Graded Competing Regional Myopic and Hyperopic Defocus Produce Summated Emmetropization Set Points in Chick

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PURPOSE. Chicks emmetropize accurately to experimentally induced myopic and hyperopic defocus. The authors investigated the emmetropization response when a specific proportion of the retina was exposed to myopic defocus while the remainder was exposed to (competing) hyperopic defocus.

METHODS. Normal chicks (14–15 days old) were fitted monocularly with a “lens-cone” device that exposed a specific proportion of the available visual field to a high-contrast grating under 10 diopters (D) of myopic defocus (with accommodation relaxed) in a series of patches. The remainder of the visual field (adjacent patches) viewed a grating under 10 D of hyperopic defocus. Groups of chicks wore a lens-cone device designed to provide a “spatial ratio” (relative proportion of visual field area) of 100:0, 50:50, 40:60, 33:67, 25:75, or 0:100 myopic versus hyperopic defocus. On-axis ocular refraction and axial ocular component dimensions were assessed after 3 and 6 days of cone wear.

RESULTS. Interocular differences in refraction (mean ± SD) at day 6 were as follows: +10.4 ± 2.5 D, +7.6 ± 3.6 D, +5.9 ± 3.7 D, +1.6 ± 2.6 D, −2.4 ± 2.7 D, and −8.9 ± 2.6 D for spatial ratios of 100:0, 50:50, 40:60, 33:67, 25:75, and 0:100 respectively. The corresponding interocular vitreous chamber depths were as follows: −515 ± 135 μm, −447 ± 137 μm, −253 ± 220 μm, −105 ± 252 μm, 230 ± 218 μm, and 592 ± 161 μm. The refraction and biometry results for the 33:67 and 25:75 groups were significantly different from those of the single defocus control groups.

CONCLUSIONS. In chicks, the on-axis emmetropization response was weighted according to the spatial ratio. Thus, as the proportion of retinal area receiving myopic defocus increased relative to that receiving hyperopic defocus, the degree of myopic eye growth was reduced. (Invest Ophthalmol Vis Sci. 2011;52:8056–8062) DOI:10.1167/iovs.10-5207

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Graded Regional Competing Defocus Produced Summated Growth

Ablated attempts in chicks to influence central refractive status by imposing peripheral defocus have had mixed success (Morgan I, et al. IOVS 2006;47: E-Abstract 3328). Although the discrepant findings from these chick studies may in part be attributable to differences in chick strain, treatment age, or experimental design, they may also be indicative of a more complex relationship between axial refractive error and peripheral defocus than has previously been assumed.

A pioneering study by Wildsoet and Schmidt used a lens-cone system with single, semitransparent target plane to try to unravel how visual emmetropization cues are decoded. In a later study, Diethier and Wildsoet used a cone system with two target planes to more precisely control the magnitudes of hyperopic and myopic defocus experienced by chicks (and to investigate the role of target contrast). They found that emmetropization was dominated by myopic defocus in this competing defocus setting (in which Maltese crosstargets produced a 50:50 spatial ratio of competing defocus). Furthermore, the regions of competing defocus appeared to influence emmetropization in an all-or-nothing manner under these conditions.

However, because visual scenes are typically composed of multiple objects at varying distances, they expose the retina to multiple levels of defocus (see Fig. 7 in Ref. 24). Thus, a “map” of retinal defocus should include several parameters: sign of the defocus, magnitude of the defocus, spatial area subtended by the retina, and eccentricity of the defocus. Among these parameters, the sign and magnitude of defocus have been extensively studied in the literature, whereas the spatial area of defocus (more correctly, the solid angle) and its eccentricity have received less attention. In a previous study, we showed that the eye can integrate simultaneously presented competing defocus signals (introduced by concentric design bifocal lenses, such that the defocused images lay on top of one another). As mentioned, the additional ability of the eye to respond to local regions of defocus demonstrates that the retina can also decode defocus in a spatially restricted manner. Together these findings prompted us to explore an alternative mode of competing defocus in which defocused images were presented to the eye as discrete, regionally distinct patches in such a way that adjacent retinal regions received defocus stimuli of opposing signs. Here we evaluated various spatial ratios of laterally separated competing defocus stimuli in this mode to test how the eye integrates this information and determines its emmetropization response and, crucially, its resultant on-axis refractive error.

