Astigmatism Associated with Experimentally Induced Myopia or Hyperopia in Chickens

Chea-su Kee1,2 and Li Deng1

PURPOSE. Astigmatism is a very common refractive error in humans, but its etiology is poorly understood. The primary purpose of this study was to determine whether alterations in visual experience would result in astigmatism in chicks.

METHODS. Longitudinal and cross-sectional data were obtained from chicks that were raised undergoing four different visual manipulations known to alter axial eye growth: form deprivation by translucent occluders, spherical defocus by −10- or +10-D lenses, and constant light. The visual manipulations began at 5 days of age and continued for a week. Age-matched groups raised without any treatment or with Velcro rings or plano lenses served as control groups. Refractions in all birds were measured with a Hartinger refractometer, and infrared photokeratometry was performed in a subset of birds at the end of the treatment period.

RESULTS. In control birds, natural astigmatism decreased in magnitude over the 7-day treatment period. In contrast, birds treated with visual manipulations developed significant amounts of astigmatism throughout the treatment period. At the end of the 7-day treatment period, whereas only 8.6% of the control chicks had refractive astigmatism ≥1 D, the percentage of treated birds that had astigmatism ≥1 D in each treatment group ranged from 66.7% to 100%. The astigmatism in the treated eyes was predominantly against-the-rule, corneal in nature, and correlated significantly with spherical ametropia of the principal meridians.

CONCLUSIONS. Visual manipulations known to induce axial ametropia also promote the genesis of astigmatism in chicks. The characteristics of astigmatism associated with spherical myopia or hyperopia in chicks is similar to those reported in humans in many respects, supporting the hypothesis that vision-dependent changes in eye growth may contribute to the astigmatism commonly found in humans. (Invest Ophthalmol Vis Sci. 2008;49:858–867) DOI:10.1167/iovs.06-1370

ASTIGMATISM

Astigmatism is a very common refractive error in which the eye's refractive power varies from one meridian to the next. In regular astigmatism, the most common form in humans,1,2 the least and the most powerful refractive meridians are separated by 90°. Of particular concern are the findings that significant amounts of astigmatism are highly prevalent in school-age children (28% in the United States [see also Ref. 4]; 23% to 58% in urban areas of Asian countries5–7), are increasing in frequency in adults after the age of 40,9–11 and are highly prevalent in American Indians12–14 and those with ocular diseases.15–16 Even with spectacle corrections, these affected populations were frequently found to have abnormal retinal electrophysiology,17 abnormal refractive development,7,18 amblyopia,19–21 and migraine headache.22 However, despite numerous clinical and animal studies focused on refractive development,23 the etiology of astigmatism is poorly understood.24

The sources of ocular astigmatism are mainly corneal and lenticular toricity.1 Of particular interest is the strong and significant correlation between total astigmatism and corneal astigmatism in humans.25–28 and macaque monkeys.29,30 However, what causes the change in ocular toricity remains unclear. Previous models for the genesis of astigmatism have centered on mechanical factors that could act directly on the cornea. For example, astigmatic errors have been shown to correlate with the specific location of eyelid abnormalities,15,31 the alterations in normal eyelid tension,32,33 and the physical relationship between the cornea and the contact lens in contact lens wearers.34–35 On the other hand, although astigmatism has been associated with abnormal optic disc shape in humans,36–38 the relationship between the genesis of astigmatism and posterior structural abnormalities has not received much attention. Given that astigmatism is associated with ametropia—39 and that alterations in ocular refraction and size are primarily a consequence of structural and molecular changes that occur at the posterior segment,39–41 it is possible that astigmatism is a passive byproduct of abnormal posterior axial eye growth. This hypothesis is in line with the suggestion that axial eye growth may alter anterior ocular structures through stretching45,46 and the fact that changes in axial length correlate significantly with changes in corneal power or lens power during early infancy.37

Exactly how abnormal axial eye growth promotes the genesis of astigmatism is unclear. However, there is ample evidence that spherical ametropia and astigmatism coexist. First, it is well known that infants frequently exhibit both spherical ametropia and astigmatism.36,46,48 Second, the magnitude of astigmatism correlates significantly with those of spherical myopia30,50–52 and hyperopia.53 Third, as predicted by the passive regulatory hypothesis just mentioned, simple astigmatism (i.e., one principal meridian is emmetropic while the other is ametropic) is less common. It has been reported that compound astigmatism (i.e., both principal meridians are ametropic) is two to three times more common than simple astigmatism.2,55 Fourth, a variety of visual manipulations known to influence postnatal axial eye growth have been...
shown to result in both axial ametropia and astigmatism in monkeys.\textsuperscript{30} The coexistence of astigmatism and spherical ametropia in these findings suggests that the mechanisms underlying astigmatism and spherical ametropia are probably related. If so, experimental conditions that lead to spherical ametropia are also likely to promote the development of astigmatism.

