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Bi-directional Corneal Accommodation in Alert Chicks with experimentally-induced Astigmatism

(Short: Corneal accommodations in astigmatic chicks)

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Abstract

This study aimed to characterize corneal accommodation in alert chicks with and without experimentally-induced astigmatism. Refraction and corneal biometry were measured in 16 chicks with experimentally-induced astigmatism (>1.00D) and 6 age-matched control chicks (astigmatism≤1.00D). Corneal accommodation was detected using a Placido-ring based videokeratography system, by measuring changes in corneal curvature from a series of consecutive images acquired from alert chicks. The correlation between the magnitudes of corneal accommodation and astigmatism was analyzed by including data from all 22 chicks. Data from all eyes showed obvious bi-directional changes in corneal accommodation. There was no significant difference in corneal accommodative changes between the fellow eyes of the treated birds, and the right and left eyes of control birds. However, positive accommodation (PA) and maximum magnitude of PA (MPA) were significantly higher in the astigmatic vs. the fellow eyes of treated chicks (mean±SE: PA=+2.24±0.44D vs. +1.26±0.20D; MPA=+7.53±0.81D vs. +4.38±0.53D, both $p<0.05$). This was not the case for negative accommodation (NA) or maximum magnitude of NA (MNA) (NA=-0.46±0.15D vs. -0.33±0.04D; MNA=-0.92±0.23D vs. -0.73±0.12D, respectively, $p>0.05$). Furthermore, higher PA and MPA were found to be correlated with higher refractive astigmatism (both $r=0.34$, $p<0.05$). These results suggest that the presence of astigmatism may interfere with accommodative function in chicks.

1. Introduction

The extent to which the cornea, the major refractive component of the eye, plays a role in accommodation is controversial. Although previous studies found 0.40D to 0.72D of corneal accommodation in humans aged between 20 to 40 years old (Pierscionek, Popiolek-Masajada & Kasprzak, 2001; Yasuda, Yamaguchi & Ohkoshi, 2003; Yasuda & Yamaguchi, 2005), negative results have also been reported (Bannon, 1946; Buehren, Collins & Carney, 2003; He, Gwiazda, Thorn et al., 2003; Read, Buehren & Collins, 2007; Rosenfield & Gilmartin, 1987). These inconsistent results may be due to methodological differences or difficulties in detecting subtle changes in corneal curvature. In contrast to the findings in humans, there is stronger evidence for corneal accommodation in several avian species, including the chicken, which has been proposed as a good model for studying corneal accommodation, because of its prominent amplitude of corneal accommodation (Glasser, Troilo & Howland, 1994; Troilo & Wallman, 1985). Previous studies showed significant corneal steepening accompanied with lenticular accommodation (Glasser, Troilo & Howland et al., 1994; Murphy, Glasser & Howland, 1995; Ostrin, Liu, Choh et al., 2011; Schaeffel & Howland, 1987; Troilo & Wallman, 1987) and the total accommodation (i.e., lenticular plus corneal accommodations) can be over 25.00D (Glasser, Troilo & Howland et al., 1994; Schaeffel, Glasser & Howland, 1988). Indeed, corneal deformation has been estimated to contribute 40.0% to 50.0% (about 6.00D to 9.00D) of the ocular accommodation (Glasser, Troilo & Howland et al., 1994; Schaeffel & Howland, 1987; Troilo & Wallman, 1987).

Nevertheless, some studies could not detect any corneal accommodation in chicks (Beer, 1892; Sivak, Hildebrand, Lebert et al., 1986).

Corneal accommodation in chicks has been reported to occur due to the contraction of a longitudinal Crampton's muscle (Walls, 1942). This muscle is the anterior portion of the striated ciliary muscle which originates at the sclera, with the scleral occiscle acting as a supporting base (Glasser, Troilo & Howland et al., 1994; Murphy, Glasser & Howland et al., 1995). A direct connection of the muscle to the corneal inner lamella creates a circumferential tension that alters corneal curvature upon muscle contraction. In empirical studies, changes in chick corneal curvature have been measured either by an infrared photokeratometer (García de la Cera, E., Rodríguez, de Castro et al., 2007; Schaeffel & Howland, 1987; Troilo & Judge, 1993) or by a modified keratometer (Irving, Sivak & Callender, 1992; Troilo & Wallman, 1987). Ocular accommodation was induced either pharmacologically by treatment with nicotine (Glasser, Troilo & Howland et al., 1994; Schmid & Wildsoet, 1997; Troilo & Wallman, 1987), or electrophysiologically by stimulation of the Edinger-Westphal nucleus (Glasser, Troilo & Howland et al., 1994; Troilo & Wallman, 1987). However, the extent to which experimental manipulations to stimulate corneal accommodation mimic the natural action of the system is still unclear.

Astigmatism is a refractive error frequently associated with myopia (or "nearsightedness") and hyperopia (or "farsightedness") in humans (Read, Collins

& Carney, 2007) and animal models (monkeys: Kee, Hung, Qiao et al., 2005); chicks: Kee & Deng, 2008). It has been hypothesized that the presence of astigmatism may facilitate the accuracy of accommodative response by utilizing the contrast cues associated with the two principal refractive meridians (Howland, 1982); thus the significant astigmatism found in infants could potentially interfere with the eye's focusing strategy and signaling pathway during early eye growth. However, despite the high prevalence of astigmatism found across different nations (see a summary figure in Kee, 2013), the functional role, if any, of astigmatism during normal and abnormal refractive development remains unclear (Kee, 2013). The present investigation had two key aims. First, we investigated whether we could detect corneal accommodation in chicks under natural viewing conditions: that is with no artificial stimulation, anesthesia, nor the use of lid retractors. Second, we sought to test the hypothesis that corneal accommodation in chicks is influenced by the level of either refractive or corneal astigmatism.

2. Methods

2.1. Animal Subjects

Twenty-two White Leghorn chicks (*Gallus gallus domesticus*) were hatched and raised in a temperature- and light-controlled animal room at The Hong Kong Polytechnic University. The light/dark cycle was 12hr/12hr (7:00am to 7:00pm) and the illumination level was about 100lux at the chicks' eye level. Food and water were provided *ad libitum*. Care and use of the animals were in compliance

with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the protocol was reviewed and approved by the Animal Subjects Ethics Sub-committee of the university.

2.2. Manipulations

Sixteen chicks treated by optical manipulations (see below) that developed >1.00D of corneal astigmatism were included in this study. Six age-matched untreated chicks served as controls. To induce astigmatism, the right eyes of the treated birds were covered, from day 5 to day 12 post-hatching, with a crossed-cylinder lens (+4.00DS/–8.00DCx45, n=3; +4.00DS/–8.00DCx90, n=3; +2.00DS/-4.00DCx180, n=3), a slit aperture (0.5mm widthx10mm height; horizontal slit, n=3; vertical slit, n=2), or a spherical spectacle lens (+15D, n=1; -15D, n=1). The fellow eyes were left untreated (we refer to these eyes as, “untreated fellow eyes”). Each lens or slit aperture was first glued to a Velcro ring with Norland Optical Adhesive (Norland Products Inc., New Brunswick, NJ, USA) and later attached to the Velcro ring’s adhesive mate, which was glued (Pattex leather contact adhesive, Dusseldorf, Germany) to the feathers around the right eye. During the treatment period, the devices were cleaned every morning. All measurements were performed at 12 days of age.

