https://doi.org/10.1016/j.visres.2011.12.011

Effects of hemiretinal form deprivation on central refractive development and posterior eye shape in chicks.

(Short: Hemiretinal form-deprivation ametropia in chicks)

[1]Chin-hung Chu, B.Sc. (Hons.) Optometry, M.A.

[2]Li Deng, Ph.D.

[1]Chea-su Kee, B.Sc. (Hons.) Optometry, M.A., Ph.D.

[1]School of Optometry, The Hong Kong Polytechnic University. Hung Hom, Kowloon, Hong Kong SAR.

[2]New England College of Optometry, 424 Beacon St., Boston, MA 02115, USA.

Word Count: 4371; Number of Figures: 5; Number of Tables: 0

Keywords: Refractive development, Eye Shape, Myopia, Astigmatism, Chickens

Corresponding author:

Chea-su Kee, Ph.D.

Voice: (852) 2766-7941; Fax: (852) 2764-6051; Email: sokee@inet.polyu.edu.hk

Proprietary interest: None

Grant support: RGC General Research Fund B-Q20K; Internal Competitive Research Grant G-YG07, The Centre of Myopia Research and The Niches Areas-Myopia Research Fund (J-BB7P) from The Hong Kong Polytechnic University.

Abstract

We determined effects of hemiretinal form deprivation (i.e., form-depriving half of the retina) on central refractive development and posterior eye shape in chicks. Seventy-seven White Leghorn chicks were randomly assigned to receive superior (SRD, "Superior Retinal Deprivation" or inferior visual field deprivation, same principle applies for the following abbreviations, n=17), inferior (IRD, n=14), temporal (TRD, n=23) or nasal hemiretinal (NRD, n=23) form deprivation monocularly from day 5 to day 26. Central refractive errors, expressed as interocular difference in spherical equivalent (M), J0 and J45 astigmatic components, were measured using Hartinger refractometer at the beginning and weekly after treatment for 3 weeks. At the end of the treatment period, eyes of a subset of birds were enucleated and eye shape profile was photographed along four different meridians. These digital images were later processed to extract axial length (AL), equatorial diameter (ED), and AL/ED. For comparison purposes, the eye shape profile was also acquired from a separate group of birds reared with monocular full-retinal form deprivation (FRD, n=10). The four hemiretinal form deprivations altered central ametropia and posterior eye shape to different degrees. The biggest contrast in M was found between SRD and IRD groups (mean±SE after 3 weeks: SRD= –4.14±0.71 D vs. IRD=+1.24±0.36 D; $p<0.05$), whereas subtle differences in J0 and J45 components were found across the four treatment groups (both $p\leq0.03$). SRD group also showed significantly higher AL/ED ratio compared to IRD and NRD groups (0.76±0.05 vs. 0.74±0.07 and 0.75±0.04; both $p\leq0.03$). Furthermore, M was significantly correlated with AL/ED

ratio in the treated eyes of hemiretinal treated chicks (r=–0.55, p<0.001). Our results suggest that mechanism regulating central ametropia can be influenced by selectively interrupting the visual experience at different parts of visual field.

1. Introduction

Access to normal visual experience is essential for normal refractive development during early eye growth. Ever since lid-sutured macaque monkeys were first reported to develop abnormally long eyeball and axial myopia (Wiesel & Raviola, 1977), the profound effects of early visual form deprivation on inducing axial elongation and refractive error development were further confirmed in other animal species tested (fish: Shen, et al., 2005; tree shrew: Sherman, et al., 1977; marmoset:Troilo & Judge, 1993; chicks: Wallman, et al., 1978; and guinea pig:Howlett & McFadden, 2006). More astonishingly, when nasal or temporal retina was obstructed by translucent occluder in chicks, only the corresponding part of the posterior globe protruded and became myopic (Wallman, et al., 1987), regardless of whether the optic nerve was intact or not (Troilo, et al., 1987). Importantly, this “local mechanism” has also been reported in infant rhesus monkeys recently; specifically, covering the temporal retina increased vitreous chamber depth and relative myopia only at the temporal side of the eyeball (Smith, et al., 2009).

Because the central region of the retina provides the finest visual acuity, it is important to learn how visual experience across the visual field affects the central refractive development. In humans, it was reported that pilots who had relative hyperopic errors in both principal power meridians at the peripheral field were more prone to myopia development (Hoogerheide, et al., 1971), suggesting that optical error signals imposed on peripheral retina may act as a cue for

regulating eye growth. In infant rhesus monkeys, covering the animal's peripheral retina by a diffuser with unobstructed central vision induced axial myopia; strikingly, the recovery from this induced myopia with unrestricted vision was virtually unaffected in the absence of an intact fovea (Smith III, et al., 2005). In chicks, it has been shown that diffusers covering different extents of peripheral retina could have a significant impact on the magnitude of axial ametropia (Irving, et al., 1995, Stone, et al., 2006). Taken together, both the presence of local mechanism and the regulating role of peripheral vision on central refractive development indicate a potential relationship between peripheral eye shape and axial ametropia. Although the classifications of ametropic groups according to estimated eye shape is not yet conclusive, it has been suggested that the incorporation of biometric parameters associated with 3-dimensional eye shape could be useful in studying refractive error development (Stone & Flitcroft, 2004).

Despite growing evidence of the interaction between eye shape and central refractive development, very little is known about the relationship between eye shape and manifest astigmatism. Given the facts that astigmatism is frequently associated with ametropic eyes (humans:Alward, et al., 1985, Guggenheim & Farbrother, 2004, Kaye & Patterson, 1997, Parssinen, 1991; monkeys:Kee, et al., 2005; chicks:Kee & Deng, 2008) and that alterations in ametropic axial growth are primarily related to structural and molecular changes that occur at the posterior segment (Rada, et al., 1994, Siegwart Jr. & Norton, 1999), it is possible that astigmatism is a byproduct of abnormal posterior eye

growth (Kee & Deng, 2008, Kee et al., 2005). This hypothesis is in line with the suggestion that axial eye growth may alter anterior ocular structures through stretching (Mutti, et al., 1998, van Alphen, 1986), and the correlation found between the changes in axial length and corneal power during early infancy (Mutti, et al., 2005). This study aimed to determine the effects of hemiretinal form deprivation on central refractive development and eye shape using chicks as an animal model.

2. Materials and Methods

2.1 Animal Subjects

White Leghorn chickens (*Gallus gallus domesticus*, n=87) were used. They were reared in a temperature controlled (~22ºC) animal facility on a 12-hour light / 12-hour dark lighting cycle, with food and water provided *ad libitum*. The average light intensity was approximately 100 lux at chick's eye level. 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 Hong Kong Polytechnic University.

