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The significance of retinal image contrast and spatial frequency composition for eye growth modulation in young chicks

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ABSTRACT

Purpose: This study sought further insight into the stimulus dependence of form deprivation myopia, a common response to retinal image degradation in young animals.

Methods: Each of 4 Bangerter diffusing filters (0.6, 0.1, <0.1, and LP (light perception only)) combined with clear plano lenses, as well as plano lenses alone, were fitted monocularly to 4-day-old chicks. Axial ocular dimensions and refractive errors were monitored over a 14-day treatment period, using high frequency A-scan ultrasonography and an autorefractor, respectively.

Results: Only the <0.1 and LP filters induced significant form deprivation myopia; these filters induced similarly large myopic shifts in refractive error (mean interocular differences \pm SEM: -9.92 ± 1.99 , -7.26 ± 1.60 D, respectively), coupled to significant increases in both vitreous chamber depths and optical axial lengths ($p < 0.001$). The other 3 groups showed comparable, small changes in their ocular dimensions ($p > 0.05$), and only small myopic shifts in refraction (<3.00 D). The myopia-inducing filters eliminated mid-and-high spatial frequency information.

Conclusions: Our results are consistent with emmetropization being tuned to mid-spatial frequencies. They also imply that form deprivation is not a graded phenomenon.

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1. Introduction

Myopia or nearsightedness is a condition in which the eye is too long for its optical power. Severe (high) myopia has been associated with visual impairment and can heavily influence the career and lifestyle choices of affected individuals (Saw, Gazzard, Shih-Yen, & Chua, 2005). Interest in understanding how visual guidance of eye growth might lead to myopia is fueled by its increasing prevalence. In the United States, approximately a quarter of the population suffers from myopia, and the prevalence is increasing (Kempen et al., 2004; Norton, 1999). Myopia has reached “epidemic” levels in some Asian countries, for example, over 90% in Taiwanese Chinese university students (Fan et al., 2004; Lin et al., 1999).

While the viewpoint that myopia has a genetic origin is apparently supported by the observed higher prevalence of myopia among the children of two myopic parents compared to children with one or no myopic parents (Mutti, Mitchell, Moeschberger, Jones, & Zadnik, 2002) and ethnicity-related differences in myopia prevalence (Katz, Tielsch, & Sommer, 1997; Wong et al., 2000), the case for homeostatic control of refractive error is strongly supported by animal studies (Wallman & Winawer, 2004). Of rele-

vance to the current study is the observation that form deprivation induces excessive ocular elongation in young animals, with similar findings in all species studied to-date (tree shrew (Sherman, Norton, & Casagrande, 1977); marmoset (Troilo & Judge, 1993); chick (Wallman, Turkel, & Trachtman, 1978); rhesus macaque (Wiesel & Raviola, 1979); mice (Schaeffel, Burkhardt, Howland, & Williams, 2004)). Likewise, pathologies leading to retinal image degradation in infants and young children, e.g., corneal opacities (Gee & Tabbara, 1988; Twomey, Gilvarry, Restori, Kirkness, Moore & Holden, 1990), cataracts (Chang, Congdon, Bykhovskaya, Munoz, & West, 2005; Rasooly & BenEzra, 1988; von Noorden & Lewis, 1987; Zhang & Li, 1996), and ptosis (von Noorden & Lewis, 1987), have been linked to the development of myopia. The consistency of this form deprivation response across different species highlights the importance of a clear (high quality) retinal image for attaining and maintaining emmetropia.

In 2 previous studies of form deprivation myopia, one in chick (Bartmann & Schaeffel, 1994), and another in monkey (Smith & Hung, 2000), diffuser density was manipulated to learn more about the visual requirements of normal refractive development, i.e., emmetropization. The general conclusion from these studies was that form deprivation myopia is a graded phenomenon, that is, the amount of myopia is directly related to the amount of retinal image contrast degradation. In the study involving chicks, increasing retinal image degradation induced increasing myopia, leading

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Bartmann and Schaeffel (1994) to conclude that “emmetropization can take place in the absence of accommodation just by maximizing the average retinal image contrast”.

The “graded response” model proposed by Bartmann and Schaeffel (1994) rests on the assumption that the underlying retina-derived growth signal encodes changes in image contrast. However, the cross-sectional nature of the data from this study is not an adequate test of the “graded response” model. For example, it is possible that the effect of retinal image degradation is simply to trigger an all-or-none response that is attenuated if and when the limit of the eye’s depth of focus is exceeded as a result of the increased ocular growth, and retinal image contrast is compromised further. This model also does not take into account the spatial frequency-dependence of emmetropization, as indicated in other studies in chicks (Diether & Wildsoet, 2005; Hess, Schmid, Dumoulin, Field, & Brinkworth, 2006; Schmid & Wildsoet, 1997). Emmetropization is predicted to fail when the contrast of critical spatial frequencies drops below threshold; here too, an all-or-none response pattern is the predicted outcome of contrast manipulations. The result reported by Bartmann and Schaeffel (1994) (i.e., increased myopia with increased retinal image degradation) is predicted for both their graded response model and the alternative all-or-none response model, although the temporal patterns of eye growth are expected to be different.

The study reported here sought further insight into the inter-relationship between retinal image degradation and eye growth regulation by tracking over time the ocular responses of young chicks exposed to different levels of retinal image contrast degradation.

