



# Ocular compensation for alternating myopic and hyperopic defocus

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## Abstract

During development, the eye grows under visual feedback control, as shown by its compensating for defocus imposed by spectacle lenses. Under normal conditions the sign and magnitude of defocus vary with viewing distance, accommodative status and other factors. To explore how periods of myopic and hyperopic defocus are integrated over time we presented rapidly alternating episodes of myopic and hyperopic defocus by sequentially illuminating a nearby scrim and the wall beyond it to chick eyes wearing lenses that put the far point between the two surfaces. We found that equal periods of myopic and hyperopic defocus generally led to compensatory hyperopia, showing that myopic defocus had a disproportionate effect. Furthermore, the degree of hyperopia depended on the frequency of alternation: low frequencies (1 cycle/30 min) resulted in more hyperopia, whereas at high frequencies (1 cycle/s) the myopic and hyperopic defocus nearly cancelled each other. If similar temporal integration effects apply to humans, they may help explain why brief accommodation events may not influence lens-compensation and why a child's total reading time may be a poor predictor of myopic progression.

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*Keywords:* Choroid; Emmetropization; Spectacle lens compensation; Refractive error

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## 1. Introduction

Decades of experimental work in animals has provided strong evidence that emmetropization, the reduction in refractive error during development, is an active, visually guided process (reviewed by Wallman & Winawer, 2004; Wildsoet, 1997). Specifically, eye length and refractive status can be altered by imposing defocus with spectacle lenses or contact lenses (chicks, Schaeffel, Glasser, & Howland, 1988; Irving, Sivak, & Callender, 1992; rhesus monkeys, Hung, Crawford, & Smith, 1995; marmosets, Whatham & Judge, 2001; guinea pigs, McFadden, Howlett, & Mertz, 2004). Under

these conditions, the eye speeds or slows its rate of elongation to grow into focus for the combined power of the spectacle lens and the eye's lens and cornea, suggesting that a feedback loop using visual cues as an error signal regulates eye growth.

One challenge faced by such a feedback control system is how to derive a useful error signal from a highly variable and often transient input (defocus, or some visual signal that depends on defocus). For example, a hyperopic eye (as usually found in young animals) will experience hyperopic defocus when looking at distant objects, but when it is focused on nearby objects, distant objects will be myopically defocused. Despite the complex pattern of input, over time animals fitted with spectacle lenses can compensate quite accurately for the power of the lens (Irving et al., 1992; Smith & Hung, 1999). How does the eye do this? Does each brief episode of blur change the momentary direction of eye growth? Do myopic and hyperopic defocus cancel, or does one predominate?

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Recent experiments suggest that the emmetropization system uses a method of integration more complex than computing a linear sum of all the blur it experiences. First, it has been shown that in chicks, as little as 2 min of lens-wear every hour can stimulate nearly as good compensation as does full-time lens-wear, and the compensation for imposed defocus of either sign is comparable, if there is no other visual input (Winawer & Wallman, 2002). In contrast, a strong asymmetry is found if the lens-wear alternates between myopic defocus imposed by positive lenses and hyperopic defocus imposed by negative lenses: the eye compensates for the positive lens, even if there is five times longer negative than positive lens-wear. In the extreme, in chicks as little as four 2-min periods of positive lens-wear per day can outweigh the effects of negative lenses worn the rest of the day (Zhu, Winawer, & Wallman, 2003). These results all suggest that the emmetropization mechanism is particularly sensitive to myopic defocus. Given that humans would almost certainly have the equivalent of these eight minutes of myopic defocus over a day, it is puzzling why myopia developing in children would not be stopped dead in its tracks. One possibility is that the asymmetries reported in the animal literature apply only to extended periods of defocus; perhaps the emmetropization mechanism either ignores very brief periods of defocus altogether or integrates them in a more balanced way.

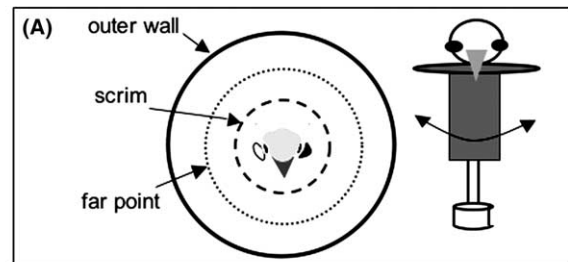
In this paper, we address the issue of how the eye's emmetropization system integrates very brief periods of defocus alternating in sign. We present results from a series of experiments in which we put chicks in a controlled visual environment for 30 min at a time. During these periods, we rapidly alternated the sign of defocus by alternately illuminating a nearby scrim (imposing hyperopic defocus) or a more distant wall (imposing myopic defocus). By doing so, we were able to address whether (1) the eye weighs short periods of positive and negative defocus equally and (2) whether the weighting depends on the frequency of alternation.

## 2. Methods

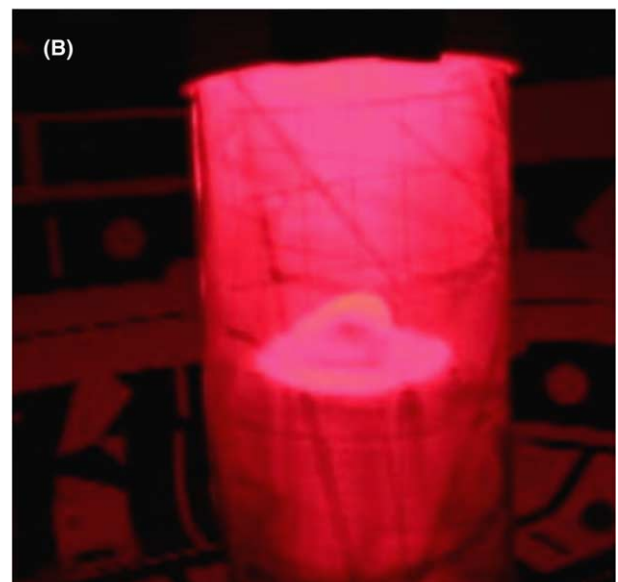
White Leghorn chickens were obtained either as eggs or 1 day after hatching from Truslow Farms (Hyline-W98-strain; Chestertown, MD), except for group 8 (Cornell K-strain White Leghorns, obtained from Cornell University, Ithaca, NY). All chicks were either 6 or 7 days post-hatching at the start of experiments, all of which lasted 3 days. At the start and end of each experiment, both eyes had their refractive error measured using a modified Hartinger Refractometer (Wallman & Adams, 1987) and their axial dimensions measured using high frequency A-scan ultrasound (Nickla, Wildsoet, & Wallman, 1998; Wallman &

