vecuronium bromide as a cycloplegic agent,[25,26] after observing significant degradation of the Hartmann-Shack images after its instillation multiple times a day (Tian Y, personal observations in a pilot study, 2004). Although previous studies in our laboratory have shown that eyes that undergo CNX surgery show near normal ocular growth patterns, they also are more hyperopic than normal.[22,23] For this reason, we included a group of control chicks that did not undergo any surgery ($n = 8$). The light general anesthesia and low light levels during measurements (described later) were used to minimize accommodative tone (albeit not eliminate it) in this group.

Measurements

Both ocular dimensions and optical aberrations were measured at regular intervals during a 3-week period, starting at 13 days of age. Ocular dimensions were measured by high-frequency A-scan ultrasonography.[8] Optical aberrations were measured with a wavefront analyzer (COAS; Wavefront Sciences, Albuquerque, NM),[27] a Hartmann-Shack wavefront-sensing device operating at the wavelength of 840 nm. Both the age range over which eyes of chicks are expected to emmetropize[28] and the quality of aberrometry images achievable were taken into consideration in choosing the starting age for measurements, the choice of 13 days being after the early rapid phase of emmetropization but before eye growth has slowed significantly.[28,29] For practical reasons, the two sets of measurements were made on separate days: A-scan ultrasonography on days 13, 16, 20, 23, and 34 and aberrometry on days 14, 17, 21, 24, and 35. On each measurement day, four sets of data were collected, at approximately 9 AM, 12 PM, 3 PM, and 7 PM. For all measurements, chicks were lightly anesthetized using isoflurane (1% in oxygen), delivered through a custom-made head-holder that stabilizes the chick's head posture. All aberration measurements were made in the dark, to prevent accommodation and to facilitate pupil dilation in eyes with intact ciliary nerves, with six good readings (Hartmann-Shack image dots sharp and uniformly distributed; optical axes of the eye and wavefront sensor aligned) being recorded per eye. CNX and control chicks were measured alternately and in each chick, the right eye was always measured before the left eye.

Data Analysis

The axial dimensions of the principal ocular components as well as the thickness of the three components making up the back wall of the eye—retina, choroid and sclera—were derived from the ultrasonography traces collected. Anterior chamber depth, lens thickness, vitreous chamber depth, choroidal thickness, and optical axial length data are presented herein. Optical axial length described the distance from the anterior corneal surface to the anterior retinal surface and is derived from the sum of anterior chamber depth (note that anterior chamber depth includes cornea thickness), lens thickness, and vitreous chamber depth, thus defining the location of the retina, the image processing tissue of the eye.

The Optical Society of America (OSA) and American National Standard Institute (ANSI) standard Zernike polynomials were used in analyzing aberration data.[30–32] Standard refraction terms—spherical equivalent refractive error (SERE, M) and primary astigmatism (J)—were derived from second-order Zernike coefficients (C_2^0, C_2^{-2}, and C_2^2) and the corresponding pupil radius (r), as shown in equations 1 and 2.

$$M = -\frac{4\sqrt{3}C_2^0}{r^2} \quad (1)$$

$$J_{180} = -\frac{2\sqrt{6}c_2^2}{r^2},\ J_{45} = -\frac{2\sqrt{6}c_2^{-2}}{r^2},\ J = \sqrt{{J_{180}}^2 + {J_{45}}^2} \quad (2)$$

Coma (C_3^{-1} and C_3^1), trefoil (C_3^{-3} and C_3^3), secondary astigmatism (C_4^{-2} and C_4^2), quatrefoil (C_4^{-4} and C_4^4), and the total third- and fourth-order aberrations are expressed in terms of root mean square (RMS) values. For simplicity, we refer to the total third- and fourth-order aberrations as total higher-order aberrations (HOA). For reporting purposes, equivalent defocus powers (EDPs) expressed in diopters, were derived from RMS values (equation 3) except for spherical aberration, where the relevant Zernike coefficient instead of the RMS is used to preserve its sign, which may be important in the context of emmetropization.

$$\text{EDP} = \frac{4\sqrt{3}\text{RMS}}{r^2} \quad (3)$$

Note that all parameters are subject to variation with pupil size. In this study, a 2-mm pupil diameter was used to analyze aberrometry data, unless stated otherwise.

Unless otherwise stated, difference data are presented to describe either changes across time or interocular differences when CNX and normal eyes are compared. Repeated-measures analysis of variance (ANOVA), combined with the Tukey-Kramer post hoc test where necessary, was applied to the data to examine the effects of age and time of day on measured parameters. The data from CNX eyes were also compared with data from normal eyes (i.e., fellows of CNX eyes as well as eyes of control animals). Statistical analyses made use of commercial software (StatView; SAS Cary, NC; and MatLab; The MathWorks, Natick, MA).

RESULTS

In this study, we tracked the ocular changes in chicks over a 3-week period, starting at 13 days of age, at which time they