2. Methods

2.1. Animals

Forty-eight White Leghorn chickens (*Gallus gallus domesticus*), obtained as hatching chicks from a commercial hatchery (Privett Hatchery, New Mexico), were used for this study. They were reared under diurnal lighting conditions (12 h on/12 h off light cycle), with access to sifted food and water ad libitum. The food was sifted to remove fine particles that may have interfered with the diffuser treatments (see below). The cage temperature was kept at approximately 30 °C. Experimental procedures were conducted in accordance to the NIH Guidelines of Animal Care and were approved by the Animal Care and Use Committee of the University of California, Berkeley.

2.2. Form deprivation myopia treatments

Transparent plano lenses were combined with each of 4 Bangerter diffusing filters (Fresnel Prism & Lens Co., AZ) spanning the available density range, generating 4 treatment groups. An additional group wore a plano lens without any filter (no form degradation) as a control. Table 1 provides the specific details of each of the 5 treatments and the number of birds randomly assigned to each. The optical devices were fitted monocularly via Velcro support rings that were glued to the chicks’ feathers around the eye with collodion adhesive, allowing the lenses to be easily removed for daily cleaning. The fellow untreated eyes served as controls in the study. Treatments were initiated in 4-day-old chicks and maintained for 14 days. This treatment duration is longer than typically used in such studies, but was used to amplify subtle intergroup differences in eye growth rates.

2.3. Measurements of treatment effects on refractive error and ocular dimensions

Refractive errors and axial ocular dimensions were measured under isofluorane anesthesia (1–2% in oxygen) using i.r. photoretinoscopy (Schaeffel, Hagel, Eikermann, & Collett, 1994) and high frequency A-scan ultrasonography (Nickla, Wildsoet, & Wallman, 1998), respectively. Baseline measurements were made before the optical devices were applied (day 0). Refractive error measurements were repeated on days 4, 9, and 14 of the treatment period. Axial ocular dimensions were assessed on days 2, 4, 7, 9, 11, and 14.

2.4. Characterization of visual effects of Bangerter diffusing filters

To demonstrate the spatial filtering properties of the Bangerter diffusing filters qualitatively, the filters were placed in turn directly over the lens of a Nikon Coolpix camera and images of grating stimuli and of chicks were captured (Fig. 1). The pho-

Table 1

Summary of experimental manipulations, including the number of chicks (*n*) assigned to each treatment group

Treatment groups	<i>n</i>
Plano lens only (no spatial contrast degradation)	11
Plano lens + 0.6 Bangerter filter	11
Plano lens + 0.1 Bangerter filter	8
Plano lens + <0.1 Bangerter filter	9
Plano lens + Light perception Bangerter filter	9

A total of 48 chickens were used, with treatments initiated at 4 days of age, after baseline measurements, and maintained for 14 days.

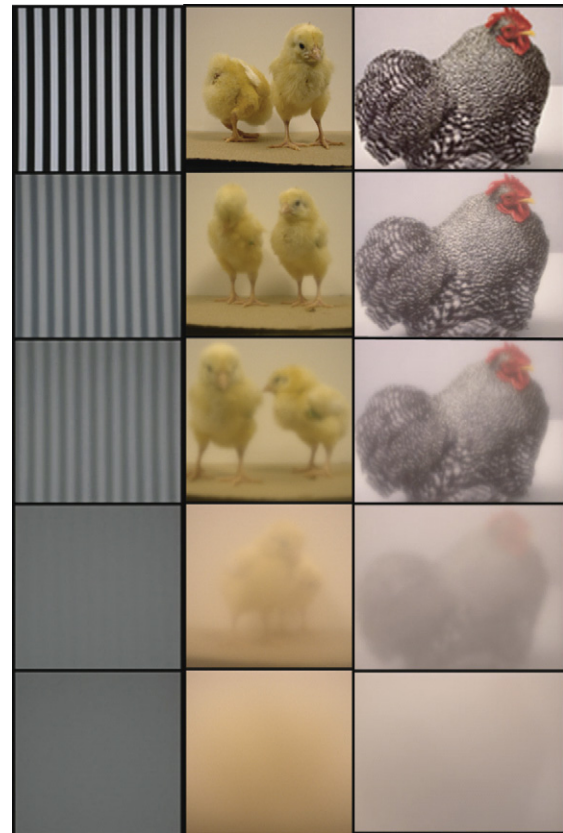


Fig. 1. Image degradation resulting from the Bangerter diffusing filters; the images displayed were recorded with a Nikon Coolpix camera with either no filter (top row), or one of the diffusing filters attached to the camera lens. There is a progressive loss of high and medium frequency detail, with the 2 densest filters transmitting only low frequency detail.

tographs were all taken at the same distance with the auto focus of the camera turned off. To characterize the spatial filtering properties of the filters, Fourier analyses were carried out on these images (Fig. 2).

To characterize the retinal image degrading effects of the Bangerter filters/lens combinations in the chicks, visual acuity was measured in 4-day-old chicks wearing the filter–lens combinations over both eyes ($n = 4$), using an already established optokinetic nystagmus paradigm (OKN (Schmid & Wildsoet, 1998)). In brief, individual chicks were placed in the center of a rotating drum (27.2 cm radius, 45 cm height), which was used to present high contrast (approximately black and white), vertically oriented square wave grating stimuli. The stimuli were mounted on the inside of the drum, rotating at a speed of 1 rpm and reversing in direction every 20 s to prevent adaptation. Six different spatial frequencies (0.08, 0.12, 0.59, 1.19, 2.37, 3.54 cycles per degree (cpd)), were tested for each of the lens–filter combinations. To control the stimulus distance, the chicks were restrained during measurements in a small, open topped container with a white neck collar that allowed relatively free rotational head movements but prevented them seeing near objects. When visible, the moving stripes elicited smooth pursuit head movements, interrupted by saccades in the opposite direction (OKN). Following (tracking) head movements lasting at least 20 s during an individual trial were interpreted as evi-