Methods

White Leghorn chicks (Gallus gallus) were obtained and bred as specific pathogen-free fertilized eggs (SPAFAgent Maharashtra, India). They were housed in an enclosure made of fine metal mesh (to minimize restriction of distant viewing) under a 12-hour light/12-hour dark cycle and given food and water ad libitum. Chicks were 14 to 15 days old at the start of experiments. All the rearing and experimental procedures were approved by the Animal Ethics Committee of the Hong Kong Polytechnic University and were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Part of this work has been published in abstract form (Tse DY, et al. IOVS 2008;49:E-Abstract 3587). In addition, unpublished biometry results from a previous study (see experiment 2 in Ref. 24) are presented and compared with those obtained here.

Lens-Cone System

A dual-plane lens-cone system (Fig. 1) was developed, based on that described by Diethier and Wildsoet. The lens we used, which was situated in front of one eye and attached to the cone using fine metal strips, was a clear polymethylmethacrylate (PMMA) lens of back vertex power of +35 D (optical zone diameter 10.0 mm, back surface radius 12.0 mm; Igel Ltd., Kuala Lumpur, Malaysia). The two target planes, each of which contained grating targets, were positioned at a dioptric distance of 10D from each of them. The visual targets printed on the distant plane focus in front of the retina, providing myopic defocus. The visual target printed on the near plane focuses behind the retina, providing hyperopic defocus. Multiple small transparent regions of the near plane allow viewing of the distant plane. (B) Distant pattern. 100% of the area is filled with gratings, providing myopic defocus. (C) Pattern for near plane. 50% of the area is filled with gratings. The remaining area is transparent, providing 50:50 myopic to hyperopic defocus ratio in terms of spatial area. (D) Near plane, showing grating pattern interleaved with a reference grid of hexagons. Every 1 of 4 hexagons was made transparent. This provides a 25:75 spatial area ratio (myopic to hyperopic defocus). (E) As above, except that 1 of 5 hexagons was made transparent. This provides a 35:67 spatial area ratio (myopic to hyperopic defocus). (F) As in D, except that 2 of 5 hexagons were made transparent. This provides a 40:60 spatial area ratio (myopic to hyperopic defocus).
analysis showed that myopic defocus was slightly increased in the periphery, with the opposite true for hyperopic defocus. The precise level of defocus introduced by each cone device was verified by performing retinoscopy on the device before it was fitted. Specifically, each of the visual targets in turn was replaced by a reflective foil, and then the retinoscopic reflex was neutralized by trial lenses placed under the +3.5 D lens (akin to a “model eye” used in teaching the retinoscopy technique. Among the 15 cones used, the degree of defocus introduced was within 1.0 D of the target value.

The distant plane of the cone (diameter 50.2 mm) was attached to the wide end (base) of the cone via a clip, to allow removal and reattachment for regular inspection (Supplementary Fig. S1, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-5207/-/DCSupplemental). The field of view through the cone system was approximately 64°. To attach the cone device to the head of a chick, a piece of fastener (Velcro, Manchester, NH) was cut into a spectacle shape that was fashioned with an additional fastener (Velcro) band so that it adhered around the eyes and around the back of the neck. This formed a hood that could be fitted to a mating piece of fastener (Velcro) glued to the head (Supplementary Fig. S2, http://www.iovs.org/lookup/suppl/doi:10.1167/iovs.10-5207/-/DCSupplemental).

**Visual Targets**

Visual targets were constructed in two stages using a graphics software package (CorelDraw; Corel Corporation, Ottawa, Canada). First, a uniform pattern made up of squares with high-contrast gratings of 0.5 cyc/deg was generated (Fig. 1B); this constituted the distance target. Second, near targets with varying spatial area ratios were constructed. The squares of the grating pattern were interleaved with transparent spaces through which the distance target would be seen (Figs. 1C–F). Making some of the hexagonal areas transparent allowed control of the spatial area ratio because these patterns were applied in the near plane of the cone device and thus allowed the eye to see to the distant plane. The transparent areas on the near target plane were evenly distributed to control for potential differential regional sensitivity in defocus detection/emmetropization.