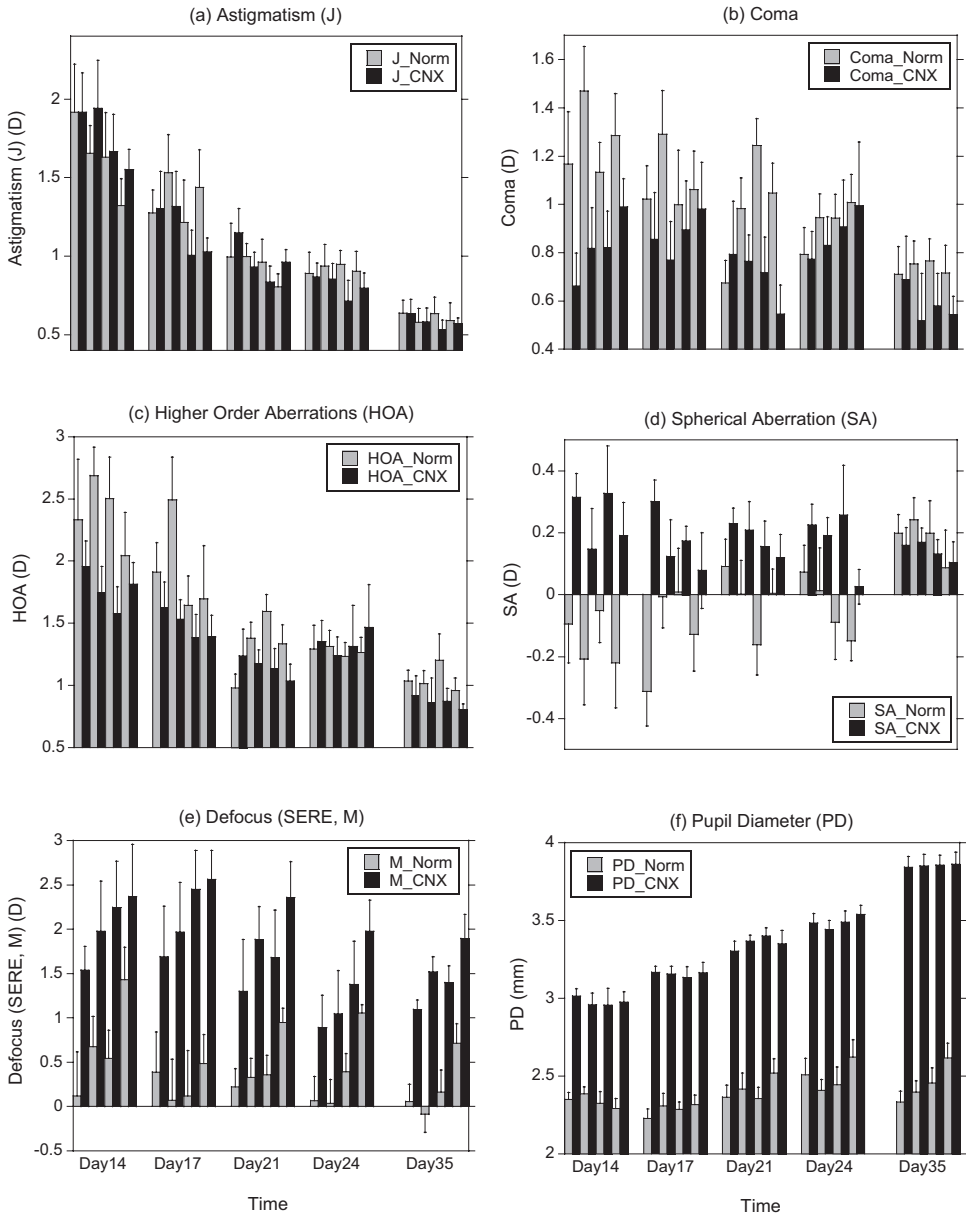

FIGURE 1. Mean aberrations and pupil sizes of the CNX eyes and right eyes of the control group. Each bar is the mean of eight eyes. (**a**) Astigmatism, (**b**) coma, (**c**) higher order aberrations, (**d**) spherical aberration, (**e**) defocus, and (**f**) pupil diameter. Each group of bars represents data collected on 1 day. Each bar represents one measurement time point (9 AM, 12 PM, 3 PM, and 7 PM, from *left* to *right*). Error bars, SE.

would have already undergone significant emmetropization but their eyes were expected to be still growing rapidly. Our data confirmed the latter, and both refractive errors and optical aberrations decreased over the same period, indicating that eyes were able to compensate for on-going ocular growth as they emmetropized. In addition to the developmental changes, significant diurnal fluctuations were observed in many of the parameters measured, both optical and dimensional (Figs. 1–4). These changes are described in detail in the following sections.

Measurement Order Effect

Because we consistently measured right eyes before left eyes, we first analyzed for order effects by using data from the control group of chicks that did not undergo surgery. Analyses revealed a significant order effect for three of the optical parameters measured: SERE, astigmatism, and coma. Specifically, compared with left eyes (OS), right eyes (OD) were more hyperopic (OD: +0.40 D; OS: −0.38 D), and had more coma (OD: 1.00 D; OS: 0.83 D), but less primary and secondary astigmatism (OD: 1.09 D and 0.35 D, respectively; OS: 1.37 D and 0.43 D, respectively; $P < 0.05$ in all cases; repeated-measures ANOVA). Possible explanations are offered in the Discussion section. These order effects placed constraints on possible interocular comparisons, with analyses of developmental and diurnal data mostly confined to the right eyes of the CNX and normal groups. No order effects were evident in the ocular dimensional data.

Developmental Changes

Data from both the lesioned right eyes of the CNX group (i.e., CNX eyes) and the right eyes of the control group were used in characterizing the developmental changes over the 3-week study period. Both groups showed decreases in spherical equivalent refractive errors (SERE) although both remained hyperopic, and these changes did not achieve statistical significance. In contrast, developmental decreases in astigmatism and most higher-order aberrations also occurred and were mostly statistical significant. For example, in the right eyes of control chicks, primary astigmatism (Fig. 1a), coma (Fig. 1b),

TABLE 1. Mean Dimensional and Optical Aberration Changes

Ocular Parameter	Control Group		CNX Group	
	Overall Changes	*P*	Overall Changes	*P*
Refractive error (SERE) (D)*	−0.481	0.60	−0.554	0.24
Astigmatism (D)	−1.021	<0.01	−1.189	<0.01
Spherical aberration (D)	0.326	<0.01	−0.104	0.74
Higher order aberrations (D)†	−1.337	<0.01	−0.908	<0.01
Coma (D)	−0.527	<0.01	−0.291	<0.01
Trefoil (D)	−1.212	<0.01	−0.748	<0.01
Quatrefoil (D)	−0.478	<0.01	−0.416	<0.01
Pupil diameter (mm)	0.211	<0.01	0.877	<0.01
Anterior chamber depth (mm)	0.366	<0.01	0.382	<0.01
Lens thickness (mm)	0.549	<0.01	0.605	<0.01
Vitreous chamber depth (mm)	0.922	<0.01	0.873	<0.01
Choroidal thickness (mm)	0.046	<0.01	0.079	0.038
Optical axial length (mm)	1.837	<0.01	1.861	<0.01

Data are shown for right eyes of control ($n = 8$) and CNX groups ($n = 8$) over the 3-week study period. Overall changes shown are the maxima of between-day differences given by Tukey-Kramer post hoc tests following repeated-measure ANOVA; the between-day differences were largest between last and first measuring days except for choroidal thickness in CNX eyes, where maximum change occurred between days 20 and 34. Negative signs indicate decreases, and positive signs indicate increases over time.

* Spherical equivalent refractive error, derived from Zernike coefficient C_2^0.

† Total third- and fourth-order aberrations.

trefoil, quatrefoil, and total higher-order aberrations (Fig. 1c) all showed statistically significant decreases over the monitoring period (Table 1). The normal left eyes of both the control and CNX groups, as well as the CNX eyes, showed similar changes that were also statistically significant. However, the right eyes of CNX and control groups showed very different trends in spherical aberration. Whereas the right eyes of the control group initially exhibited negative spherical aberration that gave way to positive spherical aberration over the monitoring period, the CNX eyes consistently exhibited positive spherical aberration (Fig. 1d). Differences in accommodative tone provide a plausible explanation for this difference (see the Discussion section).