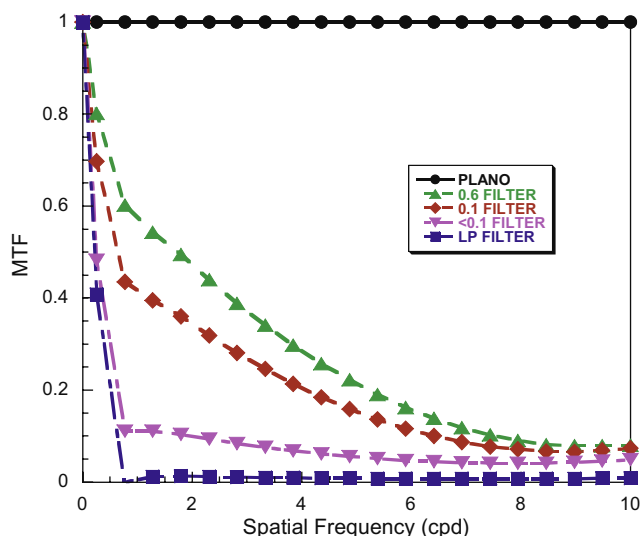


Fig. 2. Fourier analysis of the images shown in Fig. 1; MTFs compared to those derived for the plano (no filter) treatment (MTF = 1) to characterize the spatial frequency transmission properties of the diffusing filters.

dence that the stimulus was resolved. Each spatial frequency was tested 5 times with each chick, and the percentage of correct responses (head rotation in the correct direction) was recorded.

For comparison with the image degrading effects of the Bangerter diffusing filters in the chicks, their effects also were assessed in 6 human subjects using a custom-designed computer-based contrast sensitivity test (Bailey, Fitz, & Alinlubi, 2003). This test presents a flashing square stimulus of varying size and contrast on a touch-screen computer monitor divided into 4 quadrants, subjects being required to touch the quadrant in which they saw the stimulus in a forced-choice paradigm. The following spatial frequencies were tested: 0.12, 0.19, 0.48, 0.97, 2.42, 4.84, 8.35, 16.45, and 25.05 cpd. Each subject was tested monocularly, through each of the Bangerter diffusing filters, which were attached to lenses positioned in the spectacle plane. The lenses used at the spectacle plane corrected any existing refractive error. Subjects were also tested without any filter.

2.5. Data analysis

To assess the overall effects of various treatment manipulations, results were analyzed using a two-way repeated measures analysis of variance (ANOVA), followed by Tukey–Kramer post-hoc tests. Data were first normalized so that for both treated and fellow eyes, the baseline mean of each eye matched the overall baseline mean for all treated or control eyes, as appropriate. Although all axial ocular dimensions, including the thicknesses of the individual components making up the wall of the eye were recorded, the analyses described here are limited to those parameters showing significant treatment effects, i.e., changes in anterior chamber depth, vitreous chamber depth, optical axial length (calculated as the sum of anterior chamber + lens thickness + vitreous chamber depth), inner axial length (calculated as the sum of optical axial length + retina thickness + choroid thickness), and refractive error data, referenced to baseline values. The effects of both treatment and time were examined. A *p*-value of less than 0.05 was taken as an indicator of statistical significance. Linear regression analysis of refractive error changes against vitreous chamber depth changes was also undertaken.

3. Results

3.1. Biometric and refractive error data

Because of the young age of the chicks, both their treated and untreated eyes continued to grow over the treatment period. However, because of the optical quality degrading effects of the diffusing filter, we expected treated eyes to grow faster than their untreated fellows, consistent with previous reports of form deprivation myopia. The early study of Bartmann and Schaeffel also predicted a graded response to the imposed retinal image degradation, with eyes wearing the densest filters growing fastest.

The first but not the second of the above predictions was born out by our data. The treated eyes of all groups exhibited some in-

crease in optical axial length relative to the fellow untreated eyes over the 14-day treatment period (Fig. 3A), this difference in growth being statistically significant for 2 groups wearing the densest filters (<0.1 and LP), which imposed the greatest amount of image degradation ($p < 0.001$). Just 2 days into the treatment period, the increases in optical axial length for the eyes wearing these filters (<0.1 and LP) were significantly different from those wearing either no filter (plano lens) or the 0.6 and 0.1 filters ($p < 0.001$). The optical axial length changes for the latter three treatments were not significantly different from each other at any time over the treatment period ($p > 0.05$), and while the larger increases in axial length, recorded with the <0.1 and LP filter treatments, were significantly different from the plano lens group ($p < 0.05$), they were not significantly different from each other ($p > 0.05$). Treatment-related thickness changes in the retina and/or choroid did not reach statistical significance and are not reported here; the negligible changes in these tissues also is reflected in the close similarity of the trends in inner and optical axial length data (compared Fig. 3A and C).

Increased elongation of vitreous chamber depth in treated eyes accounted for the majority of the optical axial length increases; thus interocular differences for this parameter show similar trends to that described for optical axial length (compare Fig. 3A and B). Once again, the responses of treated eyes fell into two distinct groups. While all treated and untreated eyes showed vitreous chamber depth increases over the treatment period, only the LP and <0.1 filter treatment groups recorded significantly greater increases in vitreous chamber depth in their treated eyes compared to their untreated fellows by day 2 ($p < 0.001$), with the two densest filters (LP and <0.1 filter) resulting in statistically significant increases in vitreous chamber depth compared to the responses elicited with the plano, 0.6 and 0.1 filters ($p < 0.001$), which were not significantly different from each other at any time point ($p > 0.05$). The increases for the <0.1 and LP filter treatment groups were not significantly different from each other at any time point ($p > 0.05$), and both were significantly greater than that of the plano lens (control) group ($p < 0.05$).