Adams, 1987). Total ocular length was defined as the distance from the front of the cornea to the back of the sclera (unlike clinical measurements, which are made to the front of the retina, thereby not including retinal, choroidal, or scleral thickness). Measurements were made under 1.5% Halothane anesthesia, without cycloplegia, and were made at the same time of day at the start and the end of the experiment. Plastic 12 mm lenses or black plastic occluders were fitted by gluing the lens to a Velcro ring and then fixing the ring to a mating Velcro ring, glued to the feathers around the eye (for more details, see Wildsoet & Wallman, 1995).

During the experiments, chicks were housed in groups in light-proof chambers in darkness, except for eight 30-min periods each day. During four of these periods, chicks were placed in a two-drum system (Fig. 1, see below), wearing a +6 diopter spectacle lens on one eye and an opaque black occluder on the other.



schematic of apparatus



photograph of scrim from inside large drum

Fig. 1. Two-drum system. (A) Schematic: The opaque outer wall of the two-drum system was 30 cm from the drum center, and the inner scrim was 5 cm from the center. The far point falls between the two surfaces (16.7 cm for an unaccommodated emmetropic eye). Chicks were placed in the center and were rotated to encourage them to stay awake and look at the walls. (B) Photograph with inner scrim illuminated and the outer wall in the background.

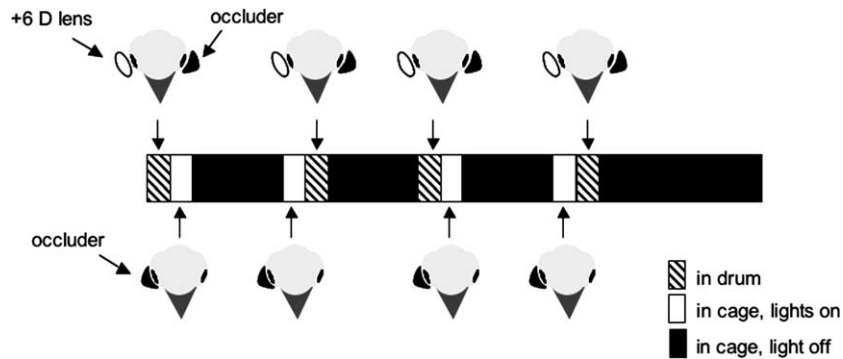


Fig. 2. Method of alternate occlusion. Chicks were kept in darkness (black regions) except for 30 min intervals, either in the two-drum system (striped) or in the cage (white). In the drum, one eye was fitted with a +6 D lens and the other eye with a black occluder (to avoid the effects of hyperopic defocus). When chicks were put in the cage with the lights on, the lens-wearing eye was covered with a black occluder and the fellow eye was uncovered. Thus, each eye had 30 min of vision and 30 min of occlusion 4 times per day. The sequence of visual episodes alternated between the time in the drum preceding the time in the cage and the reverse.

Either immediately following or immediately preceding each episode in the drum, chicks were put in the home cage with the lights on for 30 min, during which the eye that wore the lens in the drum was occluded, and the eye that was occluded in the drum now had unobstructed vision. Therefore, each bird had 30 min of vision four times per day: one eye had vision while wearing a lens in the drum, the other had vision without a lens in the cage, and both eyes had 30 min of occlusion four times per day<sup>1</sup> (Fig. 2). This protocol afforded us the advantage that each lens-wearing eye could be compared to a fellow eye that had normal visual experience. The periods of vision were spaced every 3.5 h, beginning at 9 am. For each chick, the drum/cage sequence was reversed each period, so that, for example, a chick would have drum episodes starting at 9 am, 1 pm, 4 pm, and 8 pm, and cage episodes at 9:30 am, 12:30 pm, 4:30 pm, and 7:30 pm. Every experiment was counterbalanced, so that half the birds had their first daily episode in the drum and half in the cage.

### 2.1. Two-drum system

The two-drum system consisted of an outer opaque cylinder, 30 cm in radius, the walls of which were lined with irregular black and white patterns, and an inner cylindrical metal scrim (57% of area being holes, 1600 holes/cm<sup>2</sup>), 5 cm in radius, with black patterns drawn on the scrim using ink (Fig. 1). Wearing a +6 D lens, the far point of an unaccommodated emmetropic eye would be 16.7 cm from the chick, between the two walls, leading to myopic blur (+2.7 D) when viewing

the far wall and hyperopic blur (−14 D) when viewing the nearby scrim. Although the two cylinders differed in both the saliency of the patterns (the outer was more salient to our eyes) and the degree of defocus they imposed, our objective was to study how timing affected the integration of myopic and hyperopic defocus, and we had reason to expect that the lens-power would elicit compensation in opposite directions at the two distances chosen (Park, Winawer, & Wallman, 2003, & pilot experiments). Furthermore, accommodation would reduce the hyperopic blur and increase the myopic blur, thereby reducing the imbalance. A lamp composed of multiple red LEDs rested on translucent material above the small drum, and two identical lamps rested on a larger translucent cover above the large drum. These two lamps lay on the annulus between the two drum walls. When the inner lamp alone was on, the scrim appeared nearly opaque, leading to a condition of near viewing. When the outer lamps alone were on, the scrim appeared nearly transparent and the outer wall was prominent, leading to a condition of far viewing. The illumination level of the two surfaces was matched at 1400 lux. While in the two-drum system, the chicks were slowly rotated (30°/s, reversing direction every 30 s), to encourage them to stay awake and fixate the walls. The timing parameters for all experiments are described below and summarized in Table 1.