Emmetropization serves not only to correct neonatal refractive errors but also to coordinate postnatal ocular growth to prevent the introduction of refractive errors. That the eyes grew significantly over the study period, whereas refractive errors declined, is consistent with on-going emmetropization. All three major compartments of the eye, the anterior chamber (Fig. 2a), the lens (Fig. 2b), and the vitreous chamber (Fig. 2c) grew significantly over the experimental period, in both normal and CNX eyes (Table 1). Right eyes of both the CNX and control groups also showed choroidal thickness increases during this period, although their growth patterns were different (Fig. 2e). Whereas the choroids of right eyes of control group showed a slow, linear increase in thickness with increasing age, the choroids of the CNX eyes, which were initially significantly thicker than normal (by 55 μm, $P = 0.034$, one-tailed *t*-test), thinned to less than normal (by 15 μm, day 20, $P = 0.034$, one-tailed *t*-test), before becoming again thickener than normal (by 40 μm, day 34, $P < 0.001$, one-tailed *t*-test), as is reflected in their U-shaped growth profile.

In growing eyes, some decrease in optical aberrations can be expected as a consequence of geometric scaling, quite apart from any active emmetropization process (Kisilak M, et al. *IOVS* 2005;46:ARVO E-Abstract 1971).[33] The growth (scaling)–related contributions to observed decreases in the optical aberrations were estimated for the right eyes of our control group using the following equation

$$\frac{Z_t}{Z_0} = \frac{1}{k_t^n} \quad (4)$$

where k_t is a scaling factor derived from the optical axial length data (modified from Howland[33]; in the cited paper, cornea diameters were used). Values were normalized to the optical axial length recorded on the first measurement day; thus, the minimum k_t is 1 (i.e., day 14; note there is always a 1-day discrepancy between the axial length and aberrational data; axial length data corresponding to days 13, 16, 20, 23, and 34 are used to scale aberrations measured on days 14, 17, 21, 24, and 35). The exponent n has an empirical value between 2 and 2.9; we used the middle value of 2.5. Estimates for primary astigmatism, coma, trefoil, and HOA, expressed as ratios of Z_t/Z_0, are plotted against age in Figure 3 (solid line), along with normalized raw aberration data (lines with markers). Geometric growth accounts for most (92.4%) of the observed decrease in coma but only 69.8% of the decrease in HOAs, and its contributions to the changes in primary astigmatism and trefoil are even smaller: 42.7% and 59.3%, respectively.

To understand fully the visual implications of the changes in the optical aberrations of the eyes, it is important to quantify how pupil size changed during development. The pupil diameters of the right eyes of both the CNX and control groups increased over the monitoring period—those of CNX eyes more so (pupil size increase: 0.877 mm vs. 0.211 mm respectively; Fig. 1f). Although the use of a fixed pupil size in our data analysis has merit, allowing the optical quality of equivalent optical regions to be compared, it cannot detect changes related to developmental changes in pupil size. Because aberrations increase with pupil size, the fixed pupil data reported herein are likely to overestimate the decrease in aberrations on the natural pupil and the improvements in retinal image quality with development, a point picked up in later discussion.

Effect of Time of Day

We were interested in diurnal changes in the optical parameters of the eye because of their potential ramifications for

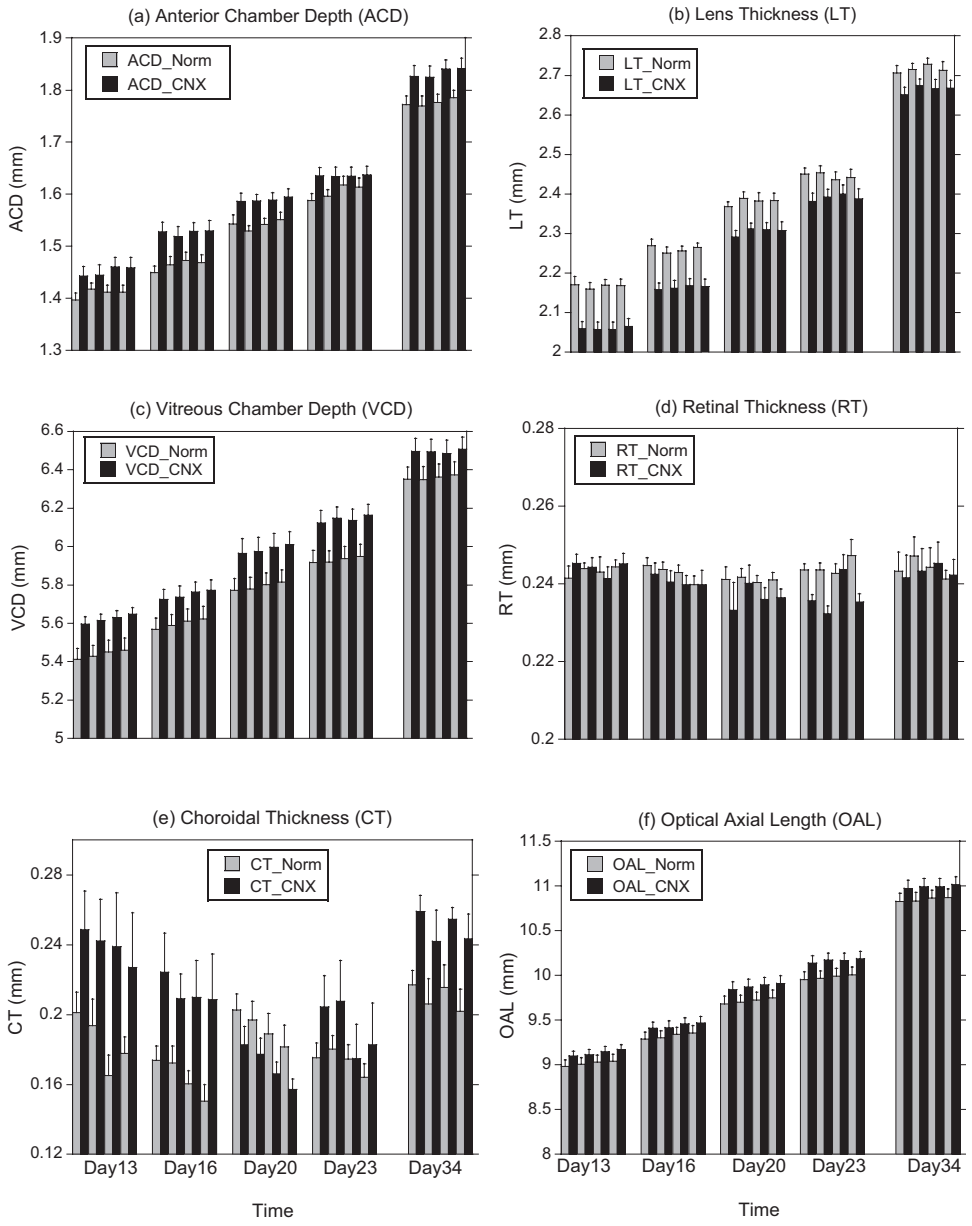

FIGURE 2. Mean ocular dimensions of the CNX eyes and right eyes of control group. Each bar represents the mean of eight eyes. (**a**) Anterior chamber depth, (**b**) lens thickness, (**c**) vitreous chamber depth, (**d**) retinal thickness, (**e**) choroidal thickness, and (**f**) optical axial length. The data are presented as described in Figure 1.