Interestingly, the two densest filters also had a growth-enhancing effect on the anterior chamber. By the end of the treatment period, the treated eyes of both the LP and <0.1 filter treatment groups showed significant increases in anterior chamber depth compared to those of plano (no filter) treatment group ($p < 0.001$), although the scale of the changes was smaller than that recorded for the vitreous chamber for both groups (mean interocular differences \pm SEM: 0.422 ± 0.112 mm and 0.523 ± 0.143 mm, respectively; Fig. 3D).

Three of the groups (no filter (plano lens), 0.6 filter and 0.1 filter), showed only very small myopic shifts in refractive error over the 14-day treatment period (less than 3.00 D), while the <0.1 and LP filter treatment groups showed large myopic shifts over the same period (mean interocular difference (\pm SEM): -9.92 ± 1.99 and -7.26 ± 1.60 D, respectively) (Fig. 4A). These refractive error changes are consistent with the described optical axial length changes; in young chicks, a 1 mm increase in axial length corresponds to approximately -15 D of myopia (Schaeffel & Howland, 1988). Qualitatively, the <0.1 and LP filter treatment groups, which showed largest increases in optical axial length, also showed the largest changes in refractive error. The only small refractive error changes in the 3 other groups (plano, 0.6 filter, 0.1 filter) also are consistent with the only small treatment-related increases in vitreous chamber depth and thus optical axial length for these groups. That changes in vitreous chamber depth are correlated with the refractive error changes ($p = 0.0328$), is also consistent with an axial origin to the refractive changes (see Fig. 4B). The <0.1 and LP groups showed steady increases in myopia over the first 9 days, refractions decreasing slightly thereafter, possibly due to an optical