2.2 Visual Manipulations

All diffusers used in this study were heat-molded using 0.5mm-thick white polystyrene plastics. A full-retinal diffuser, which had a dome shape with an internal aperture diameter of 13mm and a sagittal height of 4mm, was cut into

two equal halves to make the hemiretinal diffuser. These hemiretinal diffusers were used to cover superior, inferior, temporal or nasal retina by using the chick's pupillary center as a reference point (see Figure 1A for an illustration). Each hemiretinal diffuser, which was first glued to the hook side of a Velcro ring, was attached to the loop side of a Velcro ring that was glued to feathers around the animal's right orbit. All the left eyes were untreated and used as control.

After baseline refractions were carried out at 5 day of age, the animals were randomly assigned to receive one of the four visual manipulations: superior ("SRD", n=17), inferior ("IRD", n=14), temporal ("TRD", n=23) or nasal retinal form deprivation ("NRD", n=23). The hemiretinal diffusers were removed daily for cleaning purposes throughout the treatment period.

2.3 Refractometry

Refractive errors were measured on day 5 post-hatching and weekly after that for 3 weeks by a Hartinger refractometer (Jena Coincidence Refractometer, Model 110, Carl Zeiss Meditec, Jena, Germany) modified for small pupils (Kee & Deng, 2008, Wallman & Adams, 1987). During refractions, birds were anaesthetized with Isoflurane inhalation (1.0% - 1.5% in Oxygen) and the eye was gently held open with a custom-made speculum. Our previous study has shown that the speculum has no effect on astigmatism measurement and has only minimal effect (~0.4D) on spherical-equivalent refractive error measurement (Kee & Deng, 2008).Three sets of measurements were taken for each eye and

the average was calculated using an algorithm based on power vectors analysis (Thibos, et al., 1997). Unless otherwise stated, the data were presented as interocular differences (treated eye – untreated eye) in spherical equivalent (also known as mean ocular refraction, M), J0 and J45 astigmatic components. All the measurements were taken at about the same time of the day (10:00a.m. ± 1hour) to minimize the effects of potential diurnal variations on refractive error measurements as reported by Johnson (IOVS 2004; 45: ARVO E-abstract 4295) and Campbell (JOV 2008; 8: Fall Vision Meeting E-abstract 48).

2.4 Eye Shape Profile Imaging

Eyeball images were acquired along four different meridians to extract posterior eye shape profile. After the last refractions were performed on day 26 post-hatching (i.e., 3 weeks of treatment), subsets of chicks from each treatment group (SRD, n=9; IRD, n=8; TRD, n=10; NRD, n=11) were sacrificed by carbon dioxide asphyxiation. Each eye was first land-marked with a fine-tip marker on its sclera at 12 o'clock (superior) position, enucleated, cleared of extraocular tissues and muscles, and photographed. The setup of imaging device is illustrated in Figure 1B: the enucleated eyeball was rested on an eye holder with its anterior part facing down and its pupil center aligned with the optical axis of an alignment camera (USB camera, Polar Techno-color Ltd., HK). The alignment was judged by using the corneal reflexes of eight LEDs around the camera. Once the alignment was fixed, images of the eyeball's profiles along each of the four meridians were captured consecutively using a CCD camera (Guppy F-036B,

Allied Vision Technologies, Staltroda, Germany) by revolving the eyeball around the pupillary axis through the eye holder (Figure 1C). The acquired image was later processed using a custom MatLab algorithm (MatLab; The MathWorks, Natick, MA) to determine the eye shape parameters (Zhang, et al., 2009). In particular the posterior eye shape profile was represented by extracting ocular lengths measured from central to peripheral eccentricity up to 50°, in 5° intervals, using the corneal apex as a reference (Fig.1D). Furthermore, to determine the effects of hemiretinal form deprivation on posterior eye shape, the ratio of axial length (AL) to equatorial diameter (ED) along a particular meridian was calculated for each bird. The AL was derived from the vertical dimension enclosed by the corneal apex and a point on the posterior scleral surface, whereas the ED was derived from the widest horizontal dimension in each image (Fig.1D). For SRD and IRD birds, AL/ED data were calculated from the images acquired along the superior-inferior (vertical) plane only; for TRD and NRD birds, the AL/ED values came from the dimensions measured using the images along the temporal-nasal plane (horizontal) only. For comparison purposes, eye shape profile was recorded from a separate group of birds reared with similar protocol except that the right eyes were treated with monocular full-retinal form deprivation (FRD, n=10).

2.5 Data analysis

Statistical analyses were carried out using SAS Enterprise Guide 4.1 (SAS Institute Inc., Cary, NC). Because our primary aim was to determine the effects of

hemiretinal form deprivation on central refractive component and eye shape parameters, the data of FRD treated birds were therefore not included in the statistical tests. Repeated measure analyses (via proc mixed) were applied to test the effects of treatment, treatment duration and their interaction on treated eyes. If the interaction was statistically significant, the treatment effect was subsequently tested by one-way ANOVA and the effect of treatment duration was tested by one-way repeated measures ANOVA. In addition, if ANOVA revealed a significant difference, Tukey's post-hoc test was conducted to identify which pairs of treatment were significantly different and Dunnett's post-hoc test was conducted to test on which day the change from baseline (day 5) was significant. Pairwise Spearman correlation coefficients between AL/ED and refractive components were computed and tested for significance. Significance level was set at $\alpha=0.05$.

3. Results

3.1 Effects on inter-ocular difference in M, J0 and J45

There were significant interactions between treatment and treatment duration in M, J0 and J45 astigmatic components (all interactions, $p \leq 0.05$). Overall, both treatment and treatment duration had significant effects (all $p<0.03$) on these three refractive components.

3.1.1 Treatment Effect (by treatment week)

3.1.1.1 M

At baseline, no significant differences were found among the four treatment groups in M (ANOVA; $p=0.17$). After one week, significant treatment effects were found in M (ANOVA; $p<0.0001$). As illustrated in Figure 2, IRD group had significantly less myopic/more hyperopic M compared to the other three groups (Tukey's adjustment for pairwise comparisons, all $p\leq0.01$). In contrast, SRD group had more myopic M than the other three treatment groups (Tukey's adjustment for pairwise comparison, all $p\leq0.01$). After two weeks of treatment, significant treatment effects in M still persisted (ANOVA, $p<0.0001$), with the SRD group exhibited more myopic M compared to the other three groups of birds (Tukey's adjustment for pairwise comparison, all $p\leq0.0001$). At the end of the three-week treatment period, the treatment effects were still statistically significant (ANOVA, all $p<0.0001$): the SRD group had significantly more myopic M than the other three groups (Tukey's adjustment for pairwise comparisons, all $p\leq0.01$), whereas the IRD group had low amount of hyperopia (+1.24±0.36D), which was significantly different from SRD and NRD groups (Tukey's adjustment for pairwise comparisons, all $p\leq0.005$) but not to the TRD group (Tukey's adjustment for pairwise comparisons, $p=0.09$).