emmetropization. To be able to understand these data fully, we also recorded equivalent ocular dimensional data. Relevant data for both right and left eyes of CNX and control chicks are summarized in Table 2. Some of these data are also illustrated in Figure 4.

Both CNX eyes and right eyes of control chicks showed significant hyperopic shifts in their SERE across the day (Fig. 4a). However, most aberrations decreased across the day. For example, both HOA (Fig. 4b), and trefoil (Fig. 4d) tended to decrease across the day for both groups although normal eyes, but not CNX eyes showed an early morning increase before again decreasing; nonetheless, the diurnal fluctuations in HOA and trefoil were statically significant for both groups. Both groups showed similar diurnal decreases in astigmatism, although the changes were only significant for CNX eyes (Fig. 4c), and only the right eyes of control chicks showed significant diurnal fluctuations in coma. The same trends also were evident in the left eye data of both CNX and control groups and were statistically significant for SERE, HOA, and trefoil. There were diurnal fluctuations in spherical aberration, which was more negative in the evening, and although the patterns were similar across groups, only the changes in left eyes reached statistical significance.

Consistent with previous reports,[8] the right eyes of the control group exhibited significant diurnal fluctuations in anterior chamber depth (Fig. 4e), vitreous chamber depth, choroidal thickness (Fig. 4f), and optical axial length (Fig. 4g). Although the choroid thinned across the day, both anterior and vitreous chambers enlarged, resulting in a net increase in the optical axial length across the day. The CNX eyes exhibited similar diurnal patterns for all four parameters, the changes reaching statistical significance in all cases. CNX but not normal eyes also recorded significant diurnal fluctuations in lens thickness (Fig. 4h). This result was also unexpected, given that the ciliary muscles of these eyes were no longer innervated.

Significant diurnal fluctuations in pupil size were recorded in the right eyes of the control group but not CNX eyes, consistent with the fact that the pupils of the CNX eyes were atonic.

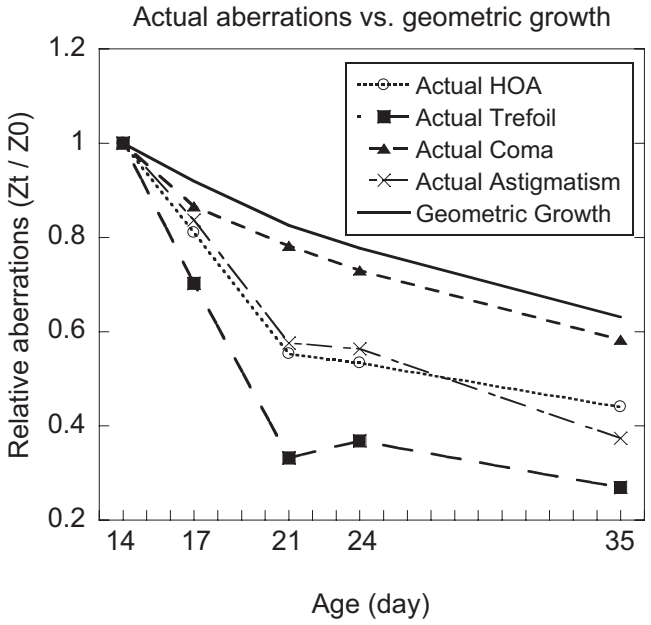

FIGURE 3. Measured decrease in aberrations over time in the right eyes of the control group. Data represent the mean of eight eyes. The estimated contribution to developmental decrease in aberration from geometric ocular growth is also shown. The *y*-axis represents the ratios of Z_t/Z_0, where Z_0 represents aberrations on day 14, and Z_t represents aberrations on day *t*. The scaling effect of ocular growth largely accounts for the developmental decrease in coma, but not in other aberrations.

Effect of CNX Surgery

As indicated earlier, part of the rationale for including CNX surgery was to avoid the need for repeated cycloplegia. However, because CNX eyes have larger than normal pupils and no accommodation, they potentially offer additional insights into the influence of optical aberrations and accommodation on emmetropization. To characterize the CNX eyes further, they were compared with the normal right eyes of the control group after we first ruled out any effect of the surgery on the fellow eyes of the CNX birds through a comparison of their left eyes with the left eyes of the control group. The left eyes of CNX and control chicks were not significantly different from each other, either in terms of their optical properties or ocular axial dimensions ($P > 0.1$ for all parameters, repeated-measure ANOVA).

In refractive terms, CNX eyes were, on average, significantly more hyperopic than were right eyes of the control group (CNX − control = +1.356 D, $P < 0.001$; Fig. 1e), and in relation to their ocular dimensions, there also were significant intergroup differences; CNX eyes had deeper anterior chambers (CNX − control = 0.048 mm, $P = 0.034$; Fig 2a), thinner lenses (CNX − control = −0.077 mm, $P = 0.006$; Fig. 2b), deeper vitreous chambers (CNX − control = 0.175 mm, $P = 0.044$; Fig. 2c) and thicker choroids (CNX − control = 0.028 mm, $P = 0.013$; Fig 2e). The difference in optical axial length (Fig. 2f) between the groups was in the same direction as the vitreous chamber difference but was reduced due to the neutralizing effect of lens thickness differences that were opposite in sign, although not statistically significant (CNX − control = 0.146 mm, $P = 0.198$).

The optical properties of CNX and normal eyes are compared under both fixed (2 mm) conditions and more natural pupil conditions. Because the CNX eyes had larger than normal pupils, the latter comparison provides a more realistic picture of differences in retinal image quality, given that aberrations typically increase with pupil size. However, the use of a standardized 2-mm pupil in aberration analyses also has merit, allowing comparison of equivalent optical zones in CNX and normal eyes and so isolation of lesion-related effects on the optical elements (i.e., corneal and lenticular).