### 2.2. Experiment 1—Single sign of defocus

To validate the two-drum system, we tested whether each of the two different illumination conditions could induce compensatory refractive changes that were opposite to each other. Two groups of chicks were placed in the drums, either with myopic defocus only (outer wall illuminated, group 1), or hyperopic defocus only (inner wall illuminated, group 2). There was no switching of illumination for either of these two groups.

<sup>1</sup> We expected the periods of occlusion to have little effect on the response to the lenses, given reports that nearly continuous occlusion is required for form-deprivation myopia (Napper et al., 1995; Smith, Hung, Kee, & Qiao, 2002).

Table 1  
Experimental conditions and results

Experiment	Group	Duration of hyperopic defocus (near illumination) (s)	Duration of myopic defocus (far illumination) (s)	Duty cycle of myopic defocus (%)	Number of animals	Refractive error (D)	Vitreous depth ( $\mu\text{m}$ )	Ocular length ( $\mu\text{m}$ )	Choroid thickness ( $\mu\text{m}$ )
1	1	0	1800	100	28	$2.37 \pm 0.48$	$-75 \pm 18$	$3 \pm 18$	$61 \pm 16$
	2	1800	0	0	5	$-2.42 \pm 1.23$	$110 \pm 26$	$88 \pm 43$	$-41 \pm 31$
2	3	900	900	50	13	$3.51 \pm 0.75$	$-111 \pm 19$	$-10 \pm 27$	$89 \pm 14$
	4	75	75	50	15	$2.85 \pm 0.63$	$-100 \pm 19$	$-19 \pm 19$	$77 \pm 13$
	5	6	6	50	15	$2.56 \pm 0.59$	$-70 \pm 24$	$3 \pm 25$	$58 \pm 17$
	6	0.5	0.5	50	13	$1.14 \pm 0.66$	$-68 \pm 18$	$-35 \pm 24$	$16 \pm 15$
3	7 <sup>a</sup>	0.5	0.5	50	7	$-1.44 \pm 1.36$	$65 \pm 23$	$96 \pm 17$	$9 \pm 12$
	8 <sup>b</sup>	900	900	50	8	$3.42 \pm 0.58$	$-76 \pm 27$	$-38 \pm 22$	$28 \pm 10$
4	9	2.5	0.5	83.3	15	$-0.76 \pm 0.36$	$9 \pm 23$	$5 \pm 25$	$4 \pm 19$
	10	1500	300	83.3	14	$2.41 \pm 0.69$	$-78 \pm 23$	$-68 \pm 26$	$29 \pm 16$

The refractive error and ocular biometry measurements are expressed as the mean relative change over the three-day experiments (the change in the lens-wearing eye minus the change in the fellow eye),  $\pm 1$  standard error of the mean.

<sup>a</sup> A plano lens was worn instead of a +6 D lens in the drum, so that the alternation was between two levels of hyperopic defocus instead of between hyperopic and myopic defocus.

<sup>b</sup> Both eyes were under cycloplegia during each episode in the drum to ensure that chicks experienced hyperopic defocus when viewing the near wall.

### 2.3. Experiment 2—Equal duration of myopic and hyperopic defocus

To assess how equal episodes of myopic and hyperopic defocus are weighted for different rates of alternation, each group of birds had alternating periods of hyperopic defocus (inner wall illumination) and myopic defocus (outer wall illumination). Within each group, the duration of periods of hyperopic and myopic defocus was equal, varying across groups in roughly equal log steps (900 s, 75 s, 6 s, and 0.5 s; Table 1).

### 2.4. Experiment 3—Control groups

A complication of our method is that if the refractive error resulting from alternating myopic and hyperopic defocus were near zero, it could either be the result of an averaging of the imposed myopic and hyperopic defocus or it could be that the flickering stimulus prevented any lens-compensation. To resolve this uncertainty we tested whether compensation could occur with rapid alternations of two degrees of hyperopic defocus. To do this, we had chicks experience rapid alternation of defocus (0.5 s each), but with a plano lens instead of a plus lens (group 7). Thus for an emmetropic eye, the outer wall (30 cm) presented 3.3 D of hyperopic defocus, and the inner scrim (5 cm) presented 20 D of hyperopic defocus.

A second possible confound is that accommodation might reduce the efficacy of the imposed hyperopic defocus at lower frequencies, but not when the sign of defocus switched twice a second. To test this hypothesis, a second control group (group 8) was run at the slowest switch rate (900 s for each viewing distance), with both eyes under cycloplegia (vecuronium bromide, 1 mg/ml;

Marzani & Wallman, 1997). The effectiveness of cycloplegia was verified by checking that the fellow eye was fully dilated when chicks were put in the cage with the lights on. This control group allowed us to test whether, during longer periods of near viewing, better accommodation might lead to less hyperopic defocus and therefore a weaker response to near viewing, compared to shorter periods of near viewing. Thus accommodation might indirectly create the frequency-dependence of the responses.

### 2.5. Experiment 4—Five times more hyperopic than myopic defocus

Because the effects of myopic defocus outweighed the effects of hyperopic defocus at all time scales in Experiment 2, the duration of hyperopic defocus during each cycle of alternation was increased for Experiment 4, so that the myopic and hyperopic defocus might cancel at short time scales. Each group had alternating periods of hyperopic and myopic defocus, either 0.5 s and 2.5 s periods (myopic and hyperopic defocus, respectively, group 9), or 5 min and 25 min (group 10; Table 1).

### 2.6. Statistics and data presentation

In all experiments, biometric measures and refractive error are primarily expressed as the “relative change”—the change in the lens-wearing eye minus the change in the contralateral control eye over the 3-day experiment. This measure minimizes the unwanted effects of batch-to-batch variation because refractive error and axial dimensions tend to be tightly correlated between the two eyes of untreated animals. For Experiment 1, in which the illumination, and hence the sign of the

imposed defocus, never changed, significance was assessed by 1-tailed paired *t*-tests within each group, and 1-tailed unpaired *t*-tests between groups. For all other groups, 2-tailed paired *t*-tests were used to assess significance within each group, and 2-tailed unpaired tests for comparisons between 2 groups. For Experiment 2, analysis of variance was used to assess significant main effects across groups, and linear regression was used to evaluate the effect of period. Multiple regression was used for the analysis of Experiments 2 and 4 combined, with period of oscillation and percentage of time with near viewing as the independent variables. The number of animals in each group is indicated in Table 1.