2.3. Measurements

Refractive status was measured under anesthesia with a modified Hartinger refractometer as described previously (Chu, Deng & Kee, 2012). After

refractometry, corneal parameters were measured in alert chicks using a custom-made videokeratography system under dim illumination without using lid retractors. To avoid the potential influence of diurnal effects (Campbell, Bunghardt, Kisilak et al., 2008; Johnson, Lytle, Troilo et al., 2004), the refractions and corneal curvature measurements were performed between 9:00am to 11:00am and 1:00pm to 5:00pm, respectively. The components of refractive errors (i.e., M, spherical equivalent; MMM, most myopic meridian; MHM, most hyperopic meridian; RA, refractive astigmatism; R-J0 and R-J45, the two astigmatic components of RA) and corneal curvature parameters (i.e., MK, mean corneal curvature; FK, flattest corneal curvature; SK, steepest corneal curvature; CA, corneal astigmatism; C-J0 and C-J45, the two astigmatic components of CA) were decomposed using power vector analysis (Thibos, Wheeler & Horner, 1997).

2.3.1. Videokeratography system (VKS)

A Placido-ring videokeratography system (VKS) was custom-built for chick eyes. The instrument comprised of a dome (80mm in radius) with an inner aperture of 12mm diameter to house a telecentric imaging system (CCD camera: Guppy AVT F-046, Edmund Optics, NJ, USA). The dome surface has 16 concentric bright rings around the inner aperture (see Figure 1A). Unlike a previous version (Xu, Kee, Zhou et al., 2009), the current system used a series of white LEDs (illumination LEDs), instead of a circular fluorescent light, to provide even illumination for the Placido-rings (see Figure 1A). To align the center of Placido-

rings with the subject's pupil center, four infrared LEDs were installed at the outer perimeter of the dome to illuminate the pupil (Fig.1A, "iris LED"). These LEDs can be switched off independently after a good alignment was achieved (Figure 1 B and C). Another four red LEDs were installed near the inner aperture to serve as fixation targets to attract chick's attention (Fig.1A, "Fixation LED"). Once the image was aligned and focused at a working distance of 80mm, the iris LEDs were switched off and a series of images were captured in multiple-shot mode (frame rate=49.4 frame per second) using the software (AVT Fire Package version 3.0) provided by the CCD camera.

To derive the common corneal biometric parameters, images of good quality (sharply focused with good alignment) were selected and analyzed via a user interface written in MatLab software (see Appendix for details). All corneal parameters were calculated from the central 2.8mm diameter because the instrumental noise was the lowest (0.18D) when compared to smaller diameters (see Appendix for details).

2.3.2. Corneal Accommodation

When the chick's attention was directed to the fixation LEDs, only the iris LEDs were switched off (i.e., the fixation LEDs were still switched on) and a series of continuous frames were captured using the multiple-shot mode as described above (500 to 1500 frames, 10.1 and 30.3 seconds duration, respectively). The fixation LEDs, located at 80mm working distance (*i.e.*, 12.5D), were the only

stimuli for positive accommodation; no stimulus was used to stimulate the negative accommodation. This procedure was performed on each eye consecutively for all birds. After excluding all distorted or disrupted images from the 500 to 1500 frames acquired from each eye, we were able to identify consecutive frames with obvious changes in Placido-ring images (*i.e.*, changes in the ring-to-ring width) while the center of the Placido rings did not appear to shift in direction. These changes could be found in all eyes within 30 to 40 consecutive frames, thus all these images were analyzed for changes in mean corneal curvature (MK) as a measure of corneal accommodation. The series of frames acquired for each eye were measured for corneal parameters and analyzed for the following statistical parameters. For each eye, the mode of MK was identified as the most frequently recorded MK. The positive (PA) and negative (NA) corneal accommodations were defined as the differences in MKs of the mode from the higher and lower values, respectively. In addition, the maximum positive (MPA) and maximum negative accommodation (MNA) were identified as the highest and lowest values from each series of frames of each eye.

The temporal pattern of corneal accommodation between the treated and fellow control eyes were examined in two ways: long intervals, and short intervals. For 4 birds (control, n=1; treated, n=3), we studied the changes in MK over approximately 300 frames per eye for 4 birds with varying degrees of refractive astigmatism (0.50D to 2.70D in their right/treated eyes, see Figure 2 for details).

These four birds were chosen because interruptions due to poor image quality, eye movement, and/or lid closure were minimal over a long interval of consecutive frames. For another 18 birds, data from shorter intervals (30 to 40 frames) were analyzed.

2.4. Data analysis

Statistical analysis was performed using SPSS 16.0 (SPSS Inc., Illinois, USA). One-way ANOVAs are used to determine if the refractive and corneal parameters are significantly different across the untreated fellow eyes of the treated birds, the right and the left eyes of control birds. One-way ANOVA was also used to determine if there were significant differences in individual parameters across the treated eyes of the treatment groups (i.e., crossed-cylinder lenses, spherical lenses, and slit apertures). Paired *t*-test was used to determine the differences between the treated and untreated fellow eyes in the treated birds. Pearson's correlation analyses are used to determine if the magnitudes of corneal accommodation in the fellow eyes (i.e., right and left eyes) are correlated, as well as whether the magnitudes of corneal accommodation and astigmatism were correlated (i.e., right and left eyes of all birds). In all tests, significance level was set at 95% level of confidence. Unless otherwise indicated, data are presented as mean and standard error (mean±SE).

3. Results

3.1. Effects of visual manipulations on refractive errors and corneal curvature

Neither the refractive (M, MMM, MHM, RA, R-J0, and R-J45) nor the corneal (MK, FK, SK, CA, C-J0, and C-J45) parameters were significantly different across the untreated fellow eyes of the treated birds, and the right and left eyes of the control birds (one-way ANOVA, all $p \geq 0.16$). As summarized in Table 1, the treated eyes, as a group, exhibited significantly higher MMM, RA, CA, and R-J0 when compared to their fellow untreated eyes (paired *t*-tests, all $p<0.05$); all other refractive (M, MHM, R-J45) and corneal (MK, FK, SK, C-J0, C-J45) parameters were not significantly different between the treated and fellow untreated eyes in the treatment groups. The magnitudes of refractive and corneal astigmatism for all eyes as a group were significantly correlated ($r=0.69$, $p<0.001$). With respect to the refractive and corneal parameters in the treated eyes, only MK, FK, and SK showed significant treatment effects (one-way ANOVAs, all $p<0.02$), with the eyes treated with spherical lenses (MK: 116.70±2.60D, FK: 115.95±2.45D, and SK: 117.50±2.80D) showing significantly flatter corneal curvature (Tukey's pairwise tests, all $p<0.05$) than those treated with crossed-cylinder lenses (MK: 121.21±0.64D, FK: 120.52±0.63K, and SK: 122.03±0.64D) or slit apertures (MK: 121.92±0.72D, FK: 121.480.84D, and SK: 122.62±0.76D). However, note that there were only two birds treated with spherical lenses, a flatter corneal curvature was found in the +15D treated eye (MK:114.1, FK:113.5, and SK:114.7) compared to the -15D treated eye (MK:119.3, FK:118.4, and SK:120.3); thus the flatter corneal curvature in this treatment group was mainly due to the +15D treated eye.