Overall, CNX eyes had larger pupils (CNX − control = 0.969 mm, $P < 0.001$; Fig. 1f). The pupils on CNX eyes not only started off larger than those of normal eyes (CNX: 2.976 mm, control: 2.339 mm; $P < 0.001$, one-tailed *t*-test), but also enlarged more over the monitoring period (CNX: 0.877 mm, control: 0.211 mm; $P < 0.001$, one-tailed *t*-test).

For a fixed 2-mm pupil size, CNX eyes exhibited relatively more positive spherical aberration (CNX − control = 0.208 D, $P = 0.04$; Fig. 1d) but there were no significant differences between the two groups for any other aberration term. Further comparisons using 3.5 and 2.0 mm pupil diameters for CNX eyes and right eyes of the control group, respectively, are shown in Figure 5 for total HOAs, expressed in terms of both RMS and EDP. Note that, although some eyes had larger pupils than the values used in these analyses, these settings allowed all eyes to be included. As expected, the CNX eyes recorded larger aberrations than the normal eyes, although statistical significance was reached with the RMS data only (for RMS, CNX: 0.669 μm, normal: 0.149 μm; one-tailed *t*-test, $P < 0.001$; for EDP, CNX: 1.51 D, normal: 1.04 D; one-tailed *t*-test, $P = 0.079$). Point-spread functions derived from data for representative CNX and normal eyes (each has the median HOA in its group), imply substantial differences in their retinal image quality (Fig. 5, images).

DISCUSSION

In seeking an explanation for the rising prevalence of myopia world-wide, interest has focused on the influence of optical aberrations on refractive development. In this study, we tracked both developmental changes and diurnal fluctuations in optical aberrations and ocular dimensions in the eyes of young chicks, to address two different but interrelated questions: (1) whether optical aberrations, similar to refractive errors, undergo emmetropization, and (2) whether there are diurnal fluctuations in aberrations that are consistent and large enough to be used by the emmetropization mechanism. Our findings are reviewed in the context of these two questions.

Emmetropization and Optical Aberrations

Are the eye's optical aberrations subject to developmental emmetropization? With pupil size fixed at 2 mm, both the CNX and the normal eyes showed significant developmental decreases in astigmatism and most HOAs over the study period, although their spherical equivalent refractive errors showed only small declines in hyperopia that did not reach statistical significance over the same period. These observations complement and extend those reported in two recent studies involving younger chicks (from hatching to approximately 2 weeks of age; Kisilak M, et al. *IOVS* 2005;46:ARVO E-Abstract 1971).[34] In both studies, HOAs were found to decrease with age, even though in one case, analyses took into account differences in pupil size (derived values similar to the EDP used in this study; Kisilak M, et al. *IOVS* 2005;46:ARVO E-Abstract 1971), and in the other case, pupil size was fixed to 1.5 mm and the RMS was used.[34] A decrease in the mean HOA-RMS of 0.09 μm (EDP, 0.63 D) from hatching to day 13 was found in the latter case. The 3-week decrease in HOA-EDP in our study was 1.34 D on 2-mm pupil diameter in normal eyes.

TABLE 2. Diurnal Changes in Ocular Components and Aberrations

Ocular Parameters	Normal Group		CNX Group	
	Diurnal Changes	*P*	Diurnal Changes	*P*
SERE (D)*				
OD	0.755	<0.01	<0.01	<0.01
OS	0.677	0.015	0.786	<0.01
Astigmatism (D)				
OD	−0.131	0.342	−0.223 (9 AM vs. 3 PM)	0.017
OS	−0.130	0.343	−0.193	0.218
Spherical aberration (D)				
OD	−0.090	0.064	−0.109	0.168
OS	−0.159	0.010	−0.159	0.025
Trefoil (D)				
OD	−0.332 (12 PM vs. 7 PM)	0.039	−0.194 (9 AM vs. 3 PM)	0.001
OS	−0.325 (12 PM vs. 7 PM)	0.013	−0.409	0.045
Higher-order aberrations (D)†				
OD	−0.317 (12 PM vs. 7 PM)	0.019	−0.159 (9 AM vs. 3 PM)	0.047
OS	−0.332 (12 PM vs. 7 PM)	0.034	−0.492	0.043
Pupil diameter (mm)				
OD	0.117	0.028	0.023	0.845
OS	0.112	0.045	−0.154	0.042
Anterior chamber depth (mm)				
OD	0.016	<0.01	0.01 (12 PM vs. 7 PM)	0.011
OS	0.015	<0.01	0.008	0.047
Lens thickness (mm)				
OD	0.002	0.980	0.012 (9 AM vs. 3 PM)	0.012
OS	0.006	0.453	0.007	0.292
Vitreous chamber depth (mm)				
OD	0.040	<0.01	0.039	<0.01
OS	0.034	0.049	0.036	<0.01
Choroidal thickness (mm)				
OD	−0.019	<0.01	−0.020	<0.01
OS	−0.020	<0.01	−0.022	<0.01
Optical axial length (mm)				
OD	0.058	<0.01	0.058	<0.01
OS	0.055	<0.01	0.053	<0.01

Data are shown for right and left eyes of CNX ($n = 8$) and control ($n = 8$) chicks. Diurnal changes represent maximum within-day changes (i.e., between two measurement time points) given by the Tukey-Kramer post hoc tests after repeated-measures ANOVA. Results are for 9 AM vs. 7 PM differences, unless otherwise indicated in the parentheses. Negative numbers indicate decreases, and positive numbers indicate increases. OD, right eyes; OS, left eyes. Diurnal changes are shown graphically for CNX and control right eyes in Figure 4.

* Spherical equivalent refractive error, derived from Zernike coefficient C_2^0.

† Total third- and fourth-order aberrations.

To address the question of whether the developmental decreases in aberrations are a product of active processes, one must first rule out geometric scaling (as a result of the eye's axial and equatorial enlargement) as an alternative explanation (Hunter J, et al. *IOVS* 2004;45:ARVO E-Abstract 4299).[33] For normal eyes, we were able to account for most of the developmental decrease in coma in terms of scaling, whereas its contribution was lower (43%–59%) for astigmatism and trefoil, as well as for HOAs (70%), based on our calculations for the right eyes of the control chicks. Thus, the possibility that optical aberrations are corrected through an active mechanism cannot be ruled out. However, it should be noted that the geometric model used in our calculations assumes isometric ocular growth yet ocular growth is not perfectly isometric, and thus our estimates may not reflect the true picture.