There is evidence that abnormal visual experience can promote astigmatism in monkeys and chickens. It has recently been demonstrated that monkeys frequently exhibit significant amounts of astigmatic errors when treated with diffusers or spherical or astigmatic lenses.\textsuperscript{29,30} More important, regardless of the treatments they receive, the characteristics of astigmatism observed in monkeys are remarkably similar, supporting the hypothesis that astigmatism may be a byproduct of abnormal eye growth. In chicks, despite their common use as an animal model for refractive-error development, effects of visual manipulations on the genesis of astigmatism have been reported in only a few studies.\textsuperscript{54–56} There has not been a systematic study on the characteristics of astigmatism associated with spherical ametropia in chickens. The primary purpose of this study was to determine whether a variety of visual manipulations, known to produce axial ametropia, would promote the development of astigmatism. We found that altered visual experience led to both spherical ametropia and astigmatism in chickens.

METHODS

Animal Subjects

Our subjects were White Leghorn chicks (\textit{Gallus gallus domesticus}, K strain, Cornell University, Ithaca, NY) raised in the animal nursery facility of the New England College of Optometry. Food and water were provided ad libitum. The light cycle was set for a 12-hour light/12-hour dark cycle, with an average light intensity of approximately 300 lux on the brooder’s floor. All the rearing and experimental procedures were reviewed and approved by the New England College of Optometry’s Institutional Animal Care and Use Committee and were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Treatment Groups

We used four visual manipulations all known to induce consistent axial ametropia in chicks. The first three treatments monocularly manipulate the visual image by either form deprivation (white translucent plastic hemispheres, or diffusers) or optical spherical defocus (+10-D and −10-D lenses; Conforma-K PMMA lenses; Conforma Laboratories, Norfolk, VA). To facilitate the removal of these optical devices for cleaning purposes, we first glued the diffuser or lens to a Velcro ring (Velcro USA, Inc., Manchester, NH) with optical adhesive (Norland Products Inc., New Brunswick, NJ) and later attached it to the Velcro’s mated fastener which was glued to the feathers around the treated eye (Collodion; Fisher Scientific, Fairlawn, NJ). The fourth treatment exposed both eyes of chicks without any optical devices to constant light in a 5000-cm\(^2\), evenly lit (±100 lux), sound-proof chamber. The treated eye was chosen based on the tagged number assigned to each animal (i.e., right eyes were used for even numbers, left eyes for odd numbers). The same rule was used for control chicks and chicks reared in constant light conditions. All the treated birds, including those from both longitudinal and cross-sectional studies, received visual manipulations starting from 5 days of age and ending at 12 days of age (i.e., the visual manipulations lasted for 7 days).

Experiment 1: Longitudinal Study

After the initial biometry measurements (described later) that were performed at 5 days of age, each chick was randomly assigned to one of four experimental groups: form deprivation by translucent diffusers \((n = 8), \) spherical defocus by −10-D \((n = 9)\) or +10-D \((n = 11)\) lenses, and constant light \((n = 10)\). Refractometry was performed on both eyes after 1, 2, 3, 4, and 7 days of the visual treatments. In a subset of chicks \((n = 29)\), keratometry was performed on the treated eye at the end of the 7-day treatment period.

Experiment 2: Cross-Sectional Study

The purposes of cross-sectional study were: (1) to determine whether the effects of visual manipulations had been affected by repeated biometry measurements in the longitudinal study and (2) to determine the contribution of corneal astigmatism to total astigmatism. Four groups of birds were reared while undergoing one of four visual manipulations: form deprivation by diffusers \((n = 11)\), spherical defocus by −10-D \((n = 10)\) or +10-D \((n = 11)\) lenses, and constant light \((n = 10)\). Both refractometry and keratometry were performed on the treated eyes at the end of the treatment period.

Control Groups

Because high magnitudes of astigmatism were found in the treatment groups throughout the treatment period in both the treated and fellow eyes (data provided later), both normal and control groups were included in the study. In addition to including age-matched untreated birds for longitudinal \((n = 11)\) and cross-sectional studies \((n = 10)\), the potential effects of the presence of the Velcro ring or PMMA lenses on refractive development were tested by rearing two additional control groups that wore either a Velcro ring \((n = 5)\) or a plano lens \((n = 9)\) over one eye from 5 to 12 days of age. Although the untreated control groups for longitudinal and cross-sectional studies underwent biometry measurements similar to those of the treatment groups, those that wore a Velcro ring or plano lens underwent refractometry measurements at 12 days of age only. At that time point, no significant differences were found in refractive astigmatism or the J0 or J45 astigmatic component either between the treated and fellow eyes or across the four groups of control birds (MANOVA, interaction \([\text{eyes} \times \text{groups}]\) and main effects; all \(P \geq 0.3\)). There were also no significant interocular differences (treated minus fellow eye) of spherical equivalent refractive error, refractive astigmatism, or the J0 or J45 astigmatic component across the four groups of birds (one-way ANOVA, all \(P \geq 0.07\)). Because of these results, the data of all normal and control birds at 12 days of age were combined and treated as a group.