3.1.1.2 J0 & J45

At baseline, no significant differences in J0 and J45 components were found among the four hemiretinal treated groups (ANOVA, all $p>0.39$). After one week, significant treatment effects were found on J0 (ANOVA, $p=0.001$) but not on J45 (ANOVA, $p=0.29$). In particular, the TRD group had more minus J0 component

compared to both NRD and SRD groups (Tukey's $p \leq 0.02$) but not to the IRD group (Tukey's $p=0.32$). On week two, significant treatment effects were found on J45 (ANOVA, both $p=0.02$) but not on J0 (ANOVA, $p=0.25$). The TRD group had J45 component significantly more minus compared to those of SRD group (Tukey's $p=0.04$). At the end of the 3-week treatment period, significant treatment effects were found on both J0 and J45 (ANOVA, both $p \leq 0.03$), the TRD exhibited more minus J0 compared to SRD with borderline significance (Tukey's, $p=0.066$) and the NRD exhibited more minus J45 compared to the SRD group (Tukey's $p \leq 0.01$).

3.1.2 Treatment Duration Effect (by treatment type)

3.1.2.1 M

Treatment duration had significant effect on M for the SRD, NRD, and TRD groups (all $p \leq 0.04$) but not for IRD group ($p=0.08$). For both SRD and NRD groups, the relative changes from baseline in M at all three time points (i.e., 1st, 2nd and 3rd weeks) were significant (Dunnett's post-hoc tests, all $p \leq 0.04$) except on the 1st week of NRD group (Dunnett's post-hoc test, $p=0.065$). For TRD group, the changes from baseline in M was significant only on the 1st week ($p=0.02$).

3.1.2.2 J0 & J45

Treatment duration had significant effects on J0 component for all (all $p \leq 0.03$) except NRD groups ($p=0.24$), and on J45 component for the NRD group only

(p=0.012). With respect to baseline, significant more minus J0 was found on 2nd week for SRD group (Dunnett's post-hoc test, p=0.02), on 1st and 3rd weeks for TRD group (Dunnett's post-hoc test, p<0.01), and on 2nd and 3rd weeks for IRD group (Dunnett's post-hoc test, p<0.007). For the NRD group, significant more minus J45 was found on 3rd week only (Dunnett's post-hoc test, p=0.03).

3.2 Posterior Eye shape Parameters

Hemiretinal form deprivations produced an enlarged eyeball in general with local expansion corresponding to the deprived region. Figure 3 illustrates the posterior eye shape profile (mean length+SE) as a function of eccentricity with reference to the corneal apex for the four hemiretinal treatment groups (half-filled symbols), a full-retinal deprived group (filled symbols), and all the fellow untreated eyes as a control group (open symbols) after the 3 weeks observation period. Eye shape profiles along the horizontal (left) and vertical (right) meridians were presented with the corresponding anatomical locations indicated on x-axes. Compared to the fellow untreated eyes, it is obvious that all hemiretinal form deprivations resulted in an overall enlargement of eyeball with a protruded area corresponding to the form-deprived region. It should be noted that this enlarged posterior segment applied to both covered *and* uncovered regions~~, which could partly be due to a general reduction in light level with the proximity of hemiretinal diffuser~~. Furthermore, the differences between hemiretinal and full-retinal form deprivations were more pronounced near the posterior pole but appeared to diminish near 50° eccentricities.

To illustrate the ocular expansion due to hemiretinal and full-retinal form deprivations at all meridians, Figure 4 plots the percentage increase in eye dimension (treated eye /fellow untreated eye) for five eccentricities (i.e., 10°, 20°, 30°, 40° & 50°) from central. For each eccentricity, percentage increase at the eight locations (two locations on each meridian) was calculated individually and averaged for each treatment group. In the polar plot, each datum represents an average increase in percentage (radius) at a particular retinal location (see Figure legend) for a treatment group. To visualize the local effects more easily, the data for the same treatment group are color coded as shown in Figure legend. Compared to full-retinal form deprivation (black lines), which produced symmetric eye expansion for virtually all measured meridians in the posterior pole from 10° to 40° eccentricities (see statistics in the following paragraph), the hemiretinal treatment groups resulted in asymmetric posterior expansions typically near the central (axial) regions but these asymmetric local effects appear to diminish gradually towards 40° eccentricity. For instance, by comparing the SRD (red lines) and IRD (green lines), one would notice much bigger expansions at, respectively, superior and inferior regions from 10° to 40° eccentricities; however, these treatment effects disappeared at 50° eccentricity. At 50° eccentricity, which was nearby the eye's equator (see Fig.1D), although both full-retinal and hemiretinal form deprivations still produced relatively bigger eye sizes compared to their fellow untreated eyes, all treatment groups exhibited larger expansion only on the temporal side of the eyeball.

To determine if individual treatments had produced asymmetric eye growth on individual meridians, for each of the five eccentricities, the differences in eye expansion between the two opposite locations (i.e., temporal – nasal; superior – inferior; superior-nasal – inferior-temporal; or superior-temporal – inferior-nasal) were calculated for each bird and the group's data were tested to see if the values were statistically significant from zero. As marked in Fig.4, any location where a symbol was inserted represented an "asymmetric expansion" along a particular meridian, e.g., asymmetric eye growths were consistently found in SRD group at superior position (superior – inferior > 0) from 10-40° eccentricities. Further analyses showed that the treatment effects of hemiretinal form deprivation on asymmetric expansion at all four meridians were statistically significant for all (one-way ANOVAs, all $p < 0.01$) except the 50° eccentricity (one-way ANOVAs, all $p > 0.19$).

Figure 5 shows the effects of hemiretinal form deprivation on M and AL/ED for the fellow untreated (open) and treated eyes (filled) at the end of the 3-week treatment period., No significant differences in M or AL/ED were found in the fellow untreated eyes across the four treatment groups (ANOVA, all $p>0.18$). In contrast, significant treatment effects were found on M and AL/ED in the treated eyes (ANOVA, $p \leq 0.006$). Similar to the results shown in Fig.2, this SRD subset also had significantly more myopic M than the other three subsets of treated birds (Tukey's adjustment for pairwise comparisons, all $p \leq 0.01$). More importantly, not

only did SRD group show significantly higher AL/ED compared to IRD and NRD groups (both $p \leq 0.03$ after Tukey's adjustment), the IRD group also had significantly smaller AL/ED compared to TRD group ($p=0.01$ after Tukey's adjustment). In addition, correlation analyses of the 38 treated eyes indicated that M (Spearman's $r=-0.55$, $p<0.001$), but not J0 (Spearman's $r=0.17$, $p=0.30$) and J45 (Spearman's $r=-0.10$, $p=0.56$), was significantly correlated with AL/ED.

4. Discussion

Our key findings were: 1) the effects of hemiretinal form deprivation on central ametropia and eye shape may vary depending on which hemiretinal was deprived; 2) the induced astigmatism showed subtle differences in magnitudes and properties across the four hemiretinal treatment groups; 3) the spherical-equivalent refractive error in these hemiretinal form deprived chicks was correlated with AL/ED ratio.