### 3. Results

#### 3.1. Experiment 1: Validation of method

With only the far wall illuminated (inducing myopic defocus), the lens-wearing eyes showed significant compensatory hyperopia. They shifted 2.1 D towards hyperopia, whereas the fellow eyes shifted 0.3 D towards myopia—a “relative difference” (change in lens-wearing eye minus change in fellow eye) of 2.4 D ( $p < 0.0001$ , Fig. 3). The compensatory hyperopia was reflected in a shortening of the vitreous chamber by 32  $\mu\text{m}$ , compared to a lengthening in the fellow eyes of 43  $\mu\text{m}$  (relative difference of  $-75 \mu\text{m}$ ,  $p < 0.001$ , Fig. 3). The difference in

vitreous chamber depths seems to have been caused by choroidal thickening in the lens-wearing eye (60  $\mu\text{m}$ , compared to 0  $\mu\text{m}$  in the fellow eye,  $p < 0.001$ , Fig. 3) and not by changes in ocular elongation (189  $\mu\text{m}$  vs. 186  $\mu\text{m}$  for lens and fellow eyes, respectively;  $p > 0.05$ ).

Illumination of only the near wall (inducing hyperopic defocus) led to the opposite pattern of results. The lens-wearing eye shifted 3.0 D towards myopia, while the fellow eye shifted only 0.6 D, though the difference was not quite significant (relative change of  $-2.4 \text{ D}$ ,  $p = 0.06$ , Fig. 3). This trend was accompanied by a significant increase of 190  $\mu\text{m}$  in vitreous expansion, more than double the 80  $\mu\text{m}$  increase in the fellow eyes ( $p < 0.01$ ). This increase in vitreous depth was mostly due to an increase in the amount of ocular elongation, 215  $\mu\text{m}$ , nearly double the 127  $\mu\text{m}$  of elongation in the fellow eyes ( $p = 0.05$ , Fig. 3). There was also a non-significant trend towards thinner choroids, with a decrease in thickness of 92  $\mu\text{m}$ , compared to a decrease of 51  $\mu\text{m}$  in the fellow eye ( $p = 0.13$ , Fig. 3).

Comparing the relative changes (change in lens-wearing eye minus change in fellow eye) between the groups subjected to myopic vs. hyperopic defocus, there were significant differences in refractive error ( $p < 0.01$ ), vitreous chamber depth ( $p < 0.001$ ), and choroid thickness ( $p < 0.05$ ), with a nearly significant difference in ocular elongation ( $p = 0.06$ ). Thus, by simply changing the illumination, we were able to induce compensation either for myopic defocus or hyperopic defocus.

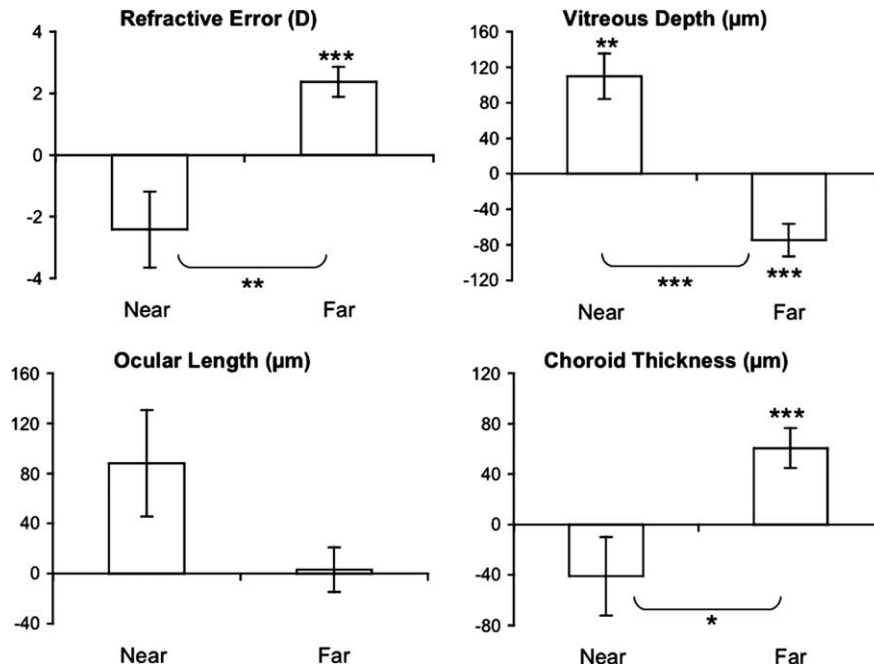


Fig. 3. Control conditions (Experiment 1). Hyperopic defocus only (near viewing) led to compensatory myopia, whereas myopic defocus only (far viewing) led to compensatory hyperopia, with significant differences between groups in refractive error ( $p < 0.01$ ), vitreous depth ( $p < 0.001$ ), and choroid thickness ( $p < 0.05$ ). Each bar reflects the mean change over three days relative to that of the fellow eye ( $\pm 1$  SEM). One asterisk (\*) indicates  $p < 0.05$ , two indicates  $p < 0.01$ , and three indicates  $p < 0.001$ . Tests within groups are paired.

### 3.2. Experiment 2: Different periods of alternation of myopic and hyperopic defocus

When the inner and outer walls were alternately illuminated for equal durations, with periods (one cycle of inner illumination followed by outer illumination) varying from 1 s (1800 cycles per drum episode; Group 6) to 30 min (one cycle per drum episode; Group 3), the myopic blur (outer wall illuminated) had the dominant effect. Pooling across groups, there was a shift of 2.4 D towards hyperopia relative to the fellow eye ( $p < 0.0001$ , ANOVA; Fig. 4). This was accompanied by inhibition of vitreous expansion, with a relative inhibition of  $87 \mu\text{m}$  ( $p < 0.0001$ , ANOVA), which was largely accounted for by choroidal thickening,  $60 \mu\text{m}$  relative to the fellow eye ( $p < 0.0001$ ); there was no significant effect on ocular elongation ( $159 \mu\text{m}$  vs.  $173 \mu\text{m}$ , lens eye vs. fellow eye;  $p > 0.05$ ).