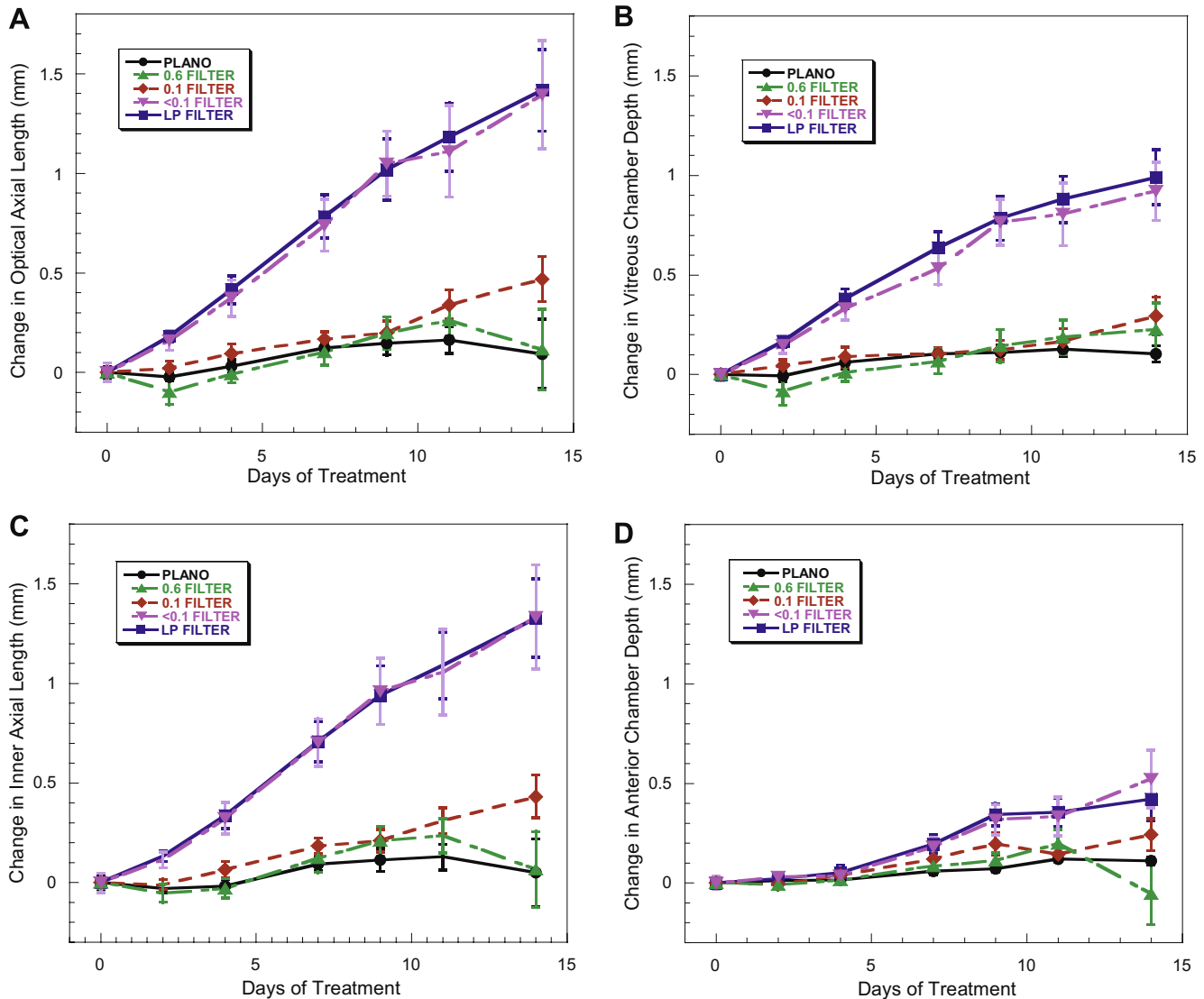


Fig. 3. Mean interocular differences \pm SEM in (A) optical axial length, (B) vitreous chamber depth, (C) inner axial length (D) anterior chamber depth, normalized to baseline values, plotted against days of treatment for each of the treatments. Only the groups fitted with the two densest (LP and <0.1) filters, showed significant increases in optical axial length. The induced changes in these two groups are similar to each other ($p > 0.05$) and significantly greater than those in the other 3 groups, i.e. with no filter, 0.6, or 0.1 filter ($p < 0.001$). The trends in vitreous chamber and inner axial length data are similar to those described for optical axial length, because increases in vitreous chamber depth account for most of the increases in optical axial length for all groups, and treatment-induced changes in retinal and choroidal thickness are minimal. There were significant increases in anterior chamber depth ($p < 0.001$) with the two densest (<0.1 and LP) filters compared to the effects of the other 3 treatments ($p < 0.001$).

scaling effect (Saltarelli, Wildsoet, Nickla, & Troilo, 2004), whereas the changes in the plano, 0.6 and 0.1 groups were much smaller and less consistent. In refractive terms, the latter 3 groups were significantly different from the <0.1 and LP groups ($p < 0.001$).

3.2. Effects of the filters on spatial frequency and contrast sensitivity

To understand the effects of the filters on ocular growth, it is important to know their effects on retinal image quality. In the OKN testing, the chicks responded incorrectly with increasing frequency, as the density of the diffusing filter increased, with the responses to the high spatial frequency patterns being affected first. Specifically, with the plano lens, the chicks correctly responded to the 3.54 cpd pattern, the highest spatial frequency tested, most of the time (more than 50% correct responses). A similar trend was seen with 0.6 filter. On the other hand, their performance for the 3.54 cpd pattern was significantly poorer with the 0.1 filter, although the chicks responded correctly most of the

time to grating frequencies of 1.19 cpd or less. With the <0.1 and LP filters, the number of correct responses mostly did not exceed chance for any of the frequencies tested. Fig. 5A shows these data; the deterioration in visual spatial resolution with increasing diffuser density is clearly seen.

Contrast sensitivity data collected from human subjects also show decreased performance with increasing filter density and there was an associated shift to the left in the high spatial frequency cut-off (Fig. 5B). The cut-off frequencies were 25.05, 8.35, 2.42 and 0.48 cpd for the 0.6, 0.1, <0.1 and LP filters, respectively, approximating the data supplied by the Fresnel Prism and Contact Lens Co., describing the relationship between filter density and visual acuity, i.e., 0.6, ~ 20 cpd; 0.1, ~ 3 cpd; <0.1, ~ 2 cpd; LP, light perception. In an equivalent study of human subjects, Smith and Hung also recorded cut-off frequencies of around 10 and less than 1 cpd for 0.1 and LP Bangerter filters, respectively.

In summary, the two measures of visual performance—spatial resolution in the chicks and the contrast sensitivity in humans—

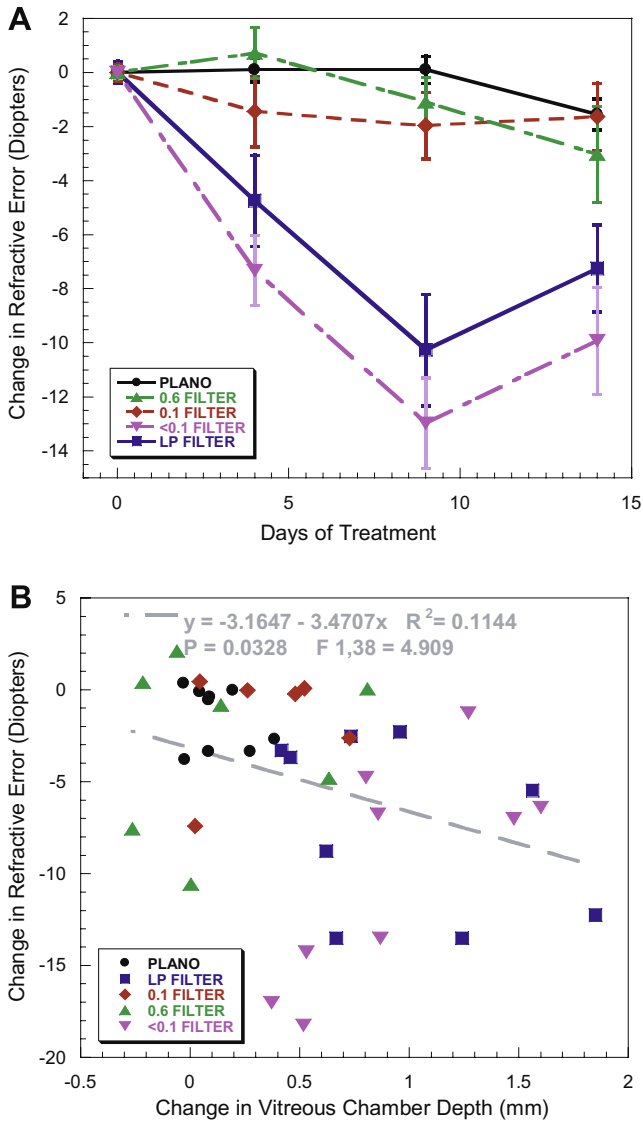


Fig. 4. Mean interocular differences \pm SEM in (A) refractive error, normalized to baseline values, plotted against days of treatment for each of the treatments. The LP and <0.1 filters induced large myopic shifts in refractive error while the other treatments induced only very small myopic shifts. (B) Refractive error changes plotted against vitreous chamber depth changes. Parameters are significantly correlated ($R^2 = 0.1144$, $F_{1,38} = 4909$, $p = 0.0328$).