Our results provide further evidence that hemiretinal form deprivation could also alter central ametropia in chicks. The magnitude of this induced myopia, however, was much smaller when compared to previous studies which partially form-deprived retina with diffuser placed 10° beyond the pupillary center or diffuser with a trapezium opening (Troilo et al., 1987, Wallman et al., 1987; see also Diether & Schaeffel, 1997), suggesting a more sensitive/plastic region within the 10° central retina. We believe that this lower magnitude of central ametropia came about because the translucent occluder we used to bisect the pupil might have exposed the treated eyes to more than half of the visual field due to eye movements and/or viewing behavior. As shown in Figs. 3 & 4, the corresponding treatment-induced changes in eye shape and dimension were more obvious at 20-30° eccentricities, whereas those changes within the 10° eccentricity were smaller in magnitude. However, it should be noted that the magnitudes of changes at corresponding areas were quite similar within 30° eccentricities

(Figures 3 & 4), suggesting that the effects of asymmetric eye movement or eye's fixating behavior, if there is any, should be minimal. Nevertheless, given the facts that chicks could exert 10-20° lateral eye movements (Schippert & Schaeffel, 2006), and that only brief periods of unrestricted vision could significantly attenuate the effects of form-deprivation or defocus-induced myopia (Kee, et al., 2007, Napper, et al., 1997, Shaikh, et al., 1999, Smith III, et al., 2002, Winawer & Wallman, 2002), it is possible that had we covered more than half of the retina, like those device used by Wallman and coworkers (Wallman et al., 1987), the changes in central ametropia and ocular dimensions would have been larger. In this respect, previous studies using occluders (Stone et al., 2006) or spherical lenses (Morgan & Ambadeniya, 2006, Schippert & Schaeffel, 2006) with central aperture (i.e., unrestricted central visual field) have consistently shown that central ametropia can be induced only if the size of the peripheral visual deprivations was big enough to cover a critical region around the central retina in chicks (see also (Irving et al., 1995)), our results provide further evidence that even if the central retina in the treated chicks might have been partially exposed to unrestricted vision, covering the four hemi-retinal sectors can still produce different impacts on central ametropia (Fig.2).

Among the four hemiretinal treated groups, IRD and SRD birds exhibited the biggest contrast in the magnitudes of central ametropia and AL/ED ratio. The differential effects of covering inferior and superior retina were also reported in previous studies using chicks (Miles & Wallman, 1990, Stone et al., 2006) and

guinea pigs (McFadden, 2002, Zeng & McFadden, 2010). Specifically, using diffusers with apertures oriented eccentrically for chicks to access inferior-nasal or superior-temporal retina, Stone et al.(Stone et al., 2006) have found that the magnitude of central myopia was much higher in birds with superior-temporal retina covered than those birds with inferior-nasal retina covered. Similarly, as reported in two abstracts, McFadden (*IOVS* 2002; 43: ARVO E-abstract 189) and Zeng and McFadden (*IOVS* 2010; 51: ARVO E-Abstract 1736) also reported that guinea pigs became more myopic when superior retina was covered with a partial diffuser. It remains unclear whether this differential susceptibility to superior-inferior retinal deprivations is related to regional variations in retinal function and/or ocular structural plasticity. At cellular level, there is evidence that the embryonic developmental pattern is distinctly different between rod and cone photoreceptor subtypes in chicks, with rods developed earlier and distributed more abundantly in the inferior retina compared to cones (Bruhn & Cepko, 1996). Furthermore, the bullwhip and mini-bullwhip cells, retinal cell types which have been proposed recently to regulate eye size in chicks (Fischer, et al., 2008), were also found to distribute asymmetrically in, respectively, ventral and dorsal circumferential marginal retinal regions (Fischer, et al., 2006). Further studies are much in need to determine whether this regional variation in cell types can influence the mechanism regulating central refractive development and eye shape. It would also be interesting to find out if the higher susceptibility to superior retinal form deprivation would lead to ocular pathologies commonly found at superior fundus (e.g., retinal hole and tear) in humans (Kanski, 1989).

The magnitude of induced astigmatism also varied depending on the retinal region receiving form deprivation, albeit its pattern is different from those shown by spherical power components (Fig.2). First, although SRD group exhibited the highest magnitudes of spherical-equivalent among the four groups of birds, the same group developed the lowest magnitude of J0 compared to other groups. Contradict to the prediction of astigmatism as a byproduct of ametropic eye growth and the direct correlations frequently found in the magnitudes of spherical-equivalent and astigmatism (Kee & Deng, 2008, Kee et al., 2005, Kisilak, et al., 2008), these results suggest that the mechanism underlying the hemiretinal form-deprivation induced astigmatism may be more complicated than previously thought. Second, the signs of J0 components were negative for all treatment groups but the signs of J45 components were somewhat varied across the treatment groups. Specifically, unlike TRD and NRD treatments, which both induced negative J45 components, the SRD treatment resulted in a positive J45 component. To our knowledge, other than the recent finding that covering the nasal visual field with either diffuser or -3D lens produced significantly higher magnitudes of manifest astigmatism in monkeys (Hung, Huang & Smith III, *IMC* 2010, Poster 44), this is the first study which shows that hemiretinal form deprivation could have significant impacts on manifest astigmatism and produced subtle differences in individual astigmatic components in chicks. It should be noted, however, that although subtle differences were found on J45 components, the magnitudes of J45 were smaller than J0 components. Based on our sample

Formatted: Left, Indent: First line: 0 cm, Don't adjust space between Latin and Asian text, Don't adjust space between Asian text and numbers

size and the data collected, we only have 80% and 68% statistical powers, respectively, to detect a maximum difference of 1.21D in J0 and a maximum difference of 0.66D in J45 across the four treatment groups. On the other hand, of those treated eyes that exhibited more than 1.0D of manifest astigmatism, the proportions of against-the-rule (axes range=60°~120°), with-the-rule (axes range= 0°~ 30° & 150°~180°), and oblique astigmatisms (observed axes=35°, 135° & 140°) were indeed quite similar after 1 week (ATR=75.9%; WTR=22.2%; Oblique=1.85%; total n=54) and 3 weeks of treatment (ATR=82.7%; WTR=13.5%; Oblique=3.8%; total n=52). How this disproportionately higher prevalence of against-the-rule astigmatism came about despite significant differential treatment effects on posterior eye shape remains uncertain. Because asymmetric ocular expansions were consistently found at 50° temporal side of all treatment groups (Fig.4), it would be interesting to study the influence of eye shape profile near equator or anterior to equator on the characteristics of astigmatism induced.