Biometry Measurements

To avoid the potential variations in refractive error due to diurnal rhythms (Johnson CA et al. \textit{IOVS} 2004;45:ARVO EAbstract 4295), all biometry measurements were performed at about the same time during the day (±2 hours). To make the measurements, each animal was anesthetized by isoflurane inhalation (1.0%-1.5%), and their eyelids were gently held apart by the same custom-made speculum. For all animals that wore a Velcro ring or plano lens underwent refractometry measurements at 12 days of age only. At that time point, no significant differences were found in refractive astigmatism or the J0 or J45 astigmatic component either between the treated and fellow eyes or across the four groups of control birds (MANOVA, interaction \([\text{eyes} \times \text{groups}]\) and main effects; all \(P \geq 0.3\)). There were also no significant interocular differences (treated minus fellow eye) of spherical equivalent refractive error, refractive astigmatism, or the J0 or J45 astigmatic component across the four groups of birds (one-way ANOVA, all \(P \geq 0.07\)). Because of these results, the data of all normal and control birds at 12 days of age were combined and treated as a group.
formed on the treated eyes at the end of the 5-day treatment period. The realignment took less than 5 minutes. The treated birds wore diffusers for each astigmatic component were derived with Fourier analysis. The three readings was collected in each eye, and the average values for each astigmatic component were derived with Fourier analysis. The short-term repeatability of Hartinger refractometry in measuring refractive astigmatism was tested by obtaining two sets of consecutive data from the treated eyes of the birds used in the experiment. The medians for corneal curvature, astigmatism, and axis were distributed, we used the Rayleigh test and Keratometry

**Refractometry**

Refractive errors of the two principal meridians were measured along the pupillary axis with a modified Hartinger's coincidence refraometer (Jena Coincidence Refractometer; Carl Zeiss Meditec, GmbH, Jena, Germany) and specified in negative correcting cylinder form. A set of three readings was collected in each eye, and the average values for each astigmatic component were derived with Fourier analysis. The short-term repeatability of Hartinger refractometry in measuring refractive astigmatism was tested by obtaining two sets of consecutive data from a separate group of diffuser-treated chicks (n = 8). Each animal was realigned between the two measurement sessions, and usually, the realignment took less than 5 minutes. The treated birds wore diffusers monocularly from 15 to 20 days of age, and refractometry was performed on the treated eyes at the end of the 5-day treatment period.

**Infrared Photokeratometry**

Corneal curvature was measured with a custom-made infrared photokeratometer similar to that described elsewhere. For each eye, a set of at least 12 individual images was acquired. Each image was first processed with a tested calibration curve to determine the corneal radius at four orientations and converted to corneal curvature using a reduced corneal refractive index of 1.333. To identify the flattest and steepest corneal curvatures (i.e., the two principal meridians), we best-fitted the corneal radii with an ellipsoid with custom software (MatLab; The MathWorks, Natick, MA). Corneal astigmatism for each animal was the difference between the flattest and steepest corneal curvatures derived from this fitted ellipsoid. Because the instrument often acquired outlier readings in each set of keratometry measurements, the medians for corneal curvature, astigmatism, and axis were used and were decomposed into astigmatic components by using Fourier analysis. The short-term repeatability for infrared photokeratometry in measuring corneal astigmatism was tested by obtaining two sets of consecutive data from the treated eyes of the birds used in the cross-sectional study (experiment 2) at the end of the 7-day treatment period (12 days of age, n = 42). Each animal was realigned between the two measurement sessions; usually, the realignment took less than 5 minutes.

**Statistical Analysis**

Statistical analyses were performed with commercial software programs (Minitab ver. 12.21, Minitab Inc., State College, PA; S-Plus, MathSoft Inc., Needham, MA; and SAS ver. 8.0, SAS Institute Inc, Cary, NC). The effects of visual manipulations across different treatment groups were tested by ANOVA. Depending on the dataset, One- or two-way ANOVA, repeated-measures ANOVA, or multivariate ANOVA (MANOVA) was used. If multifactorial ANOVA revealed a significant interaction effect, the simple effect was further examined. If the results of the ANOVA revealed a significant main or simple effect, a post hoc test was used to identify which pairs showed statistically significant differences. To test whether the axis of astigmatism was randomly distributed, we used the Rayleigh test (circular package, downloaded from the Comprehensive R Archive Network, http://cran.r-project.org/ hosted in the public domain by the Department of Statistics and Mathematics, University of Vienna, Vienna, Austria). The comparison of Pearson's correlation coefficients across different groups of birds was performed through an online statistics tool maintained by the Chinese University of Hong Kong (http://department.obs.cuhk.edu.hk/researchsupport/statmenu.asp).