indicate a steady deterioration in performance with increasing filter density, with the densest filters causing the greatest deterioration, consistent with the image degradation documented photographically. These data do not show the bimodal segregation apparent in our refraction and biometric data, a difference taken up in the discussion.

To further characterize the optical properties of the filters, MTF volume ratios were calculated for both the human contrast sensitivity data and photodocumentation (Table 2), using the following equation:

$$MTF_{VolRatio} = \frac{\int \int MTF_{filter}(u, v) du dv}{\int \int MTF_{plano}(u, v) du dv} \quad (1)$$

where MTF_{filter} and MTF_{plano} represent those of the filter and no filter condition, respectively; the latter is assumed to represent the diffraction-limited MTF; the pupil size was fixed. The MTF volume ratio is equivalent to the Strehl ratio, computed in the

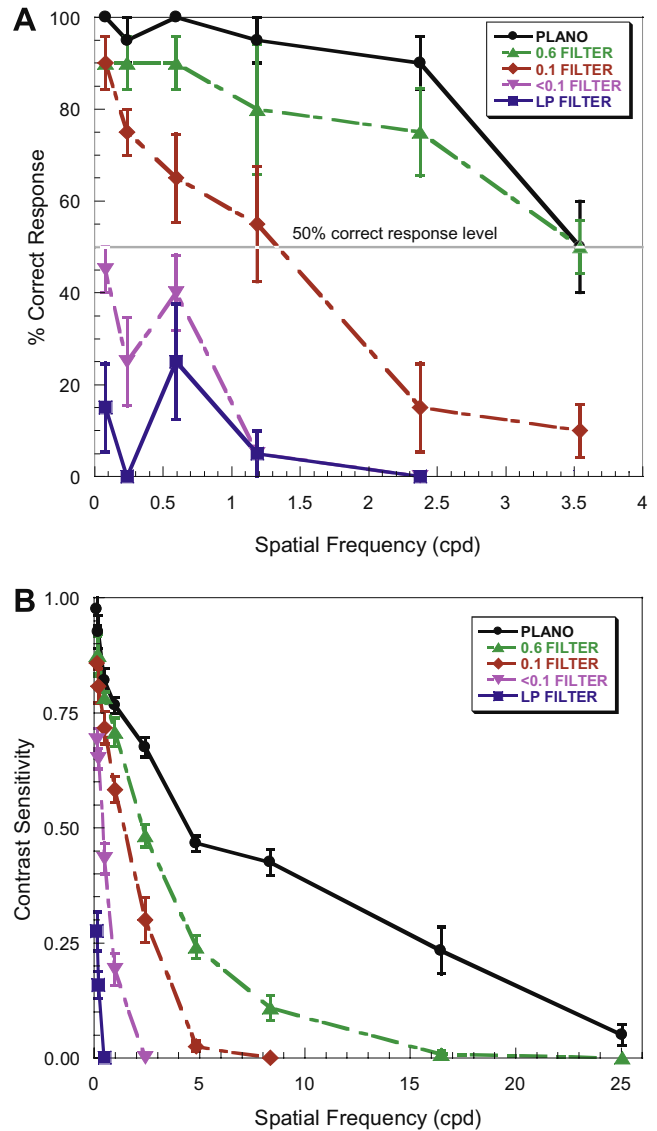


Fig. 5. (A) Effects of plano lens–filter combinations on the visual spatial resolution of normal chicks, measured using an optokinetic nystagmus paradigm and black and white grating stimuli. (B) Effects of plano lens–filter combinations on contrast sensitivity thresholds of human subjects, assessed using a custom-designed computer-based test.

Table 2

MTF volume ratios (defined in Eq. (1)) for 4 Bangerter diffusing filters (LP, <0.1, 0.1, 0.6), calculated from MTFs derived from camera images (Fig. 2) and human psychophysical data (Fig. 5B)

	0.6 filter	0.1 filter	<0.1 filter	LP filter
Camera images (objective)	36.9%	28.0%	11.8%	5.4%
Human data (subjective)	51.2%	26.9%	10.9%	6.6%

frequency domain (Tian, Arnoldussen, Tuan, Logan, & Wildsoet, 2008). Because our human contrast sensitivity data included only the small number of points (Fig. 5B), we interpolated the MTFs from splines (Bartels, Barsky, & Beatty, 1987), before applying the above equation. The MTF volume ratios calculated from the human data closely match with those derived from camera images, except for the 0.6 filter. In both cases, the MTF volume ratios for the 0.6 and 0.1 filters are at least 2-fold greater than those for <0.1 and LP filters, greater than 26%, and less than 12%, respectively. That the

human data yielded a larger MTF volume ratio for 0.6 filter than the camera images, presumably reflects the relatively greater sensitivity of the human eye to higher spatial frequencies, resulting in an overestimation of its optical effect at these frequencies.