Several human studies, using either imaging techniques (Cheng, et al., 1992, Deller, et al., 1947) or peripheral refractions (Mutti, et al., 2000), have noted a tendency for myopes and hyperopes to exhibit, respectively, more prolate and oblate eye shape (for a review see Stone & Flitcroft, 2004). However, a reanalysis of previous peripheral refractions data (collected only at 30° temporal retina, (Mutti et al., 2000) indicates that classifying refractive groups according to the geometry of eye shape has its limitation; in essence, different

kinds of eye shape could be found within each refractive group (Stone & Flitcroft, 2004). In this respect, our results showed that M was also moderately but significantly correlated with AL/ED ratio (Spearman's r= –0.55, p<0.001), indicating that myopia was associated with a more prolate/less oblate eye shape. It should be noted, however, that the AL/ED ratio was calculated based on the values acquired at the presumably most responsive meridians for individual treatment group. As reflected in Fig.4, subtle differences in eye shape at all meridians across the four treatment groups were also noted near 50° eccentricity. If AL/ED ratio was recalculated based on the averaged values of all four meridians, the correlation between M and AL/ED actually became even stronger (Spearman's r= –0.65, p<0.001), supporting the idea that 3-dimensional eye shape may be a better indicator in relating with central refractive status.

In summary, our results indicate that central refractive development can be altered to different degrees even if only half of the retina is visually deprived. The significantly higher magnitudes of central myopia associated with superior retinal form deprivation warrant further investigation. It is important to study if myopic eye growth in humans is triggered by a similar lower visual field (superior retinal) deprivation (e.g., book or desk). Equally importance is to elucidate whether and how mechanism regulating eye growth is limited by regional variations in sensory and/or mechanical structures.

5. References

Alward, W.L., Bender, T.R., Demske, J.A., & Hall, D.B. (1985). High prevalence of myopia among young adult Yupik Eskimos. *Can J Ophthalmol, 20* (7), 241-245.

Bruhn, S.L., & Cepko, C.L. (1996). Development of the pattern of photoreceptors in the chick retina. *The Journal of Neuroscience, 16* (4), 1430-1439.

Campbell, M.C.W., Bunghardt, K., Kisilak, M.L., & Irving, E.L. (2008). Diurnal rhythms of refractive error components in normal chicks. *JOV, 8* (17), #48.

Cheng, H.M., Singh, O.S., Kwong, K.K., Xiong, J., Woods, B.T., & Brady, T.J. (1992). Shape of the myopic eye as seen with high-resolution magnetic resonance imaging. *Optom Vis Sci, 69* (9), 698-701.

Deller, J., O'Connor, A., & Sorsby, A. (1947). X-ray measurements of the diameter of the living eye. *Proc R Soc Lond Series B, 134*, 456-467.

Diether, S., & Schaeffel, F. (1997). Local changes in eye growth induced by imposed local refractive error despite active accommodation. *Vision Res, 37* (6), 659-668.

Fischer, A.J., Ritchey, E.R., Scott, M.A., & Wynne, A. (2008). Bullwhip neurons in the retina regulate the size and shape of the eye. *Developmental Biology, 317* (1), 196-212.

Fischer, A.J., Skorupa, D., Schonberg, D.L., & Walton, N.A. (2006). Characterization of glucagon-expressing neurons in the chicken retina. *The Journal of Comparative Neurology, 496* (4), 479-494.

Guggenheim, J.A., & Farbrother, J.E. (2004). The association between spherical and cylindrical component powers. *Optom Vis Sci, 81* (1), 62-63.

Hoogerheide, J., Rempt, F., & Hoogenboom, W.P. (1971). Acquired myopia in young pilots. *Ophthalmologica, 163* (4), 209-215.

Howlett, M.H., & McFadden, S.A. (2006). Form-deprivation myopia in the guinea pig (Cavia porcellus). *Vision Res, 46* (1-2), 267-283.

Irving, E.L., Callender, M.G., & Sivak, J.G. (1995). Inducing ametropias in hatchlng chicks by defocus- aperture effects and cylindrical lenses. *Vision Res., 35* (9), 1165-1174.

Johnson, C.A., Lytle, G., Troilo, D., & Nickla, D.L. (2004). Chick Eyes Show A Diurnal Rhythm In Refractive Error. *Invest Ophthalmol & Vis Sci, ARVO E-Abstract 4295*

Kanski, J.J. (1989). Clinical Ophthalmology: A systematic approach. (Hong Kong: Butterworth-Heinemann Ltd.

Kaye, S.B., & Patterson, A. (1997). Association between total astigmatism and myopia. *J Cataract Refract Surg, 23* (10), 1496-1502.

Kee, C.-s., & Deng, L. (2008). Astigmatism Associated with Experimentally Induced Myopia or Hyperopia in Chickens. *Invest. Ophthalmol. Vis. Sci., 49* (3), 858-867.

Kee, C.-s., Hung, L.-F., Qiao-Grider, Y., Ramamirtham, R., & Smith III, E.L. (2005). Astigmatism in Monkeys with Experimentally Induced Myopia or Hyperopia. *Optom Vis Sci, 82* (4), 248-260.

Kee, C.-s., Hung, L.-F., Qiao-Grider, Y., Ramamirtham, R., Winawer, J., Wallman, J., & Smith, E.L., III (2007). Temporal Constraints on Experimental Emmetropization in Infant Monkeys. *Invest. Ophthalmol. Vis. Sci., 48* (3), 957-962.

Kisilak, M.L., Hunter, J.J., Huang, L., Campbell, M.C.W., & Irving, E.L. (2008). In chicks wearing high powered negative lenses, spherical refraction is compensated and oblique astigmatism is induced. *Journal of Modern Optics, 55* (4-5), 611-623.

McFadden, S. (2002). Partial occlusion produces local form deprivation myopia in the Guinea Pig eye. *Invest Ophthalmol & Vis Sci, 43* (E-Abstract 189)

Miles, F.A., & Wallman, J. (1990). Local ocular compensation for imposed local refractive error. *Vision Res, 30* (3), 339-349.

Morgan, I.G., & Ambadeniya, M.P. (2006). Imposed Peripheral Myopic Defocus Can Prevent the Development of Lens-Induced Myopia. *Invest. Ophthalmol. Vis. Sci., 47* (5), 3328-.

Mutti, D.O., Mitchell, G.L., Jones, L.A., Friedman, N.E., Frane, S.L., Lin, W.K., Moeschberger, M.L., & Zadnik, K. (2005). Axial Growth and Changes in Lenticular and Corneal Power during Emmetropization in Infants. *Invest. Ophthalmol. Vis. Sci., 46* (9), 3074-3080.

Mutti, D.O., Sholtz, R.I., Friedman, N.E., & Zadnik, K. (2000). Peripheral refraction and ocular shape in children. *Invest Ophthalmol Vis Sci, 41* (5), 1022-1030.

Mutti, D.O., Zadnik, K., Fusaro, R.E., Friedman, N.E., Sholtz, R.I., & Adams, A.J. (1998). Optical and structural development of the crystalline lens in childhood. *Invest Ophthalmol Vis Sci, 39* (1), 120-133.