**RESULTS**

**Short-Term Repeatability of Refractometry and Keratometry**

Figure 1 shows the Bland-Altman plots of total astigmatism (Fig. 1A), the J0 (Fig. 1B), and J45 (Fig. 1C) astigmatic components for refractometry (left) and keratometry repeated measurements (right). As shown, refractometry provided better repeatability than keratometry; however, no systematic relationship was found between the mean (x-axis) and the difference (y-axis) for all three astigmatic components in both instruments. The mean differences and 95% limits of agreement (in parentheses) for each astigmatic component were: total refractive astigmatism, 0.17 D (−1.39, 1.74); refractive J0, 0.21 D (−0.76, 1.18); refractive J45, −0.04D (−1.24, 1.16); total
corneal astigmatism, $-0.29$ D ($-2.43$, $1.84$); corneal J0, $-0.13$D ($-1.32$, $1.06$); and corneal J45, $0.06$ D ($-1.46$, $1.59$).

Of the repeated measurements, $87.5\%$ and $75\%$ differed by less than $1$ D for refractive and corneal astigmatism, respectively.

**Effects of Visual Manipulations on Spherical Equivalent Refractive Error**

The effects of visual manipulations on refractive status at the end of the treatment period are expressed as anisometropia (i.e., interocular difference in spherical equivalent refractive errors; treated eye minus fellow eye). Two-way ANOVA showed that there was an interaction effect between study design (longitudinal/cross-sectional) and treatment regimen ($P = 0.02$). Further statistical analyses revealed that a significant difference in anisometropia between longitudinal and cross-sectional data sets was found only in the diffuser-treated groups (post hoc test with Bonferroni correction, $P = 0.03$).

Nevertheless, at the end of the treatment period, visual manipulations produced significant effects on anisometropia across the treatment groups (one-way ANOVA, $P < 0.001$). Compared with the control group, which had an average anisometropia of $0.84$ D (95% confidence interval [CI]: $0.24$–$1.45$), diffusers (mean and 95% CI: longitudinal: $-1.14$ D, $-8.70$ to $-14.13$; cross-sectional $-8.48$ D, $-6.68$ to $-10.30$) and $-10$ D lenses (mean and 95% CI: longitudinal $-8.65$ D, $-6.73$ to $-10.53$; cross-sectional $-9.26$ D, $-7.21$ to $-11.31$) produced more myopic errors in the treated eyes (Tukey post hoc test, all $P < 0.05$); $+10$-D lenses produced more hyperopic errors in the treated eyes (mean and 95% CI: longitudinal, $+11.48$ D, $+9.79$ to $+13.17$; cross-sectional $+9.88$ D, $+8.93$ to $+10.83$; Tukey post hoc test, both $P < 0.05$); constant light, as predicted from its bilateral treatment effect, did not produce significantly different anisometropia from the control group (mean and 95% CI: longitudinal $-0.63$ D, $-1.90$ to $+0.65$; cross-sectional $-0.34$ D, $-0.96$ to $+0.27$; Tukey post hoc test, both $P > 0.05$).

More important, anisometropia induced by diffusers or $+10$- or $-10$-D lenses were in close agreement with those reported in previous studies in which similar treatment regimens were used.$^{56,61}$

**The Magnitude and Frequency of Experimentally Induced Astigmatism**

**Longitudinal Data.** At the beginning of the experiment, refractive astigmatism was not significantly different between the treated and fellow eyes or across the control and treated groups (two-way ANOVA, no interaction effect, $P = 0.77$; main effect, all $P > 0.09$). Figure 2 illustrates longitudinal changes in the magnitude of refractive astigmatism in the treated eyes for individual birds (different symbols) treated with diffusers (Fig. 2A), $-10$-D lenses (Fig. 2B), $+10$-D lenses (Fig. 2C), and constant light (Fig. 2D). In each plot, the shaded area covers the range of astigmatism found in the treated eyes of the control