Our principal finding is that the degree of induced hyperopia depended on the period of alternation (Fig. 4). In general, longer periods resulted in greater hyperopia. Specifically, there was a positive relationship between the relative change in refractive error and the period, with an increase of 0.7 D per log unit of period ( $n = 56$ ,  $p < 0.05$ , slope  $\neq 0$ , regression). A similar pattern was found in choroid thickness, with an increase in choroid thickness (relative to the fellow eye) of  $22 \mu\text{m}$  per log unit ( $p < 0.01$ , regression). There was also a non-significant trend towards greater inhibition of vitreous expansion with increasing period ( $15 \mu\text{m}$  per log

unit,  $p = 0.09$ , regression). The relation between the period and the change in ocular elongation ( $5 \mu\text{m}$  per log unit) was not significant.

### 3.3. Experiment 3—Control groups

We propose that the pattern of more hyperopia with less frequent alternation is due to an increasing imbalance in the effects of myopic and hyperopic defocus with longer episodes. Alternatively, the pattern could be due to an artifact of our apparatus such that very rapid changes in illumination prevent any compensation at all. To discern between these alternatives, we repeated the shortest period (1 s) with alternation of 3.3 D and 20 D of hyperopic defocus (birds with plano lenses, Group 7, otherwise like Group 6), so that compensatory myopia would be expected if lens-compensation was occurring despite the rapid alternation. This is essentially what we found. This group had significantly more vitreous expansion,  $65 \mu\text{m}$  more than the fellow eye, compared to  $68 \mu\text{m}$  less than the fellow eye in the group in which the sign of defocus alternated (birds wearing positive lenses;  $p < 0.001$ , unpaired  $t$ -test between the two groups, Fig. 5). This group also had a significant increase in ocular elongation,  $96 \mu\text{m}$  relative to the fellow eye, whereas the positive lens group had a  $35 \mu\text{m}$  decrease ( $p < 0.01$ , unpaired  $t$ -test between groups, Fig. 5), and a myopic shift of  $-1.4$  D shift compared to a  $+1.1$  D shift in the positive lens group, although this difference between the two groups was not significant

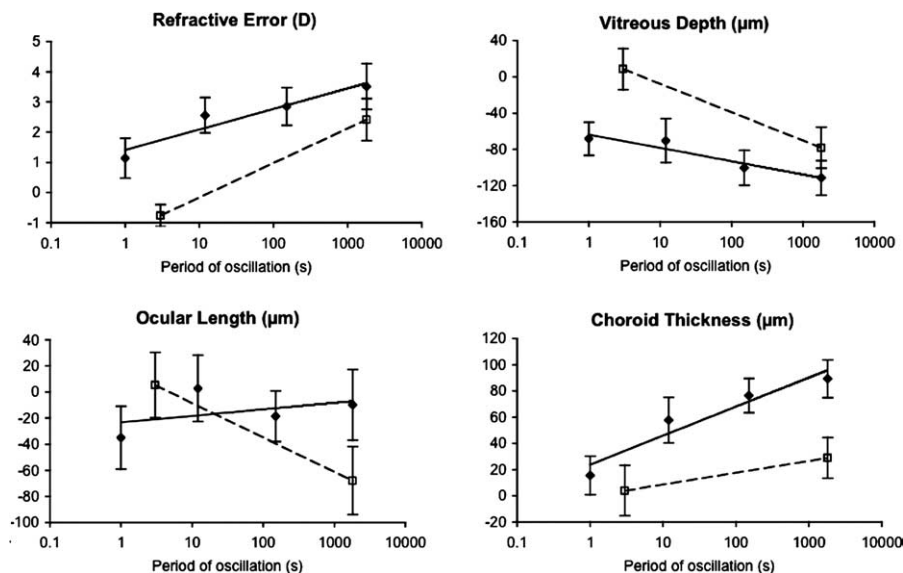


Fig. 4. The effects of the rate of alternation (Experiments 2 and 4). There was more compensatory hyperopia with lower frequency alternations whether the ratio of the duration of myopic to hyperopic defocus was 1:1 (solid lines, Experiment 2), or 1:5 (dashed lines, Experiment 4). There was also more compensatory hyperopia when the ratio was balanced (1:1) than when there was more hyperopic defocus (1:5). Multiple regressions showed that both factors, log period of oscillation and ratio of myopic to hyperopic defocus, had significant effects on refractive error, vitreous depth, and choroid thickness. There were no significant effects of either factor on ocular length.