Napper, G.A., Brennan, N.A., Barrington, M., Squires, M.A., Vessey, G.A., & Vingrys, A.J. (1997). The effect of an interrupted daily period of normal visual stimulation on form deprivation myopia in chicks. *Vision Res, 37* (12), 1557-1564.

Parssinen, O. (1991). Astigmatism and school myopia. *Acta Ophthalmol (Copenh), 69* (6), 786-790.

Rada, J.A., Matthews, A.L., & Brenza, H. (1994). Regional proteoglycan synthesis in the sclera of experimentally myopic chicks. *Exp Eye Res, 59* (6), 747-760.

Schippert, R., & Schaeffel, F. (2006). Peripheral defocus does not necessarily affect central refractive development. *Vision Research, 46* (22), 3935-3940.

Shaikh, A.W., Siegwart, J.T., Jr., & Norton, T.T. (1999). Effect of interrupted lens wear on compensation for a minus lens in tree shrews. *Optom Vis Sci, 76* (5), 308-315.

Shen, W., Vijayan, M., & Sivak, J.G. (2005). Inducing form-deprivation myopia in fish. *Invest Ophthalmol Vis Sci, 46* (5), 1797-1803.

Sherman, S.M., Norton, T.T., & Casagrande, V.A. (1977). Myopia in the lid-sutured tree shrew (*Tupaia glis*). *Brain Res, 124*, 154-157.

Siegwart Jr., J.T., & Norton, T.T. (1999). Regulation of the mechanical properties of tree shrew sclera by the visual environment. *Vision Res, 39*, 387-407.

Smith, E.L., 3rd, Huang, J., Hung, L.F., Blasdel, T.L., Humbird, T.L., & Bockhorst, K.H. (2009). Hemiretinal form deprivation: evidence for local control of eye growth and refractive development in infant monkeys. *Invest Ophthalmol Vis Sci, 50* (11), 5057-5069.

Smith III, E.L., Hung, L.F., Kee, C.-s., & Qiao, Y. (2002). Effects of brief periods of unrestricted vision on the development of form-deprivation myopia in monkeys. *Invest Ophthalmol Vis Sci, 43* (2), 291-299.

Smith III, E.L., Kee, C.-s., Ramamirtham, R., Qiao-Grider, Y., & Hung, L.-F. (2005). Peripheral Vision Can Influence Eye Growth and Refractive Development in Infant Monkeys. *Invest. Ophthalmol. Vis. Sci., 46* (11), 3965-3972.

Stone, R.A., & Flitcroft, D.I. (2004). Ocular shape and myopia. *Ann Acad Med Singapore, 33* (1), 7-15.

Stone, R.A., Pendrak, K., Sugimoto, R., Lin, T., Gill, A.S., Capehart, C., & Liu, J. (2006). Local patterns of image degradation differentially affect refraction and eye shape in chick. *Curr Eye Res., 31* (1), 91-105.

Thibos, L.N., Wheeler, W., & Horner, D. (1997). Power vectors: an application of fourier analysis to the description and statistical analysis of refractive error. *Optom & Vision Sci., 74* (6), 367-375.

Troilo, D., Gottlieb, M.D., & Wallman, J. (1987). Visual deprivation causes myopia in chicks with optic nerve section. *Current Eye Res., 6* (8), 993-999.

Troilo, D., & Judge, S.J. (1993). Ocular development and visual deprivation myopia in the common marmoset (Callithrix jacchus). *Vision Res., 33* (10), 1311-1324.

van Alphen, G.W.H.M. (1986). Choroidal stress and emmetropization. *Vision Res, 26* (5), 723-734.

Wallman, J., & Adams, J.I. (1987). Developmental aspects of experimental myopia in chicks: susceptibility, recovery and relation to emmetropization. *Vision Res, 27* (7), 1139-1163.

Wallman, J., Gottlieb, M.D., Rajaram, V., & Fugate-Wentzek, L.A. (1987). Local retinal regions control local eye growth and myopia. *Science, 237* (4810), 73-77.

Wallman, J., Turkel, J., & Trachtman, J. (1978). Extreme myopia produced by modest change in early visual experience. *Science, 201*, 1249-1251.

Wiesel, T.N., & Raviola, E. (1977). Myopia and eye enlargement after neonatal lid fusion in monkeys. *Nature, 266*, 66-68.

Winawer, J., & Wallman, J. (2002). Temporal constraints on lens compensation in chicks. *Vision Res, 42* (24), 2651-2668.

Zeng, G., & McFadden, S. (2010). Regional variation in susceptibility to myopia from partial form deprivation in the Guinea pig. *Invest Ophthalmol & Vis Sci, 51* (E-Abstract 1736)

Zhang, B., You, J., Karray, F., Kee, C.-s., & Li, Q. (2009). Astigmatism and eye shape. *International Conference on Image Processing, Computer Vision, and Pattern Recognition* (pp. 72-77). Las Vegas, USA.

Figure legends.

Figure 1. A) Hemiretinal diffusers were used to deprive half of the visual field by aligning the diffuser's edge with pupil centre. In this example, the nasal retinal is form deprived. B) Schematic diagram of the set-up of imaging system, the pupillary axis of the eyeball was aligned with the optical axis of an alignment camera guided by eight LEDs built around the camera's aperture, the eye shape profile was captured by the Guppy CCD camera. C) The profiles of the enucleated eyeball at four meridians were captured consecutively by revolving the eyeball around the pupillary axis. D). The edge of each eyeball's profile was first extracted by a custom MatLab algorithm and posterior ocular parameters were derived for eccentricities up to 50° in 5° intervals on each side.

Figure 2. Longitudinal changes (mean+/-SE) of the interocular differences in spherical-equivalent refractive error (M), J0 and J45 astigmatic components over the 3-week treatment period.