3.2. Corneal accommodation

3.2.1 Longer interval (n=4)

Figure 2 shows the temporal changes in MK over 300 consecutive frames of the right (A) and left eyes (B) for a control bird (top row) and three treated birds (bottom three rows, the right eyes were the treated eyes). The sequence of birds was arranged from top to bottom according to the magnitude of refractive astigmatism. As can be observed from this figure, the MK was frequently maintained at a particular level for all eyes, but both the treated and fellow eyes clearly showed bi-directional changes in MK from this level. In general, the changes in MK usually took a longer duration for positive (PA, about 200msec) than negative accommodation (NA, about 100msec), and the magnitudes of PA showed more variability between fellow eyes (control: RE=+1.26±0.20D vs. LE=+1.20±0.29D; treated: RE=+2.24±0.44D vs. LE=+1.20±0.29D) when compared to the NA of fellow eyes (control: RE=-0.33±0.15D vs. LE=-0.44±0.18D; treated: RE=-0.46±0.5D vs. LE=-0.39±0.11D). On the other hand, although the MPA in the four treated/right eyes (Figure 2A) were all higher than those in the untreated/left eyes (Figure 2B), there were no correlations between the magnitudes of MPA with RA or CA in these four birds. Figure 3 compares the frequency distributions of MK between the fellow eyes of the four birds; the sequence of birds followed that of Figure 2. For all eight eyes, the modes of MK occupied 45±4.6% (range: 32.0% to 65.0%) of the time, and the deviations from the mode of MK (i.e., excluding the mode) were within 1.00D in 25.2±3.3%

(range: 12.0% to 36.0%) and 12.1±3.2% (range: 4.7% to 28.0%) of the time for PA and NA, respectively.

Figure 4 shows the frequency distributions of the changes in corneal astigmatic magnitude (A) and axis (B) for the four birds in the same sequence as Figures 2 and 3. These changes were calculated by subtracting the modes of each parameter from the corresponding values. On average, the changes in corneal astigmatism during these intervals were within ±1.00D for 99.1±0.4% of the time (ranges: control/untreated fellow eyes: 99.0% to 100.0%; treated eyes: 97.2% to 98.9%), indicating that under most circumstances the corneal astigmatism contributed to at most 0.50D of changes in MK (since 1.00D cylindrical power=0.50D spherical-equivalent power). On the other hand, the astigmatic axis changed by less than ±20° for 75.2±9.1% of the time, with more variation in the control/untreated eyes than treated eyes (ranges: control/untreated fellow eyes: 22.6% to 90.9%; treated eyes: 72.7% to 97.9%), probably due to the higher instrumental noise when measuring eyes with low corneal astigmatism (see appendix and Figure 6). Although significant correlations were found between the changes in MK and astigmatic axis within the three right/treated eyes (i.e., the top three right eyes in Figure 4B), the correlations were generally low and varied in sign (Pearson's r=-0.24, +0.24, -0.36, all $p<0.001$), indicating that corneal accommodation was not correlated with a consistent pattern of change in the direction of the astigmatic axis.

3.2.1 Shorter interval (n=22)

Table 2 summarizes the magnitudes of corneal accommodative changes as well as the corresponding changes (relative to the corresponding modes) in astigmatic magnitude and axis. Except the NA in the fellow eyes of the treated group ($r=0.64$, $p<0.01$), no significant correlations between the fellow eyes were found in all other parameters for the treated and control groups ($r=0.08$ to 0.69, all $p\geq0.10$). Similar to the refractive status (Table 1), no significant difference in any of the corneal parameters was found across the untreated fellow eyes of the treated birds, and the right and left eyes of the control birds (one-way ANOVAs, all $p\geq0.11$). However, the PA (+2.24D vs. 1.26D, paired *t*-test, $p<0.05$), standard deviation of PA (0.39D vs. 0.23D, paired *t*-test, $p<0.01$), and MPA (+7.53D vs. +4.38D, paired *t*-test, $p<0.01$) were all significantly higher in the treated eyes when compared to their untreated fellow eyes. In contrast, the NA, standard deviation of NA, and MNA were not significant different between the treated and untreated fellow eyes (paired *t*-tests, all $p\geq0.29$). One-way ANOVAs showed that there was no treatment effect on any of the corneal accommodative changes (all $p\geq0.38$). Interestingly, when data from all eyes were pooled, both the PA and MPA were significantly correlated with refractive (PA vs. RA: $r=0.34$; MPA vs. RA: $r=0.34$, both $p<0.05$), but not corneal astigmatism (PA vs. CA: $r=0.13$; MPA vs. CA: $r=0.10$, both $p\geq0.41$). Figure 5 illustrates the low but significant correlation between the MPA and refractive astigmatism. On the other hand, PA was significantly correlated with NA ($r=-0.67$, $p<0.001$), but there was no correlation between MPA vs. MNA ($r=-0.06$, $p=0.71$), MPA vs. M (MPA vs. M: $r=-0.22$,

p=0.16) or MNA vs. M (r=0.08, p=0.59), nor between the maximum level of accommodation and the change in astigmatic axis (MPA vs. ΔAxis: r=-0.08, p=0.60; MNA vs. ΔAxis: r=-0.03, p=0.86; Table 2).

4. Discussion

The key findings of this study are: 1) both the control and treated eyes in alert chicks demonstrated frequent increases (PA) and decreases (NA) in corneal curvature, with PA showing much higher magnitudes than NA; 2) the magnitudes of refractive astigmatism and PA were correlated.

Non-anaesthetized chicks were capable of altering their corneal curvature to become steeper or flatter, although the magnitudes of PA and MPA were much higher than NA and MNA (Figures 2 and 3; Table 2). Despite the differences in methodologies (see Introduction section) and the age of the animals in previous studies compared with ours (4 to 10 weeks vs. 12 days), the maximum magnitudes of corneal accommodation reported in previous studies were very similar to what we found in the untreated/control chick eyes (current: 9.40D to 11.80D; Glasser, Troilo & Howland et al., 1994: 9.00D; Schaeffel & Howland, 1987: 9.00D to 10.00D; Troilo & Wallman, 1987: 10D). On average, the MPA in the untreated/control eyes ranged from 4.15D to 4.67D; only 6 out of these 28 eyes exhibited an MPA of more than 6.00D (Figure 5). Assuming that the 80mm working distance had stimulated 12.50D of total accommodation, our results

suggest that the corneal accommodation could contribute about 32.8% to 37.4% of the total ocular accommodation response.

One novel finding in this study was that astigmatic eyes appeared to show higher PA and MPA. Compared to their untreated fellow eyes, the eyes exposed to various visual manipulations not only developed significant amounts of refractive and corneal astigmatism but also exhibited higher PA and MPA (Tables 1 and 2). Furthermore, when data from all eyes in this study were pooled, the magnitudes of refractive astigmatism and PA or MPA were weakly but significantly correlated. It should be noted that during the same intervals when the corneal PA responses were observed, the changes in corneal astigmatism rarely exceeded 1.00D and the astigmatic axis did not show any consistent pattern of change (Figure 4 and Table 2). A previous study (Schmid & Wildsoet, 1997) using topical agents to stimulate (nicotine) or inhibit (vecuronium bromide) ocular accommodation in chicks also did not find significant changes in the magnitude of astigmatism (0.6D and 0.1D changes, respectively). Likewise, Schaeffel & Howland (1987) also found no significant changes in astigmatic magnitude when alert chicks were accommodating. Taken together, these results indicate that positive accommodation in chicks is accompanied with very little, if any, change in astigmatism, arguing against the presence of accommodative astigmatism in chicks. On the other hand, because astigmatism results in two line foci, it is possible that its presence may interfere with the end point of the ocular accommodative system (Howland, 1982). For instance, it is well documented

that the presence of induced astigmatism can increase the variability of accommodative behavior in humans(Stark, Strang & Atchison, 2003). Compared to the untreated fellow eyes, the astigmatic treated eyes showed a higher frequency of time spent on PA (long interval data) and an increased variability of PA (standard deviation of PA, Table 2), it is possible that these accommodative behaviors in the astigmatic eyes have produced higher magnitudes of PA and MPA (Table 2). While it should be realized that in this study corneal accommodation was captured over a separate time interval for each eye, the fact that the magnitudes of accommodative parameters were very similar across the untreated/control eyes (Table 2) indicates that the corneal accommodations we captured were representative. Thus, the higher magnitudes of corneal PA and MPA in the treated eyes are more likely to be related to the presence of significant astigmatism and not simply chance.