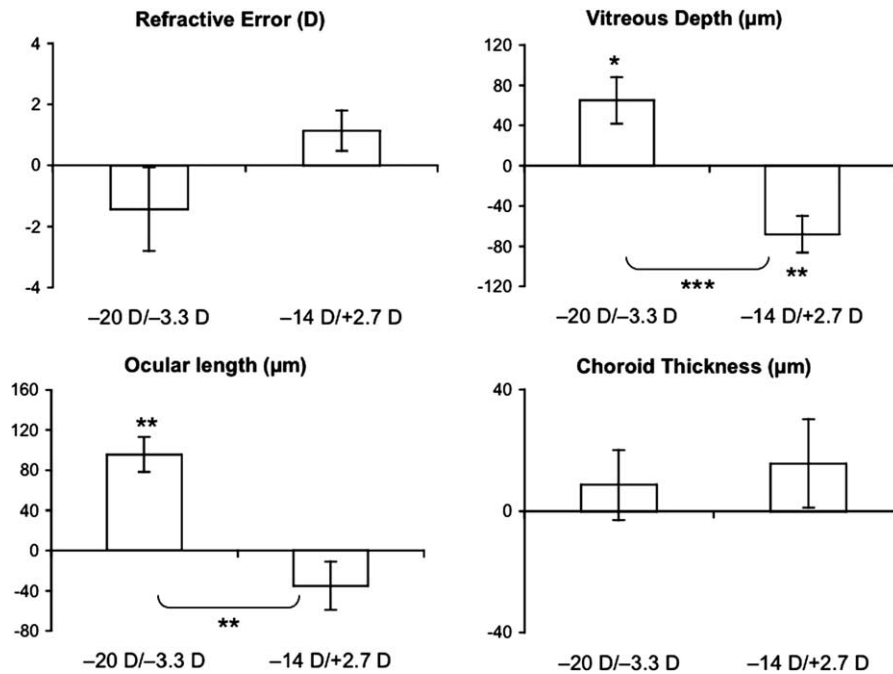


Fig. 5. Rapid light switching without alternating the sign of defocus (Experiment 3). One second periods, either with no lens (hyperopic defocus of different degrees, left bar in each plot) or positive lens (alternate myopic and hyperopic defocus, right bar in each plot). The two groups responded oppositely, with significantly different responses in vitreous depth ( $p < 0.001$ ) and ocular elongation ( $p < 0.01$ ). Positive lens group replotted from Fig. 3. Statistical tests as in Fig. 3.

( $p = 0.07$ ). Thus the rapid alternation of the scene does not prevent the eye from compensating for the defocus imposed by lenses.

Another concern was that the greater hyperopia with lower frequencies of alternation was due to accommodation. If chicks accommodated more during longer periods of hyperopic defocus, then they would experience less hyperopic defocus, which might account for the weaker response to the hyperopic half of each cycle of alternation. At high frequencies of switching, accommodation might not be activated, making defocus during the myopic and hyperopic focus more equal. To test this possibility, we repeated the longest period (30 min) with a group of birds that were under cycloplegia during each drum episode (Group 8), but otherwise were treated the same as Group 3. The groups did not differ in refractive error (relative hyperopic shift of 3.4 D vs. 3.5 D, cycloplegia vs. no cycloplegia,  $p = 0.92$ , Fig. 6), vitreous depth ( $-76 \mu\text{m}$  vs.  $-111 \mu\text{m}$ ,  $p = 0.42$ ) or ocular elongation ( $-38 \mu\text{m}$  vs.  $-10 \mu\text{m}$ ,  $p = 0.29$ ). There was, however, less choroidal thickening in the group under cycloplegia ( $28 \mu\text{m}$  vs.  $89 \mu\text{m}$ ,  $p < 0.01$ ).

#### 3.4. Experiment 4: Five times more hyperopic than myopic defocus

For a further test of the influence of episode length on the temporal integration of defocus, we attempted to cancel the greater potency of the myopic defocus

by giving five times longer episodes of hyperopic than myopic defocus during each cycle of alternation, with either 3 s cycles (group 9) or 30 min cycles (group 10). Group 9 did not shift towards hyperopia, but rather showed an approximate cancellation of the effects of myopic and hyperopic blur. There were no significant shifts relative to the fellow eye in terms of refractive error, ocular length, vitreous depth, or choroidal thickness ( $p > 0.05$ , all measures, Fig. 4).

In contrast, the group with the slower alternation compensated for viewing of the outer wall, shifting in the hyperopic direction by 2.4 D relative to the contralateral eyes, despite the preponderance of near viewing ( $p < 0.01$ , Fig. 4). The refractive shift was accompanied by a significant slowing of vitreous expansion ( $78 \mu\text{m}$  less expansion than the fellow eyes,  $p < 0.01$ ), which was mostly due to a slowing of ocular elongation ( $68 \mu\text{m}$  less growth in the lens-wearing eyes,  $p < 0.05$ ), as well as a trend towards greater choroidal thickness ( $30 \mu\text{m}$  shift relative to the fellow eyes,  $p = 0.09$ ). The shift in refractive error of the 30 min period group (relative to the fellow eyes) was significantly greater than that of the 3 s period group (2.4 D vs.  $-0.8$  D;  $p < 0.001$ ; Fig. 4), as was the inhibition of vitreous expansion ( $-78 \mu\text{m}$  vs.  $9 \mu\text{m}$ ;  $p < 0.05$ ). There were also trends towards greater inhibition of ocular elongation ( $-68 \mu\text{m}$  vs.  $5 \mu\text{m}$ ;  $p > 0.05$ ) and thicker choroids ( $29 \mu\text{m}$  vs.  $4 \mu\text{m}$ ;  $p > 0.05$ ).