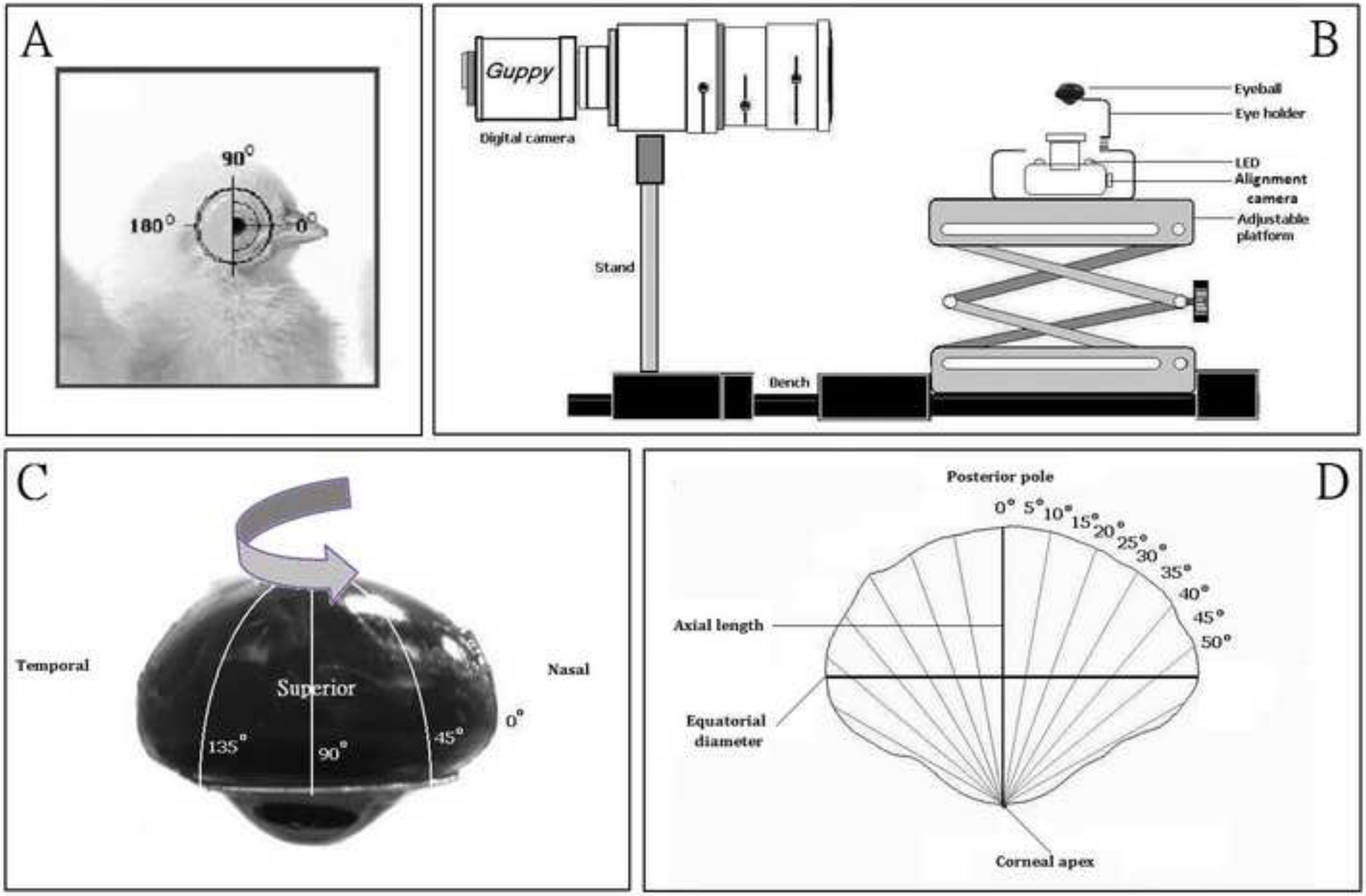
Figure 3. Posterior ocular dimension (mean+SE) as a function of eccentricities with respect to corneal apex for hemiretinal treated (semi-filled symbols) and fellow untreated eyes (open symbol). Data along the horizontal (left) and vertical meridians (right) were presented with their anatomical positions indicated on the x-axes. Data from full-retina form-deprived (filled symbols) birds were presented for reference purposes. Note that the standard errors for control eyes were small (max.=0.048) and were thus hidden by the symbols.

Figure 4. Effects of hemiretinal form deprivations on local eye shape at different eccentricities. Percentage increase in ocular dimensions for treated eyes with respect to fellow untreated eyes (treated eyes / fellow untreated eyes) are plotted for five eccentricities. Data for the five treatment groups are color coded as shown in the legend. Each ring represents a 2% increase in the treated eye relative to the fellow eye. A symbol on one side of each meridian represents a statistical significant asymmetric expansion.

Figure 5. Spherical-equivalent refractive error (M) and AL/ED ratio for treated (filled bar) and fellow untreated eyes (open bar) at the end of 3-week treatment period (mean+SE).