Compared to PA and MPA, corneal NA and MNA were much smaller in magnitudes (Figures 2 and 3, Table 2). To our knowledge, only one previous study, reported in abstract form (Troilo, Li & Howland, 1993), documented the features of MNA in chicks; approximately 4.00D of negative accommodation in 2 to 3 week-old unanesthetized chicks, but no measure was made on corneal accommodation in that study. Thus, our study provides, for the first time, clear evidence of negative corneal accommodation in alert chicks (Figures 2 and 3, Table 2). Although the magnitudes of corneal MNA in this study were only about a quarter of the negative accommodation in the previous study, both findings

support the presence of bi-directional changes in accommodative function in chicks. Further study is needed with respect to the underlying mechanism and the functional significance of this negative accommodation.

In conclusion, we detected bi-directional changes in corneal accommodation by measuring corneal changes in alert chicks. The presence of weak but significant correlations between refractive astigmatism and corneal PA and MPA suggest that the presence of astigmatism might interfere with the image quality and in turn affect the accommodative mechanism.

5. Appendix

5.1. Calibration of VKS

The radius of curvature was calibrated with five rustproof chromium steel balls (Grade 25, AISI 52100, USA) of known diameters that cover a range of corneal radii in young chicks (5/32" (3.97mm), 3/16" (4.76mm), 7/32" (5.56mm), 1/4" (6.35mm), and 9/32" (7.14mm)). A steel ball was fixed on a platform with its surface cleaned with alcohol before measurements. Five topographic images of the steel balls were taken for each ball with refocusing between measurements. Using a calibration curve (r^2=0.99) compiled from the results of all steel balls, the corneal radius of curvature (r, measured in mm) was converted into the corneal power (K, i.e., corneal curvature) using the formula K=(n-1)/r; where n=1.369 is the corneal refractive index of chicks (Mandelman & Sivak, 1983). To be able to

analyze astigmatic cornea, we further derived six biometric parameters: SK, the meridian with steepest curvature; FK, the meridian with the flattest meridian; MK, the average value of SK and FK; corneal astigmatism (CA), the dioptric difference between SK and FK; C-J0 and C-J45, the power vectors calculated from the corneal astigmatic magnitude and axis (Thibos, Wheeler & Horner et al., 1997).

Figure 6 plots the changes in meridional corneal power with respect to MK of ten chicks who exhibited a range of corneal astigmatisms. As shown, the meridional corneal powers changed smoothly through the 180° meridians, with the SK and FK separated by 90°, indicating that the corneal astigmatism found in chicks was due to a regular change in meridional corneal shape (i.e., regular astigmatism). Compared to birds with higher magnitudes of astigmatism, those with lower magnitudes exhibited slightly more variability in meridional corneal powers, probably due to the relatively higher instrumental noise when measuring lower magnitudes of change.

Images were analyzed using a algorithm written in MatLab software. Specifically, each image was first processed to enhance the rings' regions using a Gabor filtering with an adaptive thresholding strategy. After these processed rings were identified in a coarse-to-fine fashion and labelled digitally, the radial distance of each ring from the origin was detected using the Hough transform (Bryan, 2000; Duda & Hart, 1972). The radial distance was then smoothed using a median

filter and converted to radii using the method proposed by Carvalho et al. (Carvalho, Romão, Tonissi et al., 2002). The radii within three pre-selected areas, 1.50mm, 2.10mm and 2.80mm diameters of the central cornea, were segmented into 360 semi-meridians, summed, and averaged for the conventional 180 meridians according to clinical notation.

The accuracy of the instrument for measuring the three central corneal areas (1.50mm, 2.10mm, and 2.80mm diameter) were determined by calculating the difference of the measured values from the real values of three steel balls (2.78mm, 3.18mm, 3.57mm). Five images, separated by re-alignment and re-focusing, were acquired consecutively from each ball. The data of the five images were averaged using power vector analysis (Thibos, Wheeler & Horner et al., 1997) and subtracted from the real values.

5.2. Reliability and Repeatability

5.2.1. Steel balls

Repeated measures of the three steel balls showed that the accuracy of the instrument (measured value minus real value) was 0.18D for the largest tested areas (maximum differences: 1.50mm: 0.45D, 2.1mm: 0.32D, and 2.80mm: 0.18D) in all six corneal parameters. There were no significant differences across the three tested areas in MK, FK, and C-J0 astigmatic components. Although significant differences across the three tested areas were found for corneal astigmatism, SK and C-J45 astigmatic components (one-way ANOVAs,

all $p<0.001$), Tukey's post-hoc tests (all $p<0.001$) showed that the maximum differences between the two tested areas (1.50mm vs. 2.80mm) for astigmatism, SK and C-J45 were only, respectively, 0.33D, –0.32D, and –0.17D. Measurements of the astigmatic components showed a greater instrumental noise for smaller tested area (maximum differences from real value among the three steel balls: 1.50mm vs. 2.80mm: CA=–0.45D vs. –0.18D; C-J0=–0.02D vs. –0.01D; and C-J45=–0.22D vs. –0.09D). Because of the higher accuracy and lower instrumental noise with wider tested area, only data of the 2.80mm diameter central cornea were used for the analyses in this study.

5.2.2. Alert chicks eyes

Six sets of images (50 to 100 images per set) were collected from each of the treated eye for 12 birds from a separate experiment. These birds were treated monocularly with crossed-cylinder lenses and developed different degrees of corneal astigmatism (see CA results in Figure 7). Each set of images was separated by a re-alignment which often took less than 2 minutes. From each set of data, one image with good quality was manually selected, *i.e.*, there were six images from each of the twelve eyes. To see if different numbers of images would affect the outcome measures, the mean values of 5 and 3 randomly selected images from each bird were compared. Because no significant differences were found between the means of 5 versus 3 images for all six corneal parameters (i.e., SK, FK, MK, CA, C-J0 & C-J45; all $p\geq0.78$), the

repeatability of the instrument was tested by comparing the means from the first and second sets of 3 images.

The Bland-Altman plots in Figure 7 illustrate the repeatability of the six corneal parameters for these 12 treated birds. As reflected from the distributions of the six parameters, the crossed-cylinder lens treatment produced a wide range of corneal curvature and astigmatic components. Despite this significant treatment effect, the mean differences and 95% limits of agreement (in parentheses) for the six parameters were small: MK, –0.02D (+0.25, –0.25); SK, –0.03D (+0.26, –0.26); FK, –0.01D (+0.25, –0.25); CA, 0.02D (+0.21, –0.21); C-J0, 0.00D (+0.24, –0.24); and C-J45, 0.01D (+0.29, –0.29). In addition, there were no systematic changes across the dioptric ranges measured in all six parameters, and 83% of the repeated measurements differed by less than 0.25D.