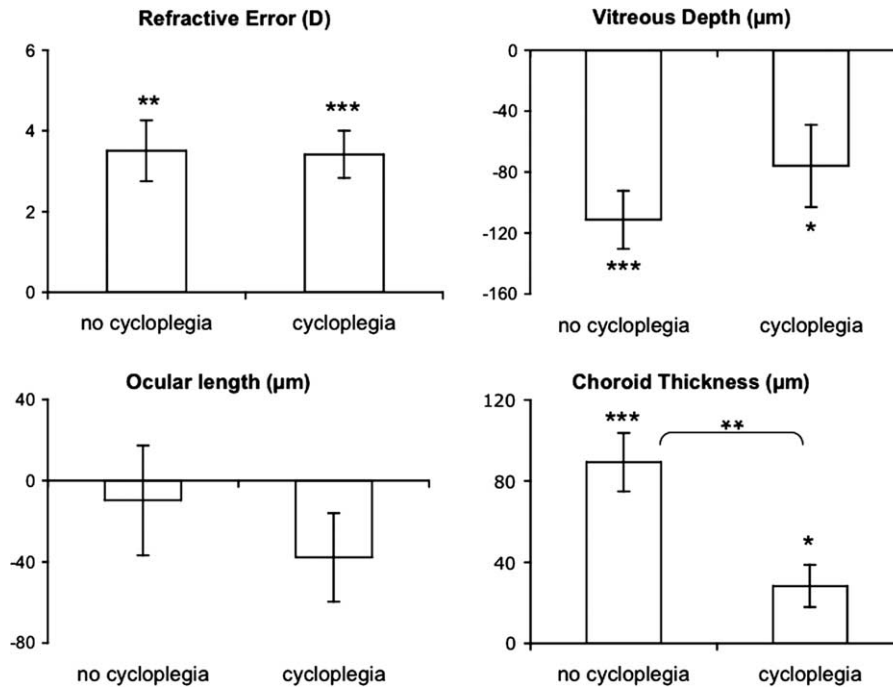


Fig. 6. Control for accommodation (Experiment 3). Fifteen minute periods of near and of far viewing per 30 min episode, either with or without cycloplegia. Both groups showed shifts towards hyperopia (compensating for the myopic defocus of the outer wall), with no significant differences between groups in refractive error, vitreous depth, or ocular length, though there was a significantly larger choroidal response in the no-cycloplegia group ( $p < 0.01$ ). No-cycloplegia group replotted from Fig. 3. Statistical tests as in Fig. 3.

### 3.5. Experiments 2 and 4 compared

Across Experiments 2 and 4, there are three principal effects: there is more hyperopia generally, there is more hyperopia with lower frequency alternations (longer periods), and this hyperopic preponderance could be eliminated at high alternation rates by increasing the fraction of each cycle during which hyperopic defocus is present (Fig. 4). Multiple regression shows all the effects to be significant: there was a significantly greater hyperopic shift in refractive error with decreasing frequency and with increasing myopic-defocus fraction of each cycle ( $p < 0.0001$ ,  $p < 0.001$ , respectively). Likewise, decreasing frequency and increasing myopic-defocus fraction were associated with more choroidal thickening ( $p < 0.01$ ,  $p < 0.001$ , respectively), and more inhibition of vitreous expansion ( $p < 0.01$ , both factors).

## 4. Discussion

Overall, our results have shown that when hyperopic and myopic defocus were alternated, first, the myopic defocus (outer wall) had the greater effect, indicated by hyperopic shifts that approximately compensate for the myopic defocus imposed by the outer wall and second, the higher the frequency of alternation, the weaker the dominance of the myopic defocus. If we presented hyperopic defocus (inner wall) for five times as long

as myopic defocus (outer wall), then the effects completely cancelled if the frequency of alternation was high, but the myopic defocus dominated if the frequency was low.

These results suggest two non-linearities in the integration of defocus: First, integration is biased in that myopic defocus tends to override hyperopic defocus when the sign of defocus is alternated, although brief episodes of either sign of defocus produce effects that are approximately equal in magnitude if only one sign of defocus is presented. Second, this bias has a frequency dependency, such that it becomes weaker with more frequent alternations.

### 4.1. Greater potency of myopic defocus

When experiments have presented animals with intermittent lens-wear or with alternating signs of lens-wear, the general finding has been that effects of myopic defocus are stronger or more enduring than those of hyperopic defocus. In the chick, for example, wearing a positive lens for only 3 h out of a 12 h day resulted in significant compensation, whereas wearing a negative lens for 9 h per day resulted in no compensation (Schmid & Wildsoet, 1996). The specific timing effects seem to be highly conserved across species: When a chick, tree shrew or monkey is fitted with a negative lens or an image degrading diffuser except for a single daily period during which the device is removed, the amount of



myopia falls off exponentially with the duration of the interruption, with a time constant of close to 1 h in each study (Smith et al., 2002). Though such experiments do not involve explicit lens-switching, the animals do develop some compensatory myopia, so that as the experiment progresses, removal of the lens or diffuser is akin to fitting the eye with a positive lens. In most cases under such a regime the partial compensation seems to stabilize before the end of the experiment (Schmid & Wildsoet, 1996; Smith et al., 2002), arguing that about 1 h per day of myopic defocus can cancel the effects of 11 h per day of hyperopic defocus.

The results of Experiment 2 also support the greater potency of myopic defocus and extend the finding to higher frequencies of alternation than are possible with manual switching of lenses, as in previous experiments. In all four groups in Experiment 2 in which a positive lens was worn, the eyes compensated, at least in part, for the myopic defocus, despite being presented with equal periods of myopic and hyperopic defocus. The greater potency of the myopic defocus is further underscored by the fact that the degree of imposed myopic defocus was less than the degree of hyperopic defocus (2.7 D vs. 14 D, though with accommodation the difference would be smaller.)

In contrast, these asymmetric effects of frequency are not generally apparent when a single, strong lens is worn continuously; in chicks (Irving et al., 1992), guinea pigs (McFadden et al., 2004) and monkeys (Graham & Judge, 1999), compensation is about as good for negative lenses as it is for positive lenses. This pattern of results is supported by Experiment 1, in which exposure to a single sign of defocus induced an approximately equal magnitude of compensation for either sign. In fact, it is possible that had the experiment lasted longer, or were there continuous defocus instead of a few brief periods per day, the compensation for hyperopic defocus imposed by near viewing might have been even greater, because the magnitude of the imposed defocus was greater for near viewing than for far.

#### 4.2. Dependency of temporal integration on the duration of defocus

The second result we report, that the imbalance between myopic and hyperopic defocus decreases with shorter periods of defocus, is a novel finding made possible only by the fact that we were able to switch the sign of defocus via the illumination thereby allowing very fast switching. This non-linearity is evident both in Experiments 2 and 4, in which the degree of induced hyperopia is greater for longer periods.

Such a non-linearity has been previously hypothesized, but never tested; specifically, chicks wearing toric lenses (Jackson cross-cylinders that present myopic defocus in one meridian and hyperopic defocus in the

other meridian) have been shown to compensate for the average refractive error of the two meridians (McLean & Wallman, 2003; Thibos, Cheng, & Phillips, 2001; but see Schmid & Wildsoet, 1997). If each small patch of retina experienced a mixture of defocus depending on the orientation of the contour it was exposed to, the first non-linearity discussed above would predict that the eye would grow to compensate for the myopic defocus, and not the average defocus. If, however, the orientation of the contours landing on a given patch of retina varied across saccades (and thus on the order of seconds or faster), then an emmetropization integrator that became linear (or close to it) with rapid oscillations might be expected to respond to the average defocus. Thus the non-linearity we report provides confirmation for this interpretation. More generally, it may also help explain why numerous lens-rearing studies have found that blocking accommodation has little effect on lens compensation (Schaeffel, Troilo, Wallman, & Howland, 1990; Schwahn & Schaeffel, 1994; Wildsoet, Howland, Falconer, & Dick, 1993): if accommodative events tend to be brief, then the change in defocus may have a minimal effect when integrated over longer periods. Minimizing the contribution of brief accommodative events to emmetropization might be useful, as accommodation might otherwise tend to reduce the effectiveness of emmetropization by eliminating the defocus that normally drives it.