Because the near plane imposed hyperopic defocus and the distant plane imposed myopic defocus, the variations of area ratio imposed a spatial ratio of competing defocus to the eye in a manner independent of eye movement.

In designing the lens-cone device, and the level of target plane defocus in particular, we considered that if the magnitude of the imposed defocus was excessively high, then the patches of defocus may have become so blurred that they were no longer regionally distinct. This risk was examined using image reconstruction techniques, and an algorithm adopted from a previous study. For an eye with an axial length of 9.2 mm and a pupil diameter of either 2 mm or 3 mm, it was found that for ± 10 D of defocus generated using the cone system described, the “near” and “far” gratings did remain relatively distinct without excessive overlapping, preserving the regional character of the imposed competing defocus pattern. It is calculated that approximately 7% and 11.5% of the retinal image spread across the border because of the blur when the pupil diameter was 2 mm and 3 mm, respectively.

**Experimental Groups and Treatment Protocol**

Refractive and biometric data were collected before the application of the lens-cone system and at day 3 and day 6 of the experiment. Retinoscopy was performed immediately after A-scan biometry so as to allow measurement along the same axis and thus reduce alignment errors. Details of the retinoscopy and ultrasound measurements have been reported previously. Animals were excluded from the experiment if the lens-cone system became detached at any time, the device was decentered so that the pupil was not aligned with the optic zone of the lens, a chick was unable to open the eye behind the device on two occasions, or the weight of a chick did not show evidence of normal growth.

The dual plane lens-cone device was applied unilaterally. The fellow eye acted as a control. Animals were divided into seven groups. Six spatial area ratios of competing defocus (myopic/hyperopic) were tested: 100:0, 50:50, 40:60, 33:67, 25:75, 0:100. An additional control...
RESULTS

In general, increasing the proportion of myopic defocus relative to hyperopic defocus in visual space reduced myopic eye growth. Emmetropization was dominated by myopic defocus if its ratio was higher than 50%, but when the contribution from myopic defocus was lower than 50%, the refractive growth was also influenced by hyperopic defocus. In this range, the relationship between refractive growth and the ratio of defocus resembled a linear function.

Refractive Error (after 6 Days of Treatment)

Refractive error at the end of the lens-cone treatment was found to vary, depending on the spatial ratio of competing defocus. The higher the proportion of myopic defocus, the more hyperopic was the refractive error. Figure 3A shows the interocular difference in refractive error (RE) for the seven spatial ratio groups after 6 days of monocular lens-cone device wear. The data are compared alongside our previous dual-power lens results. The two control groups in the present study, receiving purely hyperopic or purely myopic defocus, respectively, underwent refractive changes as predicted from previous work. The 100:0 spatial ratio group (+10 D defocus over the whole field of view of the cone) showed a mean interocular refractive error (RE) of +10.4 D; the 0:100 spatial ratio group (−10 D defocus of the full field of the cone) showed a mean interocular RE of −8.9 D. These findings indicated that, for the two single defocus conditions, refractive compensation was approximately complete by the end of the 6-day treatment period. The mean interocular RE for the competing defocus groups were +7.6 D, +5.9 D, +1.6 D, and −2.4 D for spatial ratios of 50:50, 40:60, 33:67, and 25:75, respectively. The mean interocular RE for the 33:67 and 25:75 ratio groups were significantly different from those of the single defocus controls (P < 0.01 for both). In other words, the 33:67...
and 25:75 ratio groups did not emmetropize to either of the imposed focal planes; instead, an intermediate refractive state was attained. Extrapolating from the current data, a defocus ratio of approximately 30:70 would be expected to produce an emmetropic on-axis refractive end point. For the control group of chicks fitted with a blank cone, the mean interocular RE was smaller than 0.1 D, indicating that wearing the cone device did not appreciably affect refractive development along the visual axis.