6. Acknowledgement

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Table 1

	Treated Group (n=16)		Control Group (n=6)	
	Treated Eye	Fellow Eye	Right Eye	Left Eye
M (D)	-1.95±1.55 (-12.20 to +13.21)	+0.47±0.19 (-0.38 to +1.54)	+0.67±0.32 (-0.38 to +1.71)	+0.93±0.36 (-0.38 to +2.06)
MMM (D)	-3.90±1.58 * (-13.57 to +12.68)	+0.33±0.19 (-0.90 to +1.19)	-0.43±0.32 (-0.38 to 1.54)	+0.76±0.40 (-0.90 to +1.86)
RA (D)	3.14±0.39 *** (1.05 to 6.58)	0.28±0.14 (0.00 to 1.74)	0.47±0.14 (0.00to 1.05)	0.35±0.18 (0.00 to 1.05)
R-J0 (D)	-0.94±0.35 * (-3.29 to 1.38)	-0.14±0.07 (-0.87 to 0.00)	-0.23±0.07 (-0.52 to 0.00)	-0.17±0.09 (-0.52 to 0.00)
CA (D)	1.53±0.19 *** (0.47 to 3.09)	0.59±0.08 (0.21 to 1.25)	0.57±0.20 (0.19 to 1.48)	0.75±0.19 (0.34 to 1.66)

Table 1. Refractive errors measured after 1 week of treatment or at equivalent age (P12). Data are presented as mean±SE, the range is presented in parentheses. Statistical significance between treated and fellow eyes is marked with asterisk * $P<0.05$, and *** $P<0.001$. M, spherical equivalent; MMM, most myopic meridian; RA, refractive astigmatism; R-J0, refractive J0; CA, corneal astigmatism.

Table 2

	Treated Group (n=16)		Control Group (n=6)	
	Treated Eye	Fellow Eye	Right Eye	Left Eye
Positive Accommodation				
PA (D)	+2.24±0.44 * (0.46 to 7.88)	+1.26±0.20 (0.26 to 3.38)	+1.19±0.16 (0.66 to 1.81)	+1.20±0.29 (0.37 to 2.11)
PA S.D. (D)	0.39±0.07 ** (0.07 to 1.18)	0.23±0.05 (0.04 to 0.69)	0.20±0.04 (0.10 to 0.34)	0.26±0.07 (0.08 to 0.55)
MPA (D)	+7.53±0.81 ** (3.00 to 15.70)	+4.38±0.53 (1.70 to 9.40)	+4.67±1.47 (1.80 to 11.8)	+4.15±1.16 (1.90 to 9.70)
Δ CA (D)	0.02±0.16 (-1.37 to 1.20)	-0.09±0.07 (-0.82 to 0.40)	-0.14±005 (-0.28 to -0.02)	-0.09±0.14 (-0.69 to 0.13)
Δ Axis of CA (°)	3.21±3.49 (-14.40 to 36.00)	-4.50±5.79 (-53.00 to 38.00)	-12.17±10.89 (-61.00 to 4.00)	-26.78±9.48 (-59.00 to -1.70)
Negative Accommodation				
NA (D)	-0.46±0.15 (-2.53 to -0.12)	-0.33±0.04 (-0.73 to -0.16)	-0.39±0.11 (-0.86 to -0.21)	-0.44±0.18 (-1.20 to -0.10)
NA S.D. (D)	0.07±0.02 (0.00 to 0.24)	0.06±0.01 (0.00 to 0.17)	0.05±0.02 (0.02 to 0.09)	0.06±0.02 (0.00 to 0.16)
MNA (D)	-0.92±0.23 (-3.90 to -0.20)	-0.73±0.12 (-2.30 to -0.30)	-0.73±0.19 (-1.30 to -0.40)	-0.87±0.27 (-1.70 to -0.30)
Δ CA (D)	0.09±0.17 (-0.75 to 2.12)	-0.14±0.08 (-1.02 to 0.33)	-0.09±0.17 (-0.75 to 0.27)	-0.15+0.18 (-0.36 to 0.31)
Δ Axis of CA (°)	0.03±2.64 (-15.00 to 26.00)	-3.02±4.58 (-41.00 to 15.00)	-1.83±12.78 (-47.00 to 27.00)	7.67±10.49 (-27.00 to 50.00)

Table 2. Corneal Accommodation measured after 1 week of treatment or at equivalent age (P12). Data are presented as mean±SE, the range is presented in parentheses. Statistical significance between treated and untreated fellow eyes is marked with asterisk * P<0.05, ** P<0.01. PA, positive accommodation; PA S.D., standard deviation of positive accommodation; MPA, maximum positive accommodation; MNA, maximum negative accommodation; ΔCA, change in the

magnitude of corneal astigmatism; ΔAxis of CA, change in the axis of corneal astigmatism; NA, negative accommodation; NA S.D., standard deviation of negative accommodation.

Figure 1

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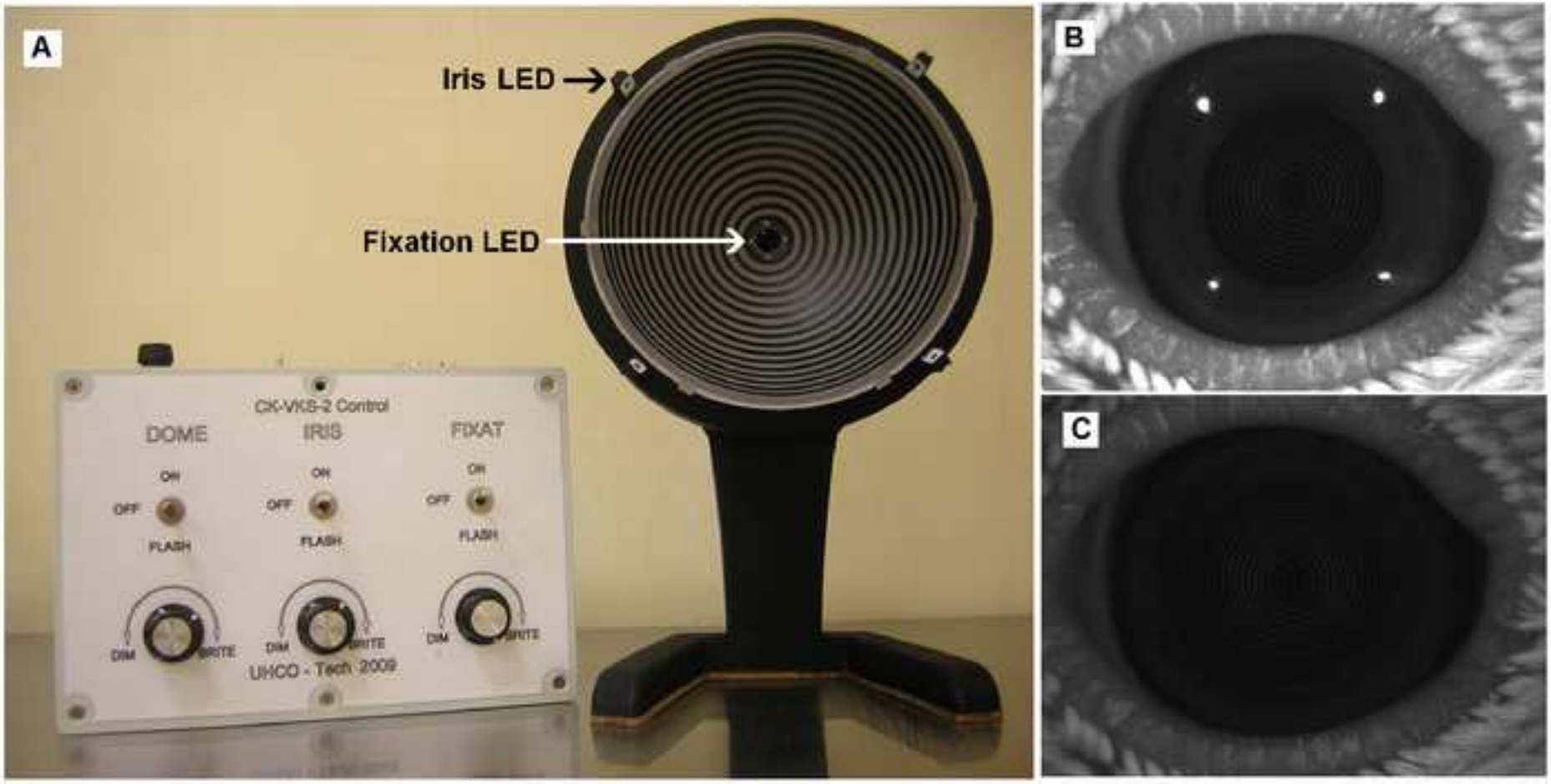