![Diagram showing longitudinal changes in refractive astigmatism](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933445/)
animals. Over time, the refractive astigmatism in control animals decreased in both magnitude and variability; in contrast, the treated animals frequently developed or maintained high magnitudes of astigmatism throughout the treatment period. Of interesting, a few birds from individual treatment groups exhibited overnight changes in the magnitude of refractive astigmatism that were quite large (e.g., Fig. 2C, bird represented by an inverted triangle). The insets in Figures 2A–D illustrate the average changes (mean ± SEM) in the magnitude of astigmatism for treated and fellow eyes of individual treatment groups. Repeated-measures ANOVA showed that astigmatism in the treated eyes were significantly higher than those of the fellow eyes in diffuser (Fig. 2A) and +10-D (Fig. 2C) groups throughout the treatment period (both \( P < 0.01 \)). In contrast, no significant differences in the magnitude of astigmatism were found between the treated and fellow eyes in (Fig. 2B) –10-D– and (Fig. 2D) constant light-treated groups (both \( P > 0.05 \)).

Figure 2E illustrates the longitudinal changes in the magnitude of refractive astigmatism (mean ± SEM) of the treated eyes for control and treatment groups. Compared to the control group, significantly higher magnitudes of astigmatism were found in the treated eyes during the 7-day treatment period in all treatment groups (repeated-measures ANOVA, \( P < 0.01 \); Dunnett post hoc test, all \( P < 0.05 \)). In addition, 28 (74%) of the 38 treated birds had the highest magnitude of astigmatism (peak astigmatism) near the middle of the treatment period (representative animals are marked with asterisks in Figs. 2A–D). This point is illustrated in plots of the average magnitudes of refractive astigmatism in the treated eyes in Figure 2F at three time points for each group of birds. The three time points were at the onset of the experiment (initial), when the highest magnitude of astigmatism occurred during the treatment period (peak data, excluding measurements at initial and end), and at the end of the treatment period (end). Repeated-measures ANOVA indicated that refractive astigmatism in the treated eyes were significantly different at these three time points for all groups of birds (including control animals, all \( P < 0.01 \)). The results of a post hoc test with Bonferroni correction indicated that, except for two comparisons marked as nonsignificant (ns) in Fig 2F, the magnitude of astigmatism at peak period and those at the other two time points were significantly different (\( P = 0.001–0.03 \)). The exceptions were the comparison between initial and peak in the control group (\( P = 0.62 \)), and the comparison between peak and end in the diffuser group (\( P = 0.20 \)). Regardless, at the end of the treatment period, all treated groups had significantly higher magnitudes of refractive astigmatism in the treated eyes than did the control group (one-way ANOVA, \( P < 0.001 \); Tukey post hoc test, all \( P < 0.05 \)). Furthermore, the magnitude of astigmatism at the end of the treatment period was significantly higher in chicks treated with diffusers than in those in the other three treatment groups (Tukey post hoc test, all \( P < 0.05 \)), but no significant differences in the magnitude of astigmatism were found between groups treated with –10, +10 D and constant light (Tukey post hoc test, all \( P > 0.05 \)).

**Cross-sectional Data.** Visual manipulations produced very similar effects on refractive astigmatism in both cross-sectional and longitudinal studies. In the treated eyes, no significant interaction effects were found between study design (longitudinal/cross-sectional) and treatment regimen for refractive astigmatism and J0 and J45 astigmatic components (MANOVA, interaction and main effects, all \( P > 0.22 \)). Similarly, no interaction effects (study design × treatment regimen) were found in the fellow eyes (MANOVA for refractive astigmatism and J45 components, all \( P > 0.3 \)) except for the J0 astigmatic component (MANOVA, \( P = 0.03 \)). Examination of the J0 astigmatic component in the fellow eyes showed that only the difference in longitudinal and cross-sectional data from the diffuser-treated groups was statistically significant (post hoc test, \( P = 0.002 \)).

Figure 3A illustrates frequency distributions of refractive astigmatism in the treated eyes at the end of the treatment period for control and treated birds from the longitudinal and cross-sectional studies. The horizontal box plots in each panel summarize the statistical values of the longitudinal and cross-sectional data sets. Whereas only 8.6% (3/35) of the control chicks had refractive astigmatism \( >1 \) D, the percentages of treated birds that had astigmatism \( >1 \) D in each treatment group ranged from 66.7% to 100%. One-way ANOVA of the cross-sectional data shows that all four visual manipulations produced significantly higher magnitudes of refractive astigmatism compared to control groups in the treated eyes at the end of the treatment period (\( P < 0.001 \); Tukey post hoc test, all \( P < 0.05 \)). Similar to longitudinal studies, diffusers produced significantly higher refractive astigmatism compared with the other three treatment groups (Tukey post hoc test, all \( P < 0.03 \)), but no significant differences in the magnitude of refractive astigmatism were found between groups treated with –10 D, +10 D, and constant light (Tukey post hoc test, all \( P > 0.7 \)).