Vitreous Chamber Depth (after 6 Days of Treatment)

Figure 3B shows the interocular difference in vitreous chamber depth (VCD) after 6 days of cone wear. The interocular difference in VCD was highly correlated with the spatial ratio of imposed competing defocus \( r = -0.96 \). The higher the percentage for hyperopic defocus, the greater was the growth of the vitreous chamber. The change in VCD difference mirrored that of the RE, suggesting that the axial change in VCD accounted for most of the change in refraction. As with RE, the mean interocular differences in VCD for the 33:67 and 25:75 ratio groups were significantly different from those of the single defocus control groups. The blank cone control group showed a change in the interocular VCD that was not significantly different from zero.

Choroidal Thickness (after 6 Days of Treatment)

The relationship between spatial ratio and interocular choroidal thickness (CHO) difference appeared to be nonlinear (Fig. 3C). Indeed, the relationship between VCD and CHO was best described as a quadratic function (regression analysis, \( R^2 = 0.67 \)). The interocular differences in CHO for the 33:67 and 25:75 ratio groups did not differ from those for either of the single defocus control groups. However, the changes in choroidal thickness for the two single defocus control groups were significantly different from one another (interocular difference in CHO, 0.275 mm and \(-0.042\) mm for the 100:0 and 0:100 spatial ratio groups, respectively; \( P < 0.01 \)). The interocular difference in CHO of the blank cone control group did not differ significantly from zero.

Distance between the Lens and Sclera (after 6 Days of Treatment)

Changes in vitreous chamber depth are related to both scleral remodeling and choroidal thickening or thinning. Therefore, a more direct representation of scleral remodeling during emmetropization can be obtained by examining the change in lens-to-sclera (LTS) distance with treatment. Figure 3D shows the interocular difference in LTS for each of the spatial ratio groups. Comparing the change in VCD (Fig. 3B) with the changes in CHO (Fig. 3C) and LTS (Fig. 3D), it was evident that changes in VCD were dominated by scleral remodeling in groups 33:67, 25:75, and 0:100, whereas choroidal thickening was also an important contributor to refractive compensation in groups 100:0 and 50:50.

Parameters at Day 3

Graded emmetropization responses similar to those described were observed in the middle of the treatment period. However, emmetropization was incomplete for both the single defocus groups at day 3 (interocular RE = +6.0 D and \(-5.5\) D for the 100:0 and 0:100 groups, respectively). In spatial ratio groups 25:75 and 33:67, chicks showed refractive and biometric changes at day 3 similar to those seen at day 6 (change in RE = +1.4 D and \(-2.6\) D, respectively; change in VCD = 0.204 mm and \(-0.035\) mm, respectively). For the most part, these changes were already significantly different from those observed in the single defocus control groups. Thus, in contrast to the single defocus groups, emmetropization in the 25:75 and 33:67 spatial ratio groups appeared to be nearly stable between day 3 and day 6, suggesting that these eyes had already reached an equilibrium state at this level of competing defocus.

Comparison with Superimposed Competing Defocus Produced by Dual-Power Lenses

The spatially separated patches of competing defocus introduced by our cone devices can be considered an alternative mode of competing defocus to the overlapping areas of myopic and hyperopic defocus produced by the concentric zone, dual-power lenses used in our previous study.24 To facilitate comparison between the two modes of competing defocus, data from our previous study are plotted side by side those from the present data in Figures 3A to 3D. Qualitatively, both paradigms showed similar trends (i.e., graded emmetropization responses). Thus, chicks treated according to both paradigms became approximately 10D hyperopic after receiving purely myopic defocus for 6 days, but refractive end points became increasingly biased toward myopia when the proportion of myopic defocus decreased from 100% to zero (Fig. 3A). Quantitatively, however, the two paradigms displayed a difference in their relative sensitivity to competing defocus that was most apparent in the 33:67 groups. Here, eyes become slightly hyperopic in the 33:67 spatial ratio cone group but significantly myopic in the 33:67 (relative image contrast) overlapping defocus dual-power lens treatment group. Hence, myopic defocus had a more dominant influence on refractive error in the spatial area paradigm than it had in the spatial contrast paradigm.