In the present study, normal eyes showed a transition from negative to positive spherical aberration over a 2- to 5-week age span, whereas in one of the related studies cited earlier,[34] spherical aberrations are reported to stabilize near zero after a period of irregular changes over the first 6 days after hatching. The larger values observed in our study in the normal eyes, which were also older, may reflect differences in measurement protocols (especially pupil size analyzed) and/or strain-related differences.[34–36] We also observed a significant difference in spherical aberration between the CNX and normal eyes. The CNX eyes consistently exhibited positive spherical aberration and had larger aberrations overall than did the normal eyes under more natural pupil conditions. Point-spread functions (PSFs) based on HOA alone (Fig. 5, inset) indicate that retinal image quality is reduced in CNX eyes (PSF for the CNX eye was much more spread out), yet the growth pattern of these eyes was near normal. Because chicks show robust responses to form deprivation, increasing their rate of eye growth to become myopic,[37] the latter result for CNX eyes suggests that their HOAs were not sufficient to derail normal eye growth regulation. That the CNX eyes showed rates of decline in hyperopia similar to those in the normal eyes and that they had more hyperopic end-point refractive errors than did normal eyes represent further evidence that eye growth had not been derailed by these HOAs. However, it must be noted that RMS and EDP are not always good predictors of visual performance, due to interactions between various aberrations and aberration–visual target interactions, as demonstrated in humans.[38–40] For the chicks in our study, the features of their cage environment are critical determinants of the effects of their aberrations on visual performance.

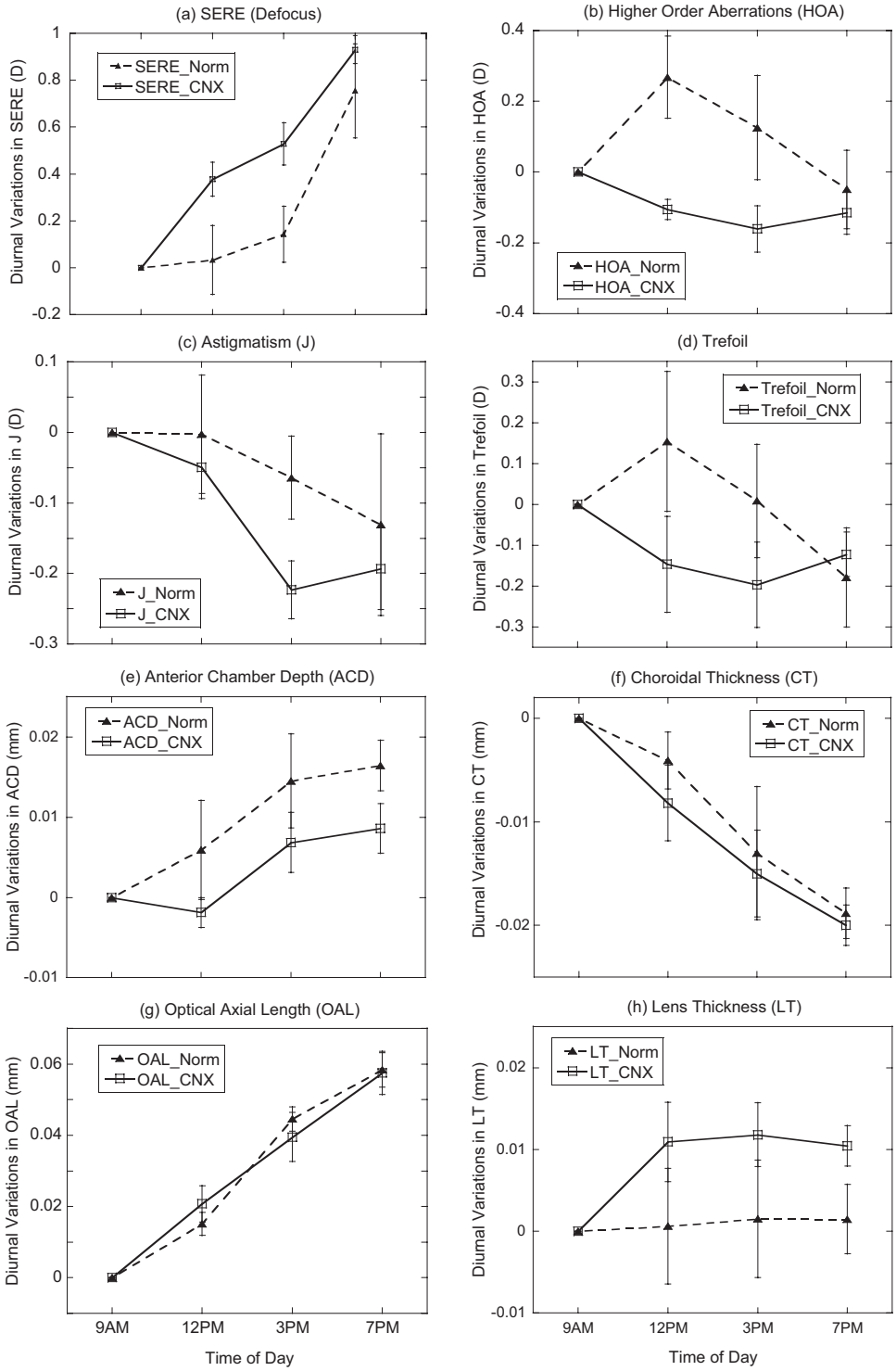

FIGURE 4. Diurnal variations in ocular aberrations and axial dimensions. (**a**) Defocus, (**b**) higher order aberrations, (**c**) astigmatism, (**d**) trefoil, (**e**) anterior chamber depth, (**f**) choroidal thickness, (**g**) optical axial length, and (**h**) lens thickness. Data points represent diurnal changes, averaged over the five measurement days (CNX eyes, $n = 8$; right eyes of control chicks, $n = 8$). The values shown are given by Tukey-Kramer post hoc tests after repeated-measures ANOVA (within-day changes from 9 AM to 12 PM, 9 AM to 3 PM, and 9 AM to 7 PM, respectively). Error bars, SE.

Diurnal Fluctuations in Optical Defocus and HOAs and Potential Significance in Emmetropization

Because the chicks in the present study were 2 weeks of age at the start of the monitoring period, it can be assumed that they had already undergone significant emmetropization. Indeed, both CNX and control groups showed only small, statistically insignificant decreases in hyperopia over the study period. However, that the emmetropization process was still operational is evidenced by the failure of these eyes to become myopic in the presence of significant eye elongation over the study period (a myopic shift of approximately −30 D is predicted, based on mean increases in optical axial lengths of 1.837 mm for right eyes of normal chicks, 1.861 mm for CNX eyes).[41] It must be assumed that this elongation serves to offset developmental changes in the eye's two optical components: flattening of the cornea and the crystalline lens.