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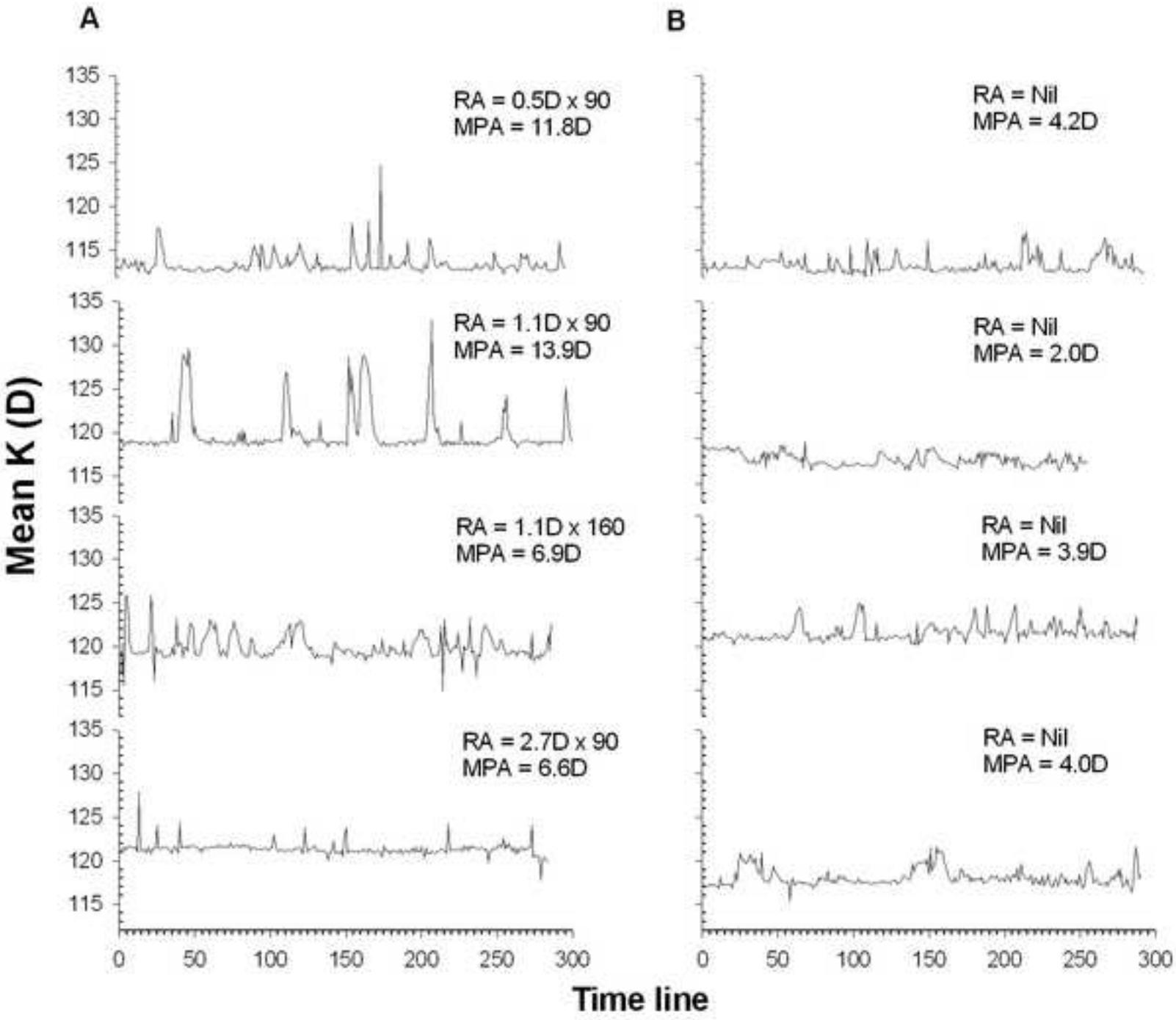

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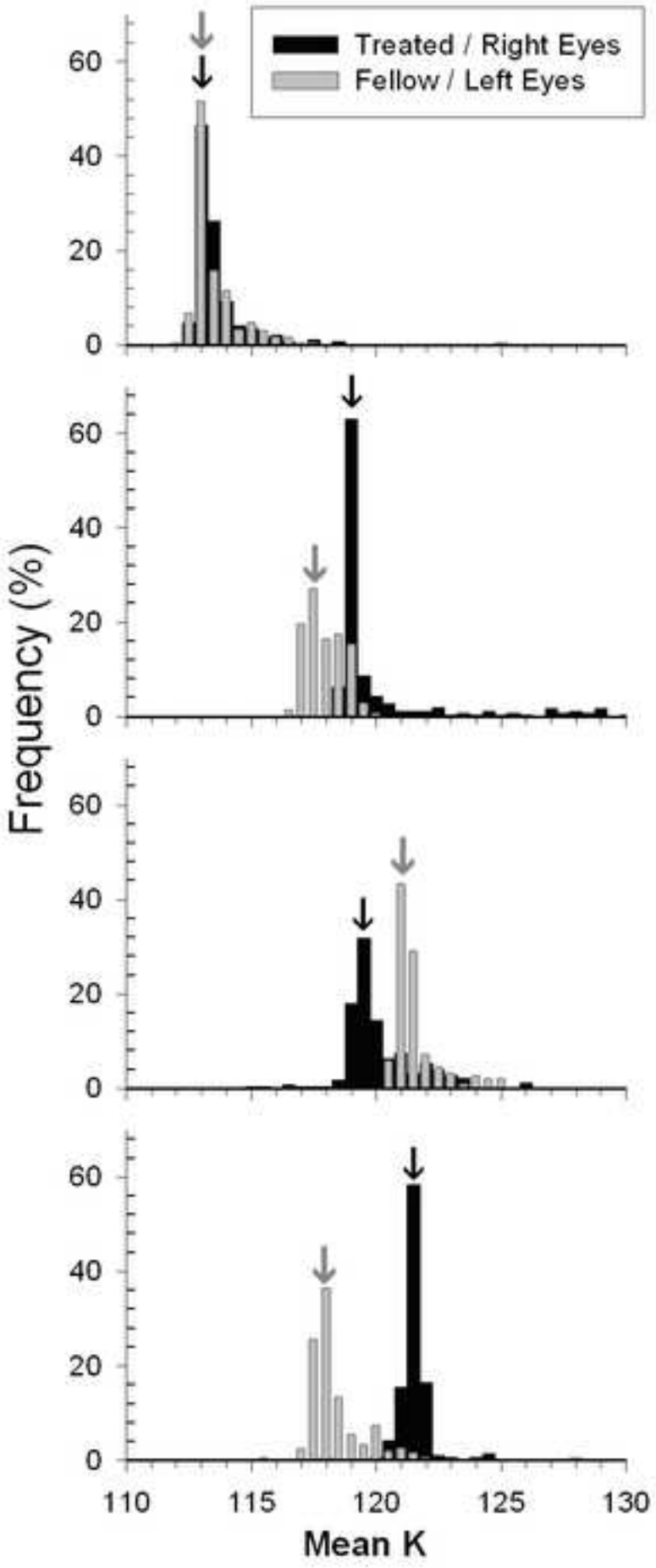