The plot in Figure 3B is similar to that in Figure 3A for the fellow eyes from all treatment groups. Although only 11.4% of control birds had astigmatism \( >1 \) D in their fellow eyes, the percentages of treated birds that had astigmatism \( >1 \) D in the fellow eyes ranged from 36.4% to 81.8% for individual treatment groups. When all nine groups of birds in Fig 3B were compared, it was found that the visual manipulations produced significant effects on refractive astigmatism and spherical equivalent refractive error in the fellow eyes at the end of the treatment period, regardless of whether the fellow eyes of constant-light groups (unlike other treatment groups, the fellow eyes in the two constant-light groups were also exposed to treatment effects) were excluded from the analysis (one-way ANOVA, all \( P ≤ 0.009 \)). A post hoc test showed that in addition to the two constant-light groups, only the diffuser group from the longitudinal study exhibited significantly higher magnitudes of refractive astigmatism in the fellow eyes than those of the control group (Tukey post hoc test, all \( P < 0.05 \)). On the other hand, a post hoc test showed that significant differences in spherical equivalent refractive errors of the fellow eyes were found between the following three pairs: each of the two constant-light groups and the control group, and the longitudinal group and the cross-sectional form-deprived groups (Tukey post hoc test, all \( P < 0.05 \)).

**Nature of Astigmatism**

**Axis of Astigmatism.** According to the axes of the correcting negative cylinder, astigmatism \( ≥1 \) D was classified as with-the-rule (180° ± 30°), against-the-rule (30° ± 30°), or oblique (31°–59°; 121°–149°). At the onset of the experiment, the axes of refractive astigmatism in the treated eyes were predominantly oriented at the 90° meridian. Of the 50 birds that had refractive astigmatism \( ≥1 \) D, 80% (\( n = 40 \)) were against-the-rule (range, 60°–117°) and 68% (\( n = 34 \)) had astigmatic axes oriented exactly at 90°. At the end of the treatment period, against-the-rule astigmatism still predominated. Of the 60 treated birds that had refractive astigmatism \( ≥1 \) D, 73.3% had against-the-rule, 25% had oblique, and 1.7% had with-the-rule astigmatism. This trend was also reflected in treated chicks that had corneal astigmatism \( ≥1 \) D in the treated eye (\( n = 64 \)): 82.8% had against-the-rule, 17.2% had oblique, and none had with-the-rule astigmatism.

The polar plots in Figure 4 illustrate the distributions of refractive (Fig. 4A) and corneal (Fig. 4B) astigmatism in the right or the left treated eyes on the treatment groups (longitu-
dinal or cross-sectional) at the end of the treatment period. Data for control animals were excluded because of the small magnitude of astigmatism. For each treated eye, the magnitude and axis of astigmatism are represented by the distance from the origin and the vector angle, respectively. As shown in the figure, regardless of the treatment regimens, the treated eyes frequently exhibited refractive or corneal astigmatism with axes near the 90° meridian, although some were oriented more obliquely. The Rayleigh test showed that the axes for refractive (mean ± SEM: right eye = 109.9 ± 4.4°, left eye = 72.3 ± 3.7°) and corneal astigmatisms (mean ± SEM: right eye = 102.5 ± 2.3°, left eye = 71.8 ± 2.9°) were not randomly distributed (all

FIGURE 3. Frequency distributions of the magnitude of refractive astigmatism in the treated (A) and fellow eyes (B) of control birds and birds treated with diffusers, -10-D lenses, +10-D lenses, and constant light at the end of the treatment period. Horizontal boxes: summary of statistical results for each study: the boundaries represent the 25th and 75th percentiles, the line within the box represents the median, and the error bars extending from the box represent the 10th and 90th percentiles.

FIGURE 4. The polar plots of refractive (A) and corneal astigmatism (B) in the right or left treated eyes at the end of the treatment period in the different treatment groups. Each data point represents the magnitude (radius) and axis (degree) of negative correcting cylinder for an individual bird. Asterisks: outliers from the left eye of the same animal: refractive, 18.3 DC × 59; corneal, 14.6 DC × 66.
Although it should be noted that the data from right or left treated eyes were collected from different birds, the average axes of the refractive or corneal astigmatism in the two eyes appeared to be mirror symmetric about the 90° axis. In addition, visual manipulations did not produce significantly different effects on refractive J0, corneal J0, and corneal J45 astigmatic components in right or left treated eyes (MANOVA, all P > 0.12). However, MANOVA with post hoc test indicated that whereas the refractive J45 component in the right eyes was significantly different only between the diffuser and constant light treatment groups (MANOVA, P = 0.002; Tukey post hoc test, P = 0.03), those in the left eye were significantly different between the diffuser and all three other treatment groups (MANOVA, P < 0.001; Tukey post hoc test, all P ≤ 0.02).