Both CNX and normal eyes were significantly more hyperopic in the evening than in the morning, with changes of similar magnitude in the two groups (−0.93 D, CNX eyes; −0.76 D, normal eyes). These results correspond closely to the

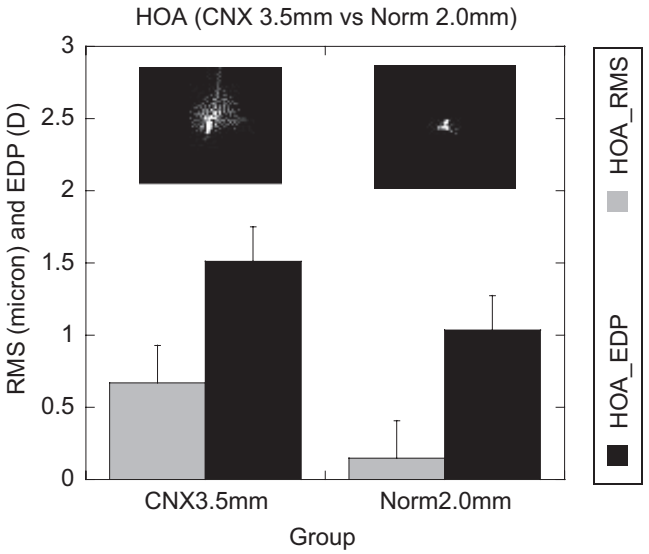

FIGURE 5. RMS and EDPs of higher-order aberrations (HOA) for CNX eyes (3.5 mm pupil diameter, $n = 8$) and right eyes of control chicks (2 mm pupil diameter, $n = 8$). Error bars, SE. *Insets*: PSFs calculated from the HOAs in representative eyes (one CNX eye and one right eye from control group; HOA-RMS represents the median for the corresponding group).

findings of another recent chick-based study in which normal eyes were found to be 0.6 D more hyperopic in the evening than in the morning (Johnson C, et al. *IOVS* 2004;45:ARVO E-Abstract 4295). In the present study, we also found significant diurnal fluctuations in some HOAs.

We asked whether observed diurnal changes in refractive errors and/or aberrations could have guided emmetropization. Experimental studies in the chick provide convincing evidence that young eyes are able to distinguish the sign of defocus and appropriately alter their eye growth to compensate for imposed focusing errors. HOAs combined with defocus produce sign-dependent differences in PSFs, and it has been speculated that eyes may make use of such differences to distinguish between myopic and hyperopic defocus during emmetropization (Hunter J, et al. *IOVS* 2003;44:ARVO E-Abstract 4341). It is known that these patterns can be distinguished perceptually,[15] although it is not known whether the retina can distinguish them. Estimates for the depth of focus for the chick eye vary from approximately 0.7 D, derived from ganglion cell density data based on a 2-mm pupil diameter to <1 D, based on an experimental emmetropization study.[42] These data place observed diurnal fluctuations in refractive error near the depth of focus limit of the normal chick eye, and so they are possibly detectible. Also, since "pure" (spherical) defocus is traditionally used to calculate depth of focus without regard to HOAs, the functional depth of focus is likely to be smaller than the 0.7 D obtained when astigmatism and HOA are also taken into account (Hunter J, et al. *IOVS* 2003;44:ARVO E-Abstract 4341).[15] CNX eyes would have a still smaller depth of focus corresponding to their larger pupils. Thus the diurnal fluctuations in defocus noted in our study are likely to have been detectable.

Accommodation, a biological focusing system like emmetropization, provides an example where fluctuations in focus can be used to guide focus adjustments; in this case, the directional signal appears to be extracted from microfluctuations in accommodation via a hunting (trial-and-error) process.[16] The autofocus systems in some digital cameras also have successfully used trial-and-error strategies to determine the correct focal plane.[43] Whether the observed diurnal fluctuations in refractive error serve a similar function for emmetropization depends on its temporal characteristics (integration time). However, as little as 2 minutes of positive lens wear is sufficient to elicit a compensatory (emmetropization) response in young chicks,[44] implying either that compensation is not trial-and-error, or that the integration time is shorter than this.

The Contradiction between SERE and Ocular Dimensional Fluctuations

Both CNX and normal eyes were significantly more hyperopic in the evening than in the morning, yet the prediction based on optical principles that their optical axial lengths would be shortest in the evening is opposite to the observed changes, with both groups recording the longest optical axial length in the evening (mean diurnal change = 0.058 mm, for both the CNX eyes and the right eyes of the control group). These apparently paradoxical results appear to be robust as similar diurnal changes in both refractive error (Johnson C, et al. *IOVS* 2004;45:ARVO E-Abstract 4295) and optical axial length[6,7] have been reported previously, albeit in independent studies, and thus their explanation is likely to be in diurnal fluctuations in one or more of the optical components of the eye.

Diurnal fluctuations in tonic accommodation can be ruled out as a possible explanation for the disparity between diurnal refractive error and optical axial length changes because CNX eyes and normal eyes showed similar trends. In search of other explanations for this result, optical modeling calculations were undertaken to estimate the refractive effects of the various diurnal dimensional changes. Because the magnitudes of refractive error changes in CNX and normal eyes were similar, these calculations were limited to normal eyes. With respect to the components contributing to optical axial length, the change in lens thickness was negligible and the increase in vitreous chamber depth (VCD), in the wrong direction to account for this disparity. Indeed, the diurnal change in SERE that must be explained increased to 1.855 D when the effect on refractive error of the VCD change of 0.04 mm was accounted for (myopic refractive error shift = 1.1 D from equation 5, based on VCD_0 of 6.0 mm, close to the average VCD on day 20). The change of 0.016 mm in anterior chamber depth (ACD), accounts for only 0.08 D of this discrepancy (equation 6, corneal radius of curvature = 3.5 mm; Padmanabhan V, unpublished data, 2005; corneal refractive index = 1.336,[45] corneal power $P_c = 96$ D; lens power $P_l = 50$ D, based on schematic eye estimations).

$$\Delta RE = \frac{1}{VCD_0} - \frac{1}{VCD_1} \quad (5)$$

$$\Delta P = P_c P_l (\Delta ACD) \quad (6)$$

These results leave fluctuations in the curvatures of one or more of the optical surfaces of the eye (anterior and posterior cornea, anterior and posterior lens) as the likely explanation for the residual error, although curvature measurements were not included in the present study. Corneal curvature fluctuations would explain why both CNX and normal eyes present the same paradox; furthermore, only a small amount of corneal flattening, e.g., from 3.50 to 3.55 mm, is needed to completely account for the diurnal hyperopic shift in SERE, because of the cornea's steep curvature and the large refractive index gradient at the anterior corneal surface. Such curvature fluctuations could result from diurnal IOP fluctuations; IOP increases across the day.[6,7] They could also result from corneal thickness fluc-

tuations,[19,46] although this second possibility has not been studied in the chick.