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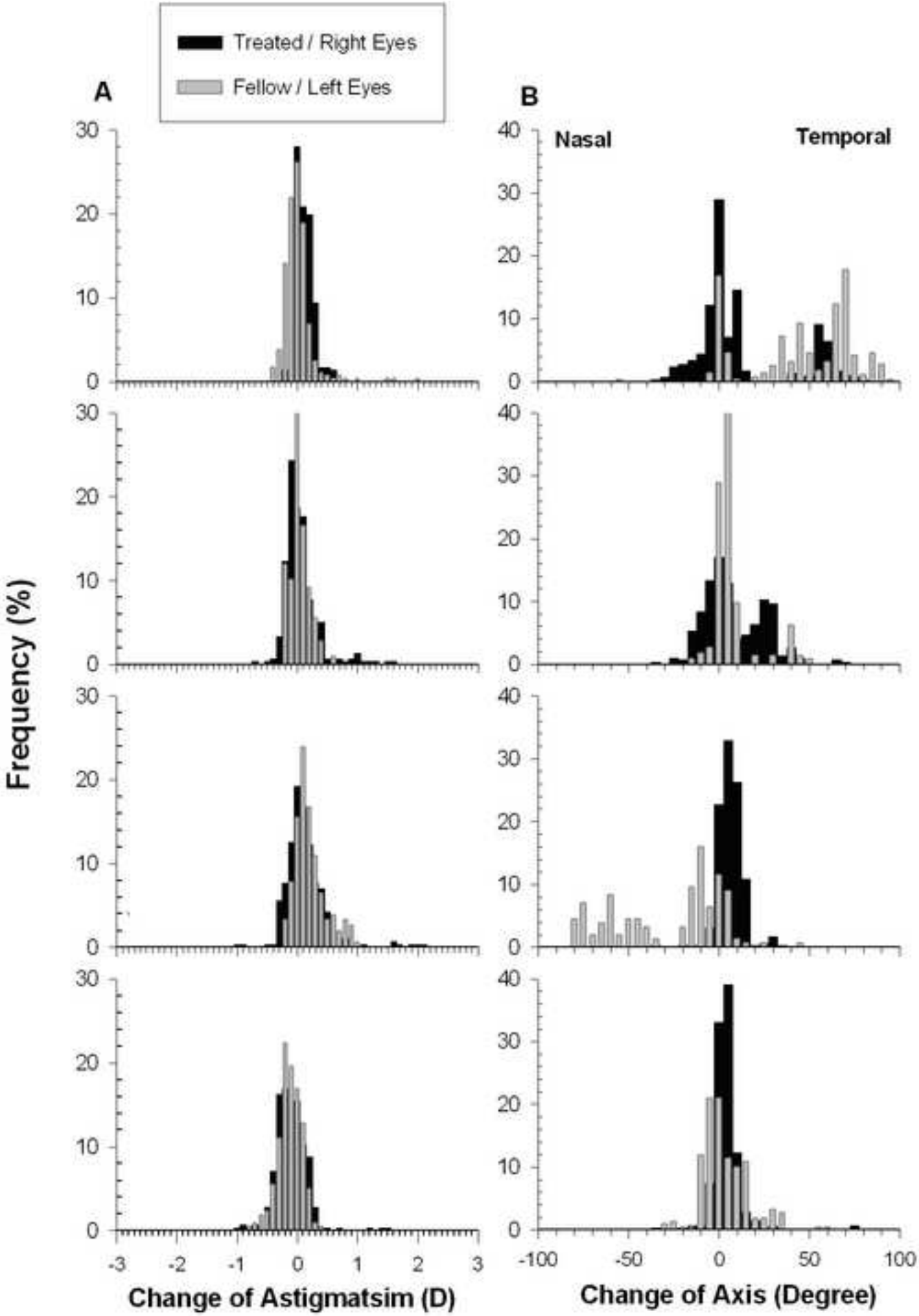

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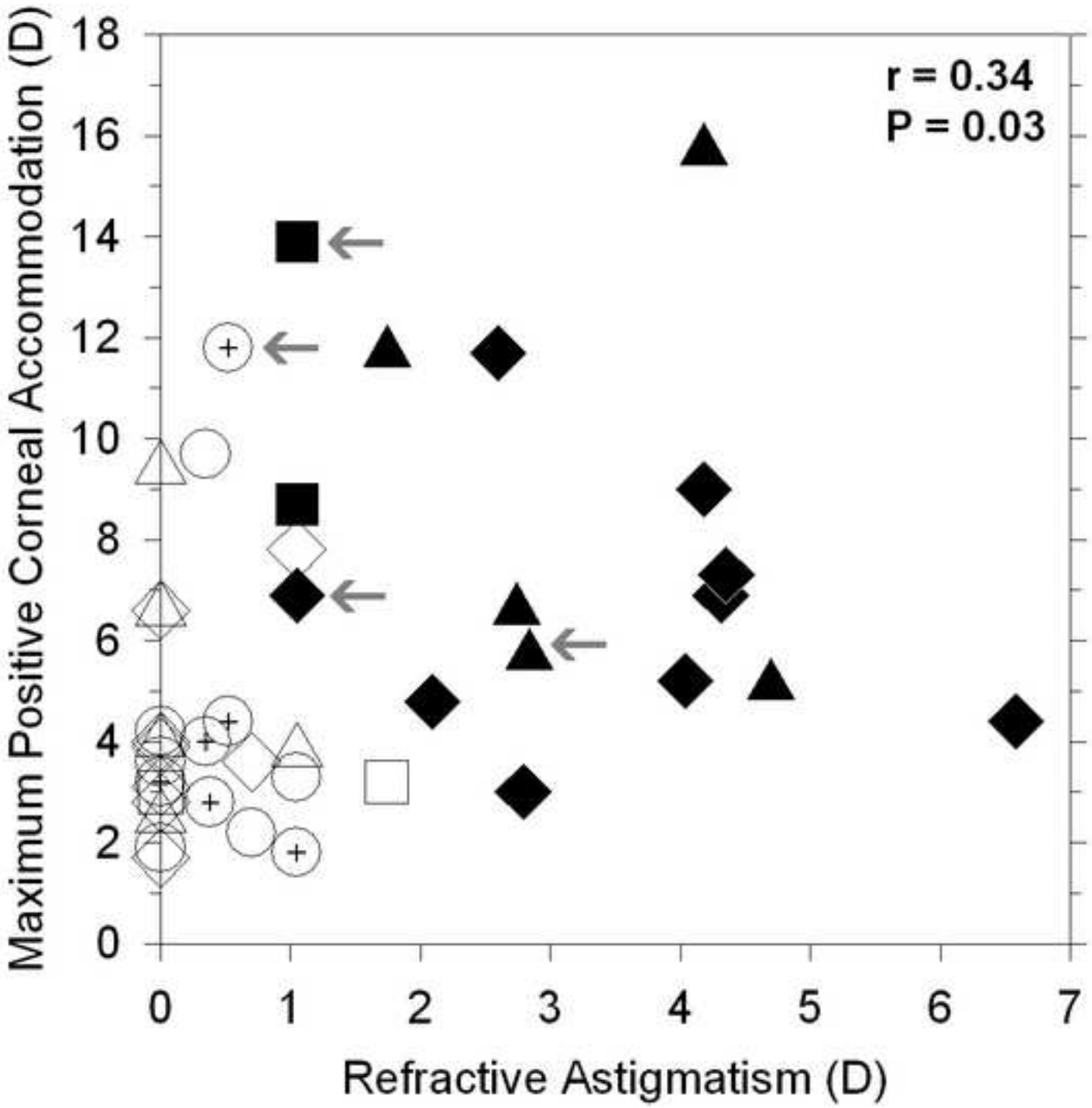