4. Discussion

The purpose of this study was to gain further insight into the mechanisms mediating form deprivation myopia. Although it is well known that retinal image degradation during early development can interfere with emmetropization, the features of the degraded retinal images responsible for the resulting myopic changes are the subject of on-going debate. Thus we measured the ocular responses of young chicks subjected to different levels of retinal image degradation.

In line with previous data (Wallman et al., 1978), we observed form deprivation myopia in our young chicks when the retinal image was sufficiently degraded. However, only two of our treatments met this condition. Specifically, of the four different diffuser treatments used in the current study, only the <0.1 and LP filter treatments induced significant myopic shifts in refractive error that were different from our control-no filter (plano lens) treatment. For these two treatments, the refractive changes reflected increased rates of ocular elongation, which were evident early in the treatment period, reaching statistical significance by day 2. There also was no difference in the growth-enhancing effects between these two (<0.1 and LP filter) treatments, even though the LP filter caused much greater image degradation (Fig. 1), and decreased contrast sensitivity more than the <0.1 filter. The negligible effects on ocular growth of the 0.1 and 0.6 filters also contrast with their effects on image quality and visual performance; both decreased the contrast sensitivity of our human subjects and the spatial resolution of the chicks, the 0.1 filter much more, consistent with its greater image degrading effect (see also Fig. 1). In summary, the ocular growth responses elicited by our 5 treatments fell into two distinct subgroups, which exhibited either only subtle refractive error changes (plano lens, 0.6 & 0.1 filters) or large myopic shifts in refractive error (<0.1 & LP filters).

What differences are there in the image degrading effects of the filters that could explain the observed dichotomy in the elicited growth response patterns? Of potential relevance is the fact that the filters used in the current study differentially affected the spatial frequency composition as well as the spatial contrast of images (e.g., see Figs. 1 and 2). For example, the <0.1 filter passed only very low spatial frequencies, while the LP filter allowed no detectable spatial information to pass through. On the other hand, both the 0.1 and 0.6 filters acted as low to medium pass filters, although they had different cut-off frequencies, estimated to be 8.35 and 25.05 cpd, respectively, based on our human contrast sensitivity data, and the 0.6 filter attenuated contrast less than the 0.1 filter at each of the spatial frequencies passed. MTF volume ratios derived from the camera images and human MTF data for these filters (Table 2) are closely matched, except for the 0.6 filter, for which a higher ratio was obtained for the human eye, presumably reflecting its greater sensitivity to higher spatial frequencies. The effects of the diffusers on retinal image quality in the chick are likely to be qualitatively similar although were not directly quantified. The limitations of the method used to obtain the filter-MTF data, which are unique to the filter-camera combination, and nonlinearities encountered in deriving system MTFs from component MTFs preclude the application of these data to obtain equivalent data for filter-chick eye combinations, although published MTF data for the chick eye are available (Coletta, Marcos, Wildsoet, & Troilo, 2003; de la Cera, Rodriguez, de Castro, Merayo, & Marcos, 2007; Garcia de la Cera, Rodriguez, & Marcos, 2006; Tian et al., 2008). Although

two other approaches have been used to quantify the ocular effects of light scatter (Navarro, 1985; Tian et al., 2008), both have their limitations and cannot be applied to the current study.

A dependence of ocular growth regulation on either spatial frequency or contrast can explain our results, given the differences between the filters noted above. However, based on previous studies, it seems likely that differences in the spatial frequency content of the retinal images, and thus the cut-off frequencies of the filters, are more important than differences in retinal image contrast. For example, Schmid and Wildsoet (1997), showed that exposure for 20 min per day to spatial frequencies within the range of 0.086 and 4.3 cpd, but not higher or lower spatial frequencies, were sufficient to inhibit myopic changes in chicks form deprived with white opaque diffusers. This result was interpreted as evidence that emmetropization is tuned to mid-spatial frequencies. This same study showed that even low contrast stimuli (i.e. 9%), could inhibit form deprivation myopia under these conditions. In another closely related study, restricting visual experience by reducing spatial frequency information prevented recovery from form deprivation myopia and induces myopia in normal chicks (Diether, Gekeler, & Schaeffel, 2001). Further evidence for the spatial frequency-tuning of emmetropization is contained in studies by Diether and Wildsoet (2005) and Hess et al. (2006). In the former study, improved compensation to myopic defocus was observed when a 1.2 cpd striped filler pattern was incorporated in the target stimulus and the contrast threshold for compensation to imposed myopic defocus was found to be dependent on the target spatial frequency composition. In the latter study, argument is made for tuning of emmetropization to high spatial frequencies, although this conclusion is based on only one data point, representing the highest spatial contrast used.

A critical role of retinal image contrast in eye growth regulation was proposed by Bartmann and Schaeffel in an early study in chicks, in which the amount of form deprivation myopia was found to be correlated with the amount of retinal image degradation (Bartmann & Schaeffel, 1994). This study included 4 handmade “occluders”—one heavily and one slightly frosted (translucent plastic foil, frosted by emery paper), one clear and one black. Bartmann and Schaeffel proposed a simple mechanism for emmetropization in which the rate of eye growth was tuned to retinal image contrast. However, our results do not follow the predictions of this model—of increased rates of axial elongation with the 0.6 and 0.1 filters, as well as differences between the growth-enhancing effects of all filters. Instead, our results suggest that emmetropization is relatively insensitive to retinal image contrast degradation, consistent with the findings of studies by Diether and Wildsoet (2005) and Schmid and Wildsoet (1997). It is possible that this apparent insensitivity of emmetropization to contrast degradation is an effect of retinal contrast adaptation, which would lessen the effect on retinal activity of switching from the 0.6 filter to the 0.1 filter. Such effects have been noted in both chick-based studies (Diether & Schaeffel, 1999) and human-based studies (George & Rosenfield, 2004). Unfortunately, the hand made origin of the devices used in the earlier study by Bartmann and Schaeffel (1994), and its cross-sectional nature, limit the scope of comparative analyses. Nonetheless, it is noteworthy that both frosted filters recorded similarly reduced modulation transfer values in the mid-frequency range (~2–4 cpd), and a closer inspection of corresponding axial length data revealed minimal difference across corresponding treatment groups (Table 1: 9.6 ± 0.24 mm, heavily frosted vs. 9.52 ± 0.6 mm, lightly frosted; right eyes). Finally, while we cannot rule out the possibility that ocular growth in the chick is subject to contrast-modulation, our data suggest a very narrow operating range, between that imposed by the 0.1 and <0.1 filters.