DISCUSSION

The Nature of Visual Stimuli in the Present Experiment

Our cone-lens devices exposed regions of the retina to patches of competing defocus that were evenly distributed over the central and peripheral visual field. Thus, unlike some previous studies that investigated the relative importance of the central versus peripheral retina in response to defocus, the present study attempted to examine the retina’s regional sensitivity as an averaged response from widely distributed areas receiving either myopic or hyperopic defocus.

We chose not to disable eye movements in our experiments, partially for practical reasons and partially to avoid eliminating optical cues directly associated with such movements, which are potentially useful to the emmetropization system.26 Therefore, a patch of defocus of a particular sign might have been imposed on a certain region of retina at some times but might also have swept across the retina at other times. At every instant in time, however, the overall ratio of competing defocus would have remained unchanged. In addition, as with most other lens defocus studies, accommodation in the treated eye was not disabled, particularly because it had been reported to be crucial for compensation to myopic defocus under competing defocus conditions.25

Relationship to Previous Studies

As mentioned, the competing defocus introduced by the cone-lens devices differed from that produced by the concentric dual-power lenses we described previously.24 In the cone-lens paradigm, adjacent local retinal regions are subjected to defocus stimuli of opposite sign. In the dual-power lens paradigm, the competing defocus stimuli are pitted against each other at
the same retinal locations. The latter competing defocus paradigm was achieved using lenses with a series of refractive annuli that focused incoming light onto 1 of 2 independent image shells, separated longitudinally with respect to the visual axis. By changing the proportion of positive and negatively powered annuli, different ratios of myopic versus hyperopic defocus could be introduced.

The present findings suggest that the relationship between the spatial extent of imposed competing defocus and the resultant emmetropization response is linearly additive over the central 60° of the chick visual field. Our findings extend previous observations in which very large portions of the visual field have been shown to have local responses to visual deprivation. 14, 15 Crucially, when the visual field was exposed to approximately equally sized areas of myopic and hyperopic defocus, the emmetropization response was dominated by myopic defocus. This suggests that a small region of myopic defocus is able to counteract a larger region of hyperopic defocus. A similar bias toward myopic defocus being a “stronger” emmetropization cue than hyperopic defocus has been reported previously. This includes conditions in which competing defocus stimuli were presented to the whole retina superimposed on top of one another. 24 During intermittent lens wear, 27 as alternating exposure to defocus 26, 28 (Morgan I. IOVS 2003;44: ARVO E-Abstract 1988) or as opposing astigmatic blur. 50 Our present results also demonstrated that the 25:75 and 33:67 groups had an almost stable refractive error between days 3 and 6. Given that myopic and hyperopic defocus, respectively, trigger “stop” and “go” growth responses when presented independently, this stabilization in growth—despite the presence of ongoing opposing defocus stimuli—suggests that eye growth had reached an “equilibrium” set point of intermediate refractive status, where these stimuli were balancing each other.

It has been shown that the peripheral retina plays a dominant role in guiding emmetropization because it can determine refractive error along the visual axis in eyes subjected to peripheral form deprivation or hyperopic defocus, even in the presence of clear central vision. 51 Our current findings provide support that this result is explained by the fact that the peripheral retina covers a substantial part of the visual field, thereby outweighing the contribution from the macula.

Potential Underlying Mechanisms

The mechanism by which the retina decodes defocus information remains elusive. Intuitively, it seems possible that a defocused image is recognized by some unknown subtypes of retinal neuron whose receptive fields are specifically stimulated by a defocused image. Similar mechanisms have been shown to be responsible for decoding other visual features, such as color and movement. 52–54 The receptive field of such neurons may be so robust that opposing astigmatic blur cues 50 are processed similarly to competing spherical defocus, so that in both cases they lead to summed responses with a slight hyperopic bias. Similarly, there may be systems of neurons that can detect superimposed defocused images through independent sensing channels and then integrate the combined information.