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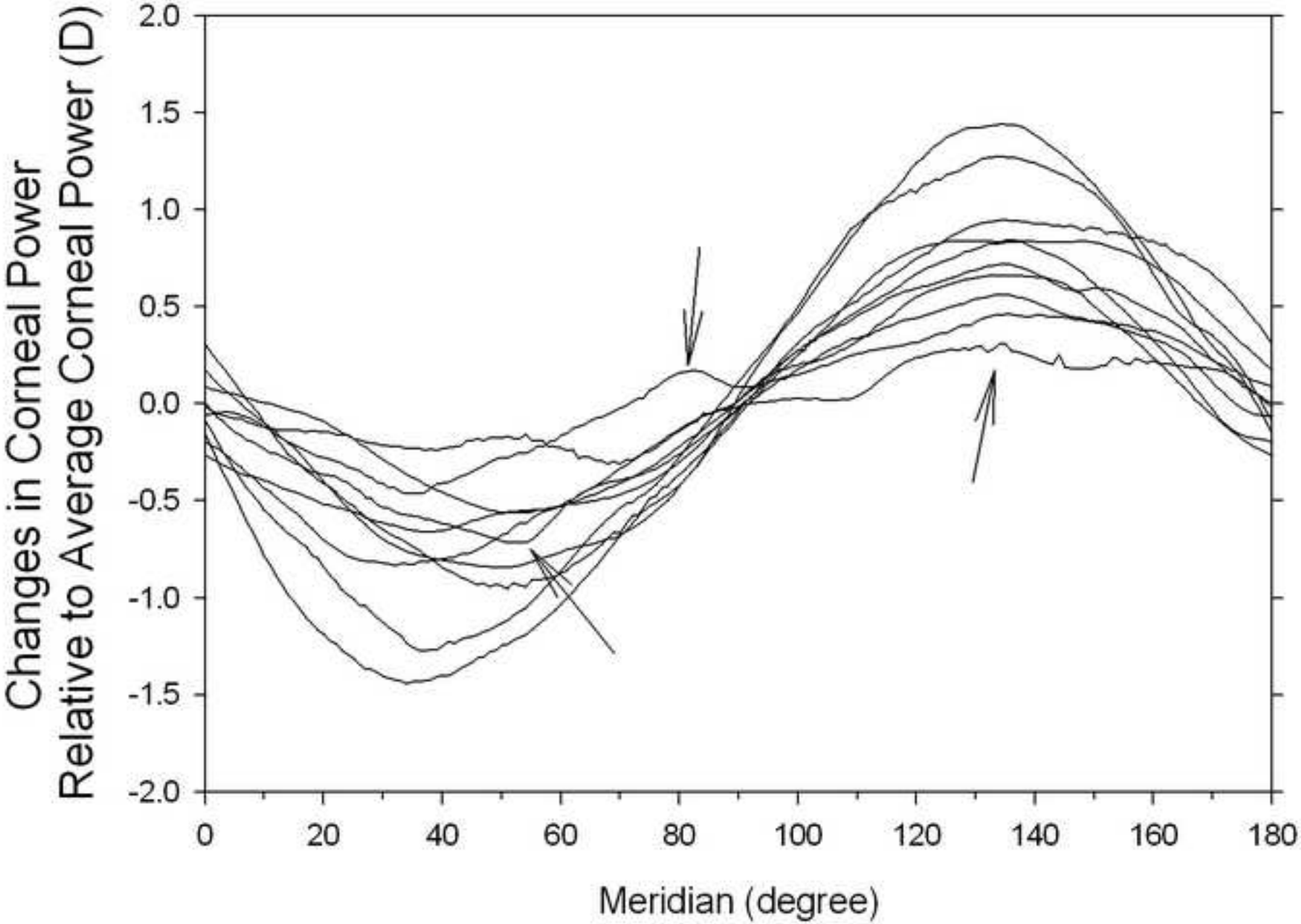

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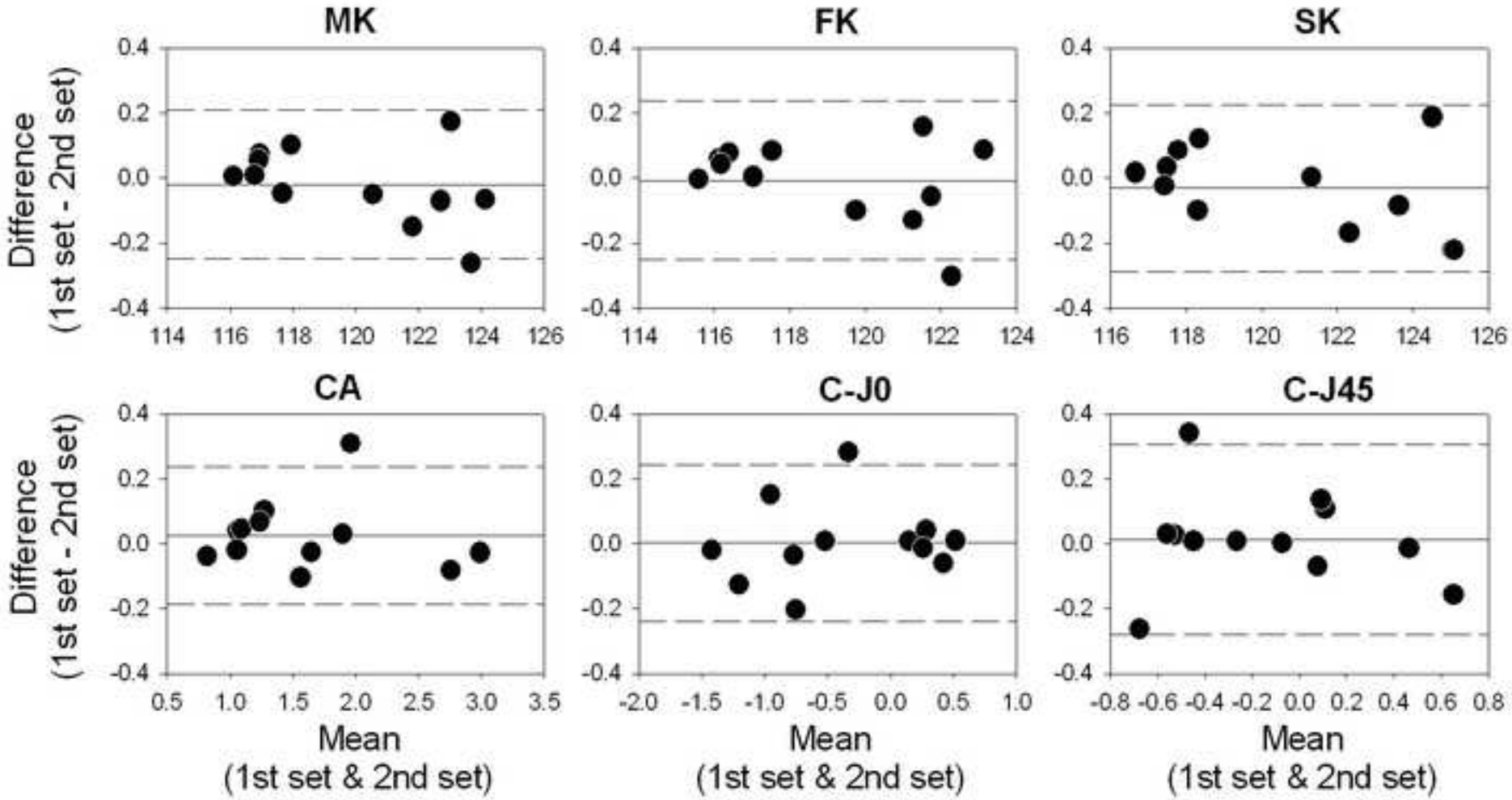

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