The more balanced integration (i.e. less hyperopia) with more rapid switching is not due to lens compensation being impeded by the frequent illumination transients, even though flicker can impair emmetropization, and the reduction in the compensation for negative vs. positive lenses depends on the frequency and the duty cycle of the illumination (Schwahn & Schaeffel, 1997). In our experiments we observed good compensation for alternations of 2 degrees of hyperopic defocus even with 1 Hz oscillations, the shortest period used for any of the other experiments. Thus, we interpret the decrease in the hyperopia with shorter periods as a change in the weighting of myopic vs. hyperopic defocus as a function of duration of defocus.

The asymmetry is also not due to the eye only being able to reduce blur via accommodation when the frequency was low enough. Our second control experiment shows that chicks prevented from accommodating by cycloplegia still showed a strong bias to compensate for the myopic defocus even with the slowest switching rate, 15 min each of near and far viewing. Furthermore, the initial control experiment for the apparatus, in which chicks viewed only the near or the far wall, also suggests that accommodation does not necessarily interfere with emmetropization, as the chicks viewing only the near wall for 30 min periods showed a clear pattern of compensation, with more than a twofold increase in the rate of vitreous chamber expansion, despite the fact that in

principle, they could have cleared the hyperopic defocus by accommodating.

#### 4.3. Ocular components of compensation

A puzzle about the anatomical changes observed was that there was little inhibition of ocular elongation (16  $\mu\text{m}$  of inhibition,  $p > 0.05$ ) across all the groups in which the refractions shifted significantly towards hyperopia (groups 1, 3, 4, 5, 8, and 10), whereas usually ocular elongation and refractive error are highly correlated in lens-rearing experiments. Even the myopic defocus control group (group 1) showed no inhibition of ocular elongation. In a previous study in which lens-power, drum diameter, and frequency and duration of lens-wear were identical to the conditions for the myopic defocus control group in this study, significant inhibition of ocular elongation did accompany the compensatory hyperopia (Park et al., 2003). The lack of slowed elongation in this study might be explained either by the presence of the nearby scrim (even when the outer wall was illuminated, the inner scrim was faintly visible, potentially inducing hyperopic defocus), or by the paradigm of alternate occlusion. The former explanation would require that simultaneous myopic defocus (from the far wall) and hyperopic defocus (from the scrim) would block the elongation response but not the choroidal response. A follow-up control study suggests that this is not the case: Chicks given positive lens-wear in the two-drum system when only the outer wall was illuminated (similar to group 1), but without alternate occlusion, did show significantly slowed ocular elongation (136  $\mu\text{m}$  increase in the lens-wearing eyes vs. 223  $\mu\text{m}$  in the fellow eyes,  $p < 0.01$ ). Given the reports discussed above in which very brief periods of positive lens-wear or unobstructed vision outweighed day-long negative lens-wear or form-deprivation, it seems highly unlikely that the short periods of occlusion per se would significantly interfere with the response to the positive lenses. Instead, it may be that the occlusion of the fellow eye during lens-wear, perhaps in combination with the presence of the inner scrim, affected the viewing pattern of the chick and thereby the normal inhibitory mechanism.

Nonetheless, the compensatory hyperopia in these experiments was axial in nature, as all six groups with hyperopic shifts showed significant inhibition of vitreous chamber expansion; this inhibition was principally due to choroidal thickening, except for group 10, which did show an inhibition of ocular elongation. Dissociations between the two mechanisms of compensation have been reported in a few previous studies (Kee, 1998; Park et al., 2003; Winawer & Wallman, 2002). Moreover, such dissociations might be more common than suspected, as many laboratories report axial length as the distance from cornea to retina, thereby confounding changes in choroidal thickness with changes in

length of the whole globe. Further studies are necessary to clarify the differential requirements for choroidal and scleral (eye-length) compensation for lenses.

#### 4.4. Relation to emmetropization

Overall, the results we report here provide further evidence that emmetropization depends on a non-linear integration of defocus. These non-linearities may reflect adaptations to natural viewing conditions in two ways. First, it may be that for eyes near emmetropia, sustained periods of hyperopic defocus are normally much more common than sustained periods of myopic defocus, perhaps especially for young animals if they mostly look at near objects. If so, an integrator that weighed myopic and hyperopic defocus equally would drive an emmetropic eye to myopia. Second, it may be that over very short time scales, an emmetropic eye may have a more balanced quantity of myopic and hyperopic defocus. This might be so if gaze tends to shift from near to far objects (leading to a transient myopic defocus) as often as from far to near objects (leading to transient hyperopic defocus). Thus, an optimal strategy might be to weigh myopic vs. hyperopic defocus more equally during brief episodes than during long episodes. Such an argument is, of course, necessarily speculative as little is known about the pattern of defocus experienced under natural conditions. However, the fact that animals of all species (Smith, 1998), including humans in societies with predominantly outdoor lives (Morgan & Rose, 2005), tend to reduce refractive errors as they develop argues that these non-linearities are well suited to guide emmetropization under natural conditions. Conversely, the results may help explain why the total amount of time a child spends reading (presumably related to the total duration of hyperopic defocus) is not a good predictor of the degree of myopia the child develops (e.g., Mutti, Mitchell, Moeschberger, Jones, & Zadnik, 2002; Saw et al., 2000). If similar non-linearities hold in humans, then factors such as the frequency and duration of short breaks may be just as important as the total time spent reading or doing other nearwork.

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