Why might emmetropization be tuned to mid-spatial frequencies? From an optical perspective, the removal of high spatial fre-

quencies will increase the depth of focus of the eye. Mid-spatial frequency-tuning of emmetropization would thus be protective against small degrees of retinal image degradation, for example, due to short term fluctuations in higher order optical aberrations (Tian & Wildsoet, 2006), and also makes sense in terms of the spatial frequency content of natural images, which show a $1/f$ energy distribution (Field & Brady, 1997).

The dichotomous nature of our results involving Bangerter filters to manipulate retinal image quality bear a striking resemblance to data from a study in chick using the same filters in combination to optical defocus by Park, Winawer, and Wallman (2003). Similar inhibitory effects on ocular elongation were recorded with a +7 D lens used alone, or in combination with either a 0.4, 0.2 or 0.1 filter. In contrast, the addition of a <<0.1 Bangerter filter produced the opposite response—increased ocular elongation and myopia. Thus these data are consistent with our findings although not directly comparable. Interestingly, the 2 moderate density filters (0.1 and 0.2), attenuated but did not eliminate the expected defocus-induced choroidal thickening response, suggesting that there are separate visual signals driving ocular elongation and choroidal growth, with different spatial frequency and/or contrast tuning.

Are there species-related differences in the ocular response of young animals to form deprivation? In a related study involving monkeys, Smith and Hung (2000), used Bangerter filters, as in the current study, with some overlap in the choice of filters used in our two studies (0.4, 0.1, & LP compared to 0.6, 0.1, <0.1 & LP). They found the degree of retinal image degradation required to trigger form deprivation myopia to be relatively low in monkeys and the amount of myopia present at the end of the treatment period to be correlated with the amount of image contrast reduction. As described, these results appear to bear more resemblance to the data reported for chick by Bartmann and Schaeffel (1994), than with the data described here. However, the responses of the monkey eyes were quite variable. For example, as judged by induced anisometropia, one of 5 monkeys tested with the densest (LP) filter and one of 5 monkeys tested with the 0.1 filter failed to respond, while 2 of 3 monkeys wearing the lightest filter (0.4) “out-responded” 3 monkeys of the 0.1 filter group and 2 monkeys of the LP group and approximately matched the response of another monkey in the latter group. Inter-animal differences in naturally occurring optical aberrations, as observed in a previous study by the same group (Ramamirtham, Kee, Hung, Qiao-Grider, Roorda & Smith, 2006), may account for the above differences, the retinal image degradation experienced by each animal reflecting contributions from its own optical aberrations and the diffuser worn. MTF volume ratios estimated from reported MTF functions showed up to 2-fold differences between eyes.

For the two groups in the current study showing significant increases in myopia over the 14-day treatment period, both showed a plateauing of the refractive changes after day 9, although interocular differences in vitreous chamber depths continued to increase steadily over the remainder of the treatment period. This plateauing is likely to represent an optical artifact tied to how refractive errors are calculated, as previously reported by Saltarelli et al. (2004).

Although both diffusers and negative defocusing lenses cause eyes to become myopic, some studies suggest that the mechanisms mediating form deprivation and lens-induced myopia are different (Choh, Lew, Nadel, & Wildsoet, 2006; Kee, Marzani, & Wallman, 2001; Schaeffel, Hagel, Bartmann, Kohler, & Zrenner, 1994; Yew, 2004). For example, in a chick study by Kee et al. (2001), negative lenses induced more rapid ocular elongation than diffusers, and the response to the former also had an earlier onset; similarly, scleral proteoglycan synthesis was found to increase more rapidly in eyes wearing negative lenses than those wearing diffusers. That the less

dense diffusers used in the current study did not induce significant myopia yet low power defocusing lenses do (both stimuli are likely to have similar effects on retinal image quality), lends further support to the argument that different mechanisms are involved. However, it is noteworthy that the rate of initial compensation to imposed optical defocus is apparently independent of lens power (Wildsoet & Wallman, 1997), another example of an all-or-none response. Understanding the similarities and differences between form deprivation- and lens defocus-induced myopia is critical to understanding the underlying mechanisms for the more common forms of human myopia, i.e., juvenile- or late-onset myopia, and the myopia induced by early occluding pathology.

In summary, our study suggests that the amount of image degradation required to trigger form deprivation myopia in chicks is relatively high; they further suggest, although do not prove, that supra-threshold stimuli trigger increased eye growth as a fixed (all-or-none) response. This threshold represents a MTF volume ratio between 26% and 12%. Our data are also consistent with middle spatial frequency information playing an important role in eye growth regulation and thus emmetropization in the chick.

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