**Refractive versus Corneal Astigmatism.** Both refractometry and keratometry data were available for 29 birds in the longitudinal study and all 52 birds in the cross-sectional study. The total magnitude and the J0 and J45 components for refractive and corneal astigmatisms in the treated eyes of these birds were used for Pearson’s correlation analysis. Because the Pearson’s correlation coefficients were not significantly different across the four treatment groups (comparing two or more correlation coefficients, P > 0.055), the data were combined. As a group, all three refractive and corneal astigmatic components were moderately but significantly correlated (Fig. 5, r = 0.65, 0.44, and 0.58 for total astigmatism and the J0 and J45 components, respectively, n = 81, all P < 0.003), suggesting that the astigmatic errors were largely corneal.

**Astigmatism and Spherical Aberrometry.** To determine the association of astigmatism with spherical aberrometry (i.e., refractive powers of the two principal meridians), data from the treated eyes at 12 days of age from all control and treatment groups were divided into hyperopic (spherical equivalent refractive error > 0) or myopic subgroup (spherical refractive error < 0). Table 1 summarizes the results from Pearson’s correlation analyses. In general, refractive/corneal total astigmatisms correlated more strongly with the most ametropic meridians than the least ametropic meridians in both myopes and hyperopes. Even though the coefficients were low in many conditions, the degrees of association between the total astigmatism and the two principal meridians were comparable to

![Figure 5](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933445/)
those reported in large-scaled human studies using a similar analytical approach.39

**DISCUSSION**

Our main findings were (1) all four visual manipulations produced both spherical and astigmatic errors in chickens; and (2) the astigmatism was largely corneal, its axes frequently oriented near 90° and bilaterally mirror symmetric, and its magnitude significantly correlated with those of spherical ametropia.

Although all four visual manipulations in the present study produced astigmatism of similar characteristics, the magnitude of astigmatism at the end of the treatment period was significantly higher in chicks treated with form deprivation than in those treated with spherical defocus or constant light. Unlike birds treated by spherical defocus and constant light, which showed a tendency to decrease in the magnitude of astigmatism toward the end of the treatment period, form-deprived chicks appeared to maintain high degrees of astigmatism throughout the treatment period (Fig. 2F). Furthermore, compared with the other three treatment groups at the end of the treatment period, the distribution of the magnitudes of astigmatism was more widely spread (Fig. 3A), and the refractive J45 component appeared to differ under certain conditions in the form-deprived birds. Although it has been suggested that spherical defocus and form deprivation regulate refractive development through different neural feedback conditions,62–64 our results provide further evidence that form deprivation also produces a larger impact on ocular toricity compared with both hyperopic and myopic defocus, at least after a week of visual manipulation. Our data showed that refractive astigmatism correlated moderately but significantly with corneal astigmatism, but the fact that form deprivation did not produce significantly different corneal astigmatism compared with other visual manipulations suggests that other factors may have contributed to the higher ocular toricity in form-deprived eyes. However, because +10- and −10-D spherical lenses were the only powers that we tested, we cannot rule out the possibility that spherical lenses of different powers would lead to higher magnitudes of astigmatism within the same time frame.

Altered visual experience has consistently been shown to produce spherical equivalent refractive errors in a variety of animal species,86 but its effect on ocular toricity has been noted in only a few studies. In chicks, Schmid and Wildsoet84 have reported that the magnitude of natural against-the-rule astigmatism significantly decreased after hatching (8.2 D) and stabilized by approximately 3 weeks of age (0.6 D). In addition, form deprivation for 2 weeks produced astigmatism that had different axis orientation from those of the fellow untreated eyes (0.3 D with-the-rule astigmatism vs. 2.7 D against-the-rule astigmatism); rearing chicks in constant light led to a developmentally increase, rather than a natural decrease, in the magnitude of against-the-rule astigmatism.54 The effects of high-power spherical defocus on the genesis of astigmatism were noted by Irving et al.56 and more recently by Kislak et al. (IOVS 2006;47:ARVO E-Abstract 1799). The high-power spherical defocus was created by either switching to spherical lenses of equal power (10 D) but opposite sign in the middle of a 2-week treatment period (e.g., +10 D replaced by −10 D) or imposing −30-D spherical defocus (Kislak ML et al. IOVS 2006;47; ARVO E-Abstract 1799). In both cases, significant amounts of astigmatism (2–4 D); Kislak et al.: 1.5 D of J45) were found in association with a change in spherical ametropia. Last, several investigators have reported that imposing astigmatism with astigmatic lenses resulted in higher than normal amounts of astigmatism,54,57,66 although two studies, published as abstracts, found no significant effects of imposed astigmatism on ocular astigmatism (Thibos LN et al. IOVS 2001;42:ARVO Abstract 324, and Laskowski FH et al. IOVS 1996;37:ARVO Abstract 3140). Although differences in experimental design (e.g., strain of birds, the onset and duration of treatment regimens) preclude further comparison with previous studies, our results provide strong evidence that the four visual manipulations commonly used in experimental eye research can promote the development of astigmatism, at least in the strain of birds that we used.57 The presence of significant amounts of astigmatism during ametropic eye growth underscores the importance of identifying and characterizing the astigmatic components when using this common animal model in refractive development research.