Significance of Observed Differences between CNX and Normal Eyes

In terms of eye growth and refractive development, the CNX and normal eyes generally showed similar behavior, although CNX eyes tended to have thinner than normal crystalline lenses and they also were consistently more hyperopic than normal. The optical axial length result is consistent with other studies[6,23] and is likely to be product of emmetropization, increased ocular elongation serving to offset the expected decrease in lens power resulting from the CNX surgery. The latter interpretation also is consistent with the lens thickness data (i.e., lens thinning associated with the loss of ciliary muscle tone).

Reduced ciliary muscle tone in CNX eyes may also underlie observed differences in spherical aberration between CNX and normal eyes. In humans, increasing accommodation is linked to a shift from positive to negative spherical aberration.[24] Based on this finding and because CNX eyes cannot accommodate, one would predict them to exhibit positive spherical aberration as observed consistently over the study period. According to the same line of argument, the change from initially negative spherical aberration to positive spherical aberration in normal eyes over the study period suggests a developmental decline in ciliary muscle tone in these eyes.

The finding that the pupils of the CNX eyes were larger than normal is an expected outcome of the lesioning surgery, which eliminates the neural input to the iris sphincter muscle. However, the finding that the pupils of the CNX eyes enlarged at a faster rate than normal was unexpected. It implies that the CNX eyes were expanding faster equatorially than normal, and, although relevant parameters were not measured in the present study, this interpretation is consistent with results from a study involving ciliary ganglionectomy in chicks.[47] A growth modulatory role for the ciliary nerve and/or ciliary muscle tone is implied.

Finally, CNX and normal eyes also exhibited different developmental profiles in relation to choroidal thickness. In CNX eyes the choroid thinned early in the 3-week monitoring period and then thickened again, whereas the choroids of normal eyes showed slow incremental growth. The early choroidal thinning in CNX eyes may represent a compensatory response to hyperopic blur,[48] since these eyes were also more hyperopic than normal. However, although these eyes remained more hyperopic than normal, there was no late increase in hyperopia corresponding to the later choroidal thickening. The latter response may reflect the loss of a tonic influence on choroidal thickness mediated by the choroidal branch of the ciliary nerve, and revealed when compensatory scleral growth changes, which lag behind choroidal changes,[41] reduce the defocus on the retina and thus reduce the influence of defocus on choroidal thickness. This interpretation rests with the assumption that the CNX surgery disrupted the innervation to the choroid, a possibility consistent with the report of choroidal thickening in eyes undergoing ciliary ganglionectomy.[6] Differences in the ages of chicks in the present study and the latter cited study[6] are the likely explanation for why altered diurnal choroidal thickness rhythms were reported after ciliary ganglionectomy only.

Possible Measurement Artifacts

It is generally assumed that the two eyes of normal animals will have similar ocular dimensions, refractive errors, and aberrations, as is the case in most humans.[49] This was also true of the axial ocular dimensions in our normal birds. However, we found significant interocular differences in the SEREs and some optical aberrations in normal birds that we speculate to be a product of the gaseous (isoflurane) anesthesia used for all measurements. The interocular difference in SEREs in CNX birds is also consistent with this explanation. Its value (1.989 D), is close to the sum of the SERE differences between (1) the right eyes of CNX and normal groups (mean = 1.356 D, an index of the lesioning effect) and (2) the right and left eyes of normal chicks (mean = 0.783 D). That we observed this order effect only in refractive error and aberrational data presumably reflects the much longer time required for aberrometry compared to ultrasonography (i.e., 15 minutes vs. <5 minutes). The last measured (left) eyes were relatively myopic, presumably reflecting a relative increase in parasympathetic tone with the deepening of anesthesia over this time, although other explanations, including blood flow changes in the ciliary body, cannot be ruled out.

The assumption that the two eyes of normal chicks have similar refractive errors and aberrations was confirmed by conducting aberrometry once on eight 3-week old normal chicks. The procedures were the same as described in the Methods section except that right eyes were measured first in half of the chicks and left eyes first in the other half. No significant interocular differences were found ($P > 0.1$ in all cases, two-tailed *t*-test) in SERE (mean ± SD: OD = 0.41 ± 0.80 D, OS = 0.34 ± 1.05 D), astigmatism (OD = −1.25 ± 0.64 D, OS = −1.32 ± 0.47 D), coma (OD = 0.96 ± 0.39 D, OS = 0.90 ± 0.53 D), trefoil (OD = 1.03 ± 0.68 D, OS = 1.21 ± 0.66 D), spherical aberration (OD = −0.01 ± 0.05 D, OS = −0.01 ± 0.02 D), second astigmatism (OD = 0.32 ± 0.13 D, OS, 0.42 ± 0.20 D), quatrefoil (OD = 0.56 ± 0.40 D, OS = 0.60 ± 0.30 D) and total HOA (OD = 1.69 ± 0.52 D, OS = 1.75 ± 0.74 D).

Although observed diurnal changes in SERE were in the wrong direction to be explained as an artifact of the multiple daily measurements (i.e., hyperopic instead of myopic), we collected further confirmatory data from the right eyes of nine additional 4-week-old normal chicks that were measured using the same procedures as described in the Methods section but only twice daily, once in the morning (~10 AM) and once in the evening (~7:00 PM). These additional data show the same diurnal trends as described earlier for normal eyes (i.e., more hyperopic refractive errors; change in SERE = 0.68 D), thinner choroids (by 0.03 mm), and longer optical axial lengths (by 0.05 mm) at the end of the day. There were also significance changes in trefoil (−0.29 D), and HOAs (−0.31 D) over the day ($P < 0.05$ in all cases, one-tailed paired *t*-test).

Conclusion

The eyes of young chicks rapidly elongate between 2 and 5 weeks of age, yet they are able to avoid corresponding myopic shifts in their refractive errors, irrespective of whether accommodation is functional or not, implying that it is not a fundamental prerequisite for emmetropization, as expressed by the maintenance of emmetropia in eyes that are still elongating as part of normal development. Over the same age span, there are decreases in astigmatism and higher-order aberrations that can only partly be explained by geometric scaling, raising the possibility of active emmetropization as an alternative explanation for these changes. There are also diurnal fluctuations in spherical equivalent refractive error and some higher-order aberrations that are independent of age and accommodation and that may be used to decode the eye's refractive error status during emmetropization, a possibility that warrants further investigation.

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