



Temporal constraints on lens compensation in chicks

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Abstract

If the effective focal length of a growing eye is modified by spectacle lenses, the eye compensates by altering its growth, thereby keeping images in focus, a process we presume is similar to normal emmetropization. Using chicks, we have investigated how much visual exposure the eye needs to exhibit the two principal components of ocular compensation: altered rate of elongation (a scleral mechanism) and altered choroidal thickness. We have found that surprisingly small amounts of vision through spectacle lenses can elicit robust scleral and choroidal compensation if other visual feedback is limited by keeping the animals in the dark when not wearing lenses. Furthermore, we have found that the amount of vision necessary to induce these responses can be summarized as three rules: First, several brief daily episodes are more effective than a single or a few longer daily episodes, even if the total amount of vision is the same. Second, extremely brief episodes, even if very frequent, are relatively ineffective. Third, when plus and minus lenses are worn successively on the same eye, the plus lens has the dominant effect, even if the minus lens is worn five times longer than the plus lens. In addition, we have shown that the elongation rate and choroidal thickness responses are dissociable, such that brief, infrequent lens-viewing produces only an elongation response in the case of plus lens-wear and only a choroid response in the case of minus lens-wear. We thus show that the emmetropization system does not integrate defocus in a simple, linear fashion. These non-linearities, if present in children, might explain why, although education and reading show an epidemiological correlation with myopia, the total time spent reading and doing other nearwork by individual children generally does not predict the degree of myopia. It may therefore be necessary to quantify more complex temporal patterns of nearwork over the day in order to measure the impact of nearwork on eye growth.

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1. Introduction

It is clear from work on animals that the elongation of the eye during early life is under feedback control using visual signals. It has been shown in the chick (Schaeffel, Glasser, & Howland, 1988), tree shrew (Norton & Siegart, 1995), and monkey (Hung, Smith, & Crawford, 1994) that the eyes compensate for artificial alteration of the plane of focus by spectacle lenses, such that the normal growth in the distance from cornea to retina slows in response to positive lenses and speeds in response to minus lenses. As a result, in either case the imposed refractive error disappears over time. In both

chicks and monkeys, wearing spectacle lenses results in accurate compensation for a large range of imposed defocus: in chicks, spectacle lenses between -10 and $+15$ D induced a compensation that eliminated an average of 97% of the refractive error over 1 week (Irving, Sivak, & Callender, 1992), and in macaques, lenses between -2 and $+8$ D caused compensation that eliminated 78% of the induced refractive error over 10–23 weeks (Smith & Hung, 1999). Similar compensatory eye length changes have been found in fish for which the focal plane is altered by altering the wavelength of the ambient light (Kroger, Hirt, & Wagner, 1999; Kroger & Wagner, 1996). The refractive compensation in chicks and primates consists of two components: changes in the rate of elongation of the whole eye, as measured from cornea to sclera, and changes in the thickness of the choroid (Hung, Wallman, & Smith, 2000; Norton & Kang, 1996; Siegart & Norton, 1