The characteristics of astigmatism associated with experimentally induced myopia or hyperopia found in this study are similar to those reported in infant monkeys.30 First, the astigmatism found in both species was largely corneal in origin. Second, the dynamic changes in the magnitude of astigmatism (i.e., an initial transient increase followed by a decrease toward the end of treatment period; Fig. 2), were frequently found in both species.29,30 Third, the astigmatic axes in the two eyes appeared to be mirror symmetric, although the average astigmatic axes in both eyes were slightly more obliquely oriented in monkeys50 than those in chickens. These three similarities in the characteristics of astigmatism were found in both species treated by form deprivation or spherical defocus. Furthermore, the yoking effects of visual manipulations on the fellow eye’s astigmatism found in this study have also been reported in monkeys that were treated with cylindrical lenses.56 However, in contrast to chicks, monkeys did not develop a significant amount of astigmatism under constant light,50 neither did monkey eyes exhibit dramatic changes in ocular components caused by constant light, as previously reported in chicks.58–71 Although it has been speculated that monkeys may be shielded from the constant light effect by a thicker skull and/or by their covering their heads with their arms,70,71 it is unclear at this point what is responsible for the different effects of constant light on eye growth between these two species. Nevertheless, the key point is that the presence of significant astigmatism is associated with abnormal axial eye growth in both species.

The natural and experimentally induced astigmatism found in chickens is qualitatively similar to those reported in humans. First, similar to what we found in chicks, human astigmatism is primarily corneal.1,25,26,72–75 Second, many studies26,76–83 have shown that the prevalence of significant amounts of astigmatism (>1.0 D) is high in human infants immediately after birth, but decreases to the adult level by school age (for a summary figure, see Ref. 84). This natural decrease in the magnitude of infantile astigmatism was also noted in this study (Fig. 2E) and in a previous study83 using chicks. Third, as observed in our birds, the astigmatic axes reported in clinical studies85–87 (see Ref. 88 for contradictory findings) tends to be more obliquely oriented in myopic populations. In our treated birds, the average refractive astigmatic axes were 109.9° and 72.3° for the right and left treated eyes, respectively. These findings are supported by the results of a 3-year longitudinal study of the relation between myopic progression and astigmatic axis in 238 myopic children from third to fifth grades of primary school.51 Specifically, comparing the data collected at the onset and end of study, Parssinen51 noted that the percentage of astigmatic axes within the range of 91° to 149° in right eyes increased from 14.3% to 20.2%, and axes within the range of 31° to 89° in left eyes increased from 11.3% to 18.5%. Likewise, Fulton et al.90 have shown that myopic children with oblique astigmatism were, on average, more myopic than those without oblique astigmatism (i.e., against- or with-the-rule astigmatism). Fourth, similar to our finding in chicks, the mag-
nitude of refractive astigmatism has been shown to correlate significantly with the magnitude of the spherical power of the principal meridian, although the coefficients were low for both species. Another study on chicks also reported a correlation between experimentally induced astigmatism and spherical ametropia, although moderate levels of astigmatism, like those found in our study, were associated with much more myopic refractions (1.5 D at J45 with 29 D of myopia; Kisilak ML et al. IOVS 2006;47;ARVO E-Abstract 1799). Nevertheless, the many similarities of astigmatism between chickens and humans suggest that visual experience that alters axial eye growth may contribute to the genesis of astigmatism in humans.

In summary, we have demonstrated that four visual manipulations, all known to alter axial eye growth, promote the development of astigmatism in an animal model commonly used for refractive development. Because none of the four treatment regimens imposed astigmatic errors, the induced astigmatism cannot be considered as an active, direct compensatory ocular response. On the other hand, since all four visual manipulations produced astigmatism with very similar characteristics, it is possible that the induced astigmatism is a byproduct of abnormal axial eye growth. Although it is unclear how and to what extent abnormal visual experience alters ocular toxicity, the availability of chickens as an animal model provides great opportunities to elucidate the regulatory mechanisms underlying astigmatism.

Acknowledgments

The authors thank David Troilo and Debora Nickla for careful reading of an early draft of the manuscript.

References