Possible mechanisms accounting for the integrated emmetropization response may include one or more of the following: (1) The retina may function as a series of small emmetropization units in which each unit has the capability to decode defocus information and store it locally, such as through synaptic plasticity (and, in addition, to integrate such information over time). The retina could mediate choroidal and scleral changes by the release of signaling molecules locally; the cumulative effect of mixing such signaling molecules produced by each emmetropization unit would then produce summed physical changes in the underlying sclera and choroid. The multiple units together would enable the eye to “image process” the varying spatial nature of defocus in visual scenes and, ultimately, to manifest an integrated emmetropization response. (2) Eye or head movements would facilitate mixing of signaling molecules under the mechanism of alternating defocus. In this scenario, specific retinal areas would have a higher chance of being exposed to a particular type of defocus for a prolonged period if the defocus stimulus occupied a large region of visual space. The spatial ratio of defocus is somehow converted into a correlated temporal ratio as the eye moves. (3) Defocus information may be decoded by the retina, resulting in the release of local growth signals. Lateral diffusion of the growth signals could occur during the process of transmission and transduction to the choroid and sclera. Mixed signaling molecules arriving at the sclera would then produce an integrated response. (4) There may be lateral interactions of defocus signals at the level of the neural retina. Some neurons, such as retinal amacrine cells, have lateral anatomic networks that may integrate signals from multiple regions. Glucagonergic amacrine cells (chicks), GABAergic amacrine cells (mammals), and vasoactive intestinal polypeptide-releasing amacrine cells (mammals) have been reported to respond to defocus. 55–57 They have receptive fields of various sizes that may contribute to lateral integration over different extents of retinal surface area.

Implications

It has been documented that defocus signals can be integrated over time 28, 29, 38–40 or when competing defocus stimuli are presented to the same retinal location. 24 The present data imply that the effect of defocus on emmetropization is quantitatively related to the spatial area subtended by the region or regions of defocus. Although the underlying mechanism is unknown, the data suggest that, in chicks, increasing the proportion of myopic defocus relative to hyperopic defocus in visual space offers a route to reducing (or even reversing) myopic eye growth. In other words, our findings imply that the profile of spatial defocus—as dictated by the physical distribution of visual objects in an environment—is important to the process of emmetropization. Given that when near objects are viewed in an outdoor environment a high proportion of visual space will be seen under myopic defocus, our results may also be relevant to the phenomenon that children who spend more time outdoors tend to have less myopia. 41, 42

Future Directions

It is still a mystery how the retina can discern the signs and magnitude of defocus. Further work is needed to unravel the neurobiology and biochemistry of this feat. Determining the smallest patch of defocus that the retina can recognize may shed light on the decoding mechanism. If some specific retinal neuron subtypes are critically involved in decoding defocus or any integrative processing, their receptive fields, densities, and distributions in the retina may be important in determining regional differences in sensitivity to myopic or hyperopic defocus. Furthermore, if the density of "emmetropization-responsive" cells is higher in a particular region of retina, this region may contribute more weight per unit area to determining the refractive status of the wider local area. Another possible approach to investigating this multidimensional problem would be to determine the area of the retina, centered on the macula, required to override the peripheral retina. Determining the relative balance of these regions may be important in describing the differential regional sensitivity to defocus across the retina.
In conclusion, this study provides evidence that adjacent patches of myopic and hyperopic defocus are integrated quantitatively either at the level of the retina, choroid, or sclera, to guide emmetropisatory eye growth. Our findings highlight the importance of the spatial properties of defocus in visual scenes in guiding ocular growth. Further work is needed to determine other factors that contribute to the spatial distribution of defocus and to determine how these relate to variables such as posture, size of reading materials, distribution of background objects, partial spectacle correction, and so on, that may conspire to affect the development of myopia in children.

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References

42. Ashby R. The role of light intensity in controlling ocular development in chickens. 13th International Myopia Conference; July 26–29, 2010; Tübingen, Germany.