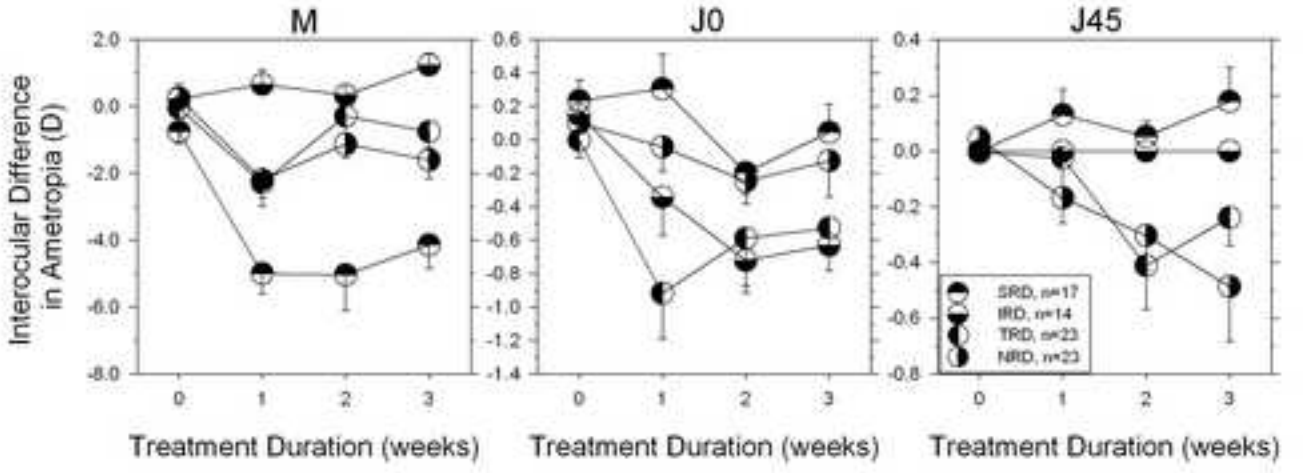
Figure

Click here to download high resolution image



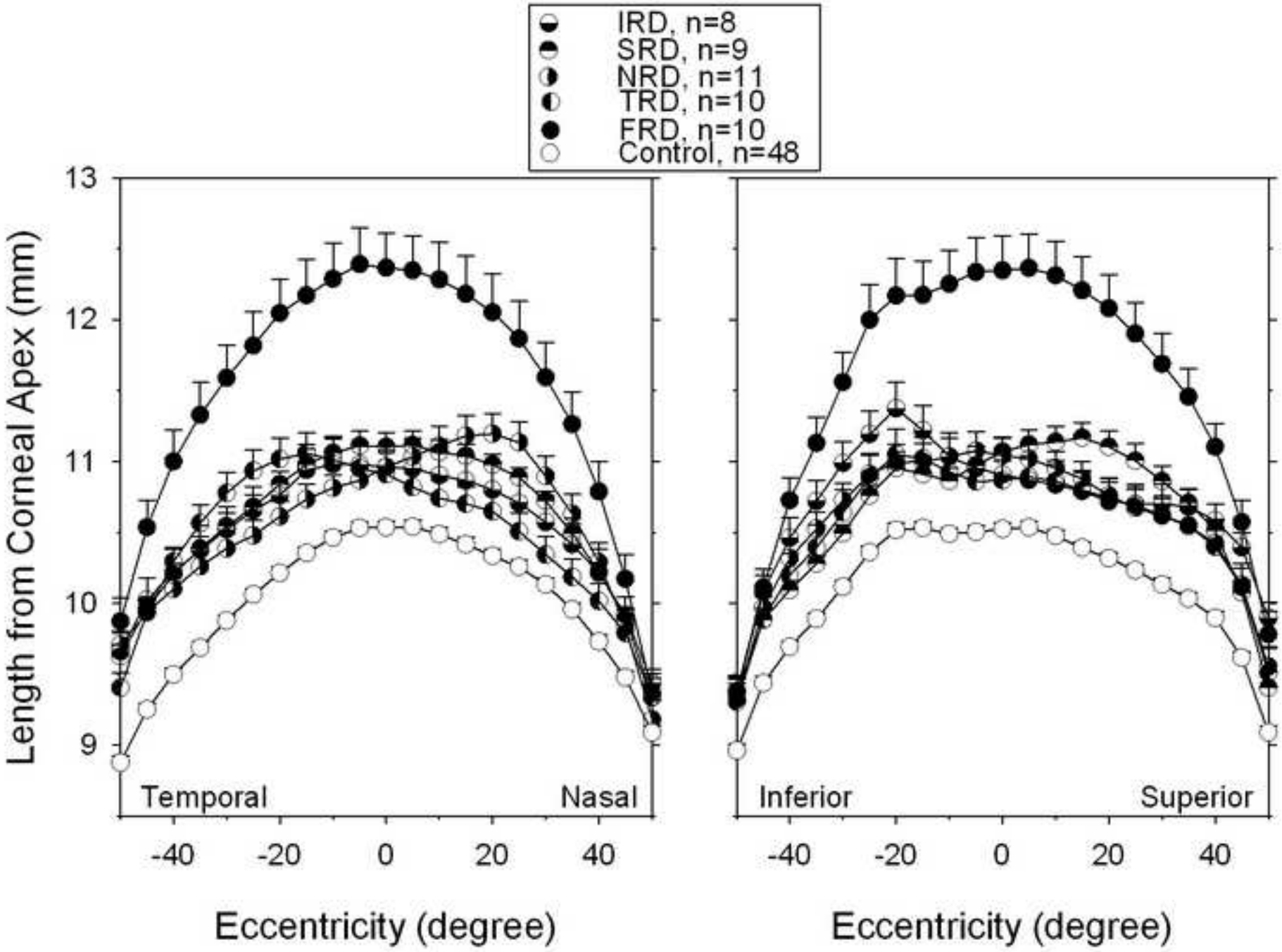
Figure

Click here to download high resolution image



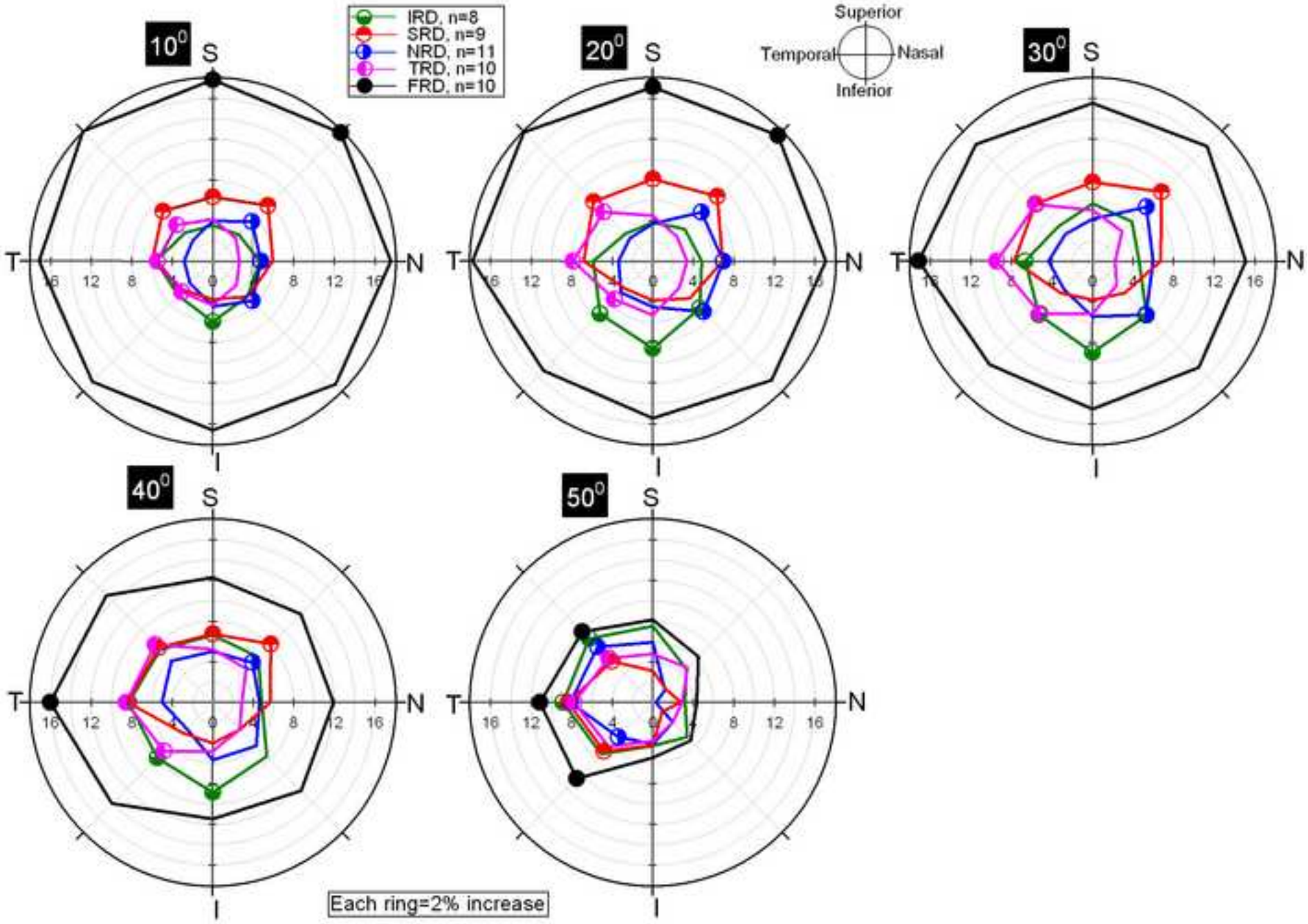
Figure

Click here to download high resolution image



Figure

Click here to download high resolution image



Figure

Click here to download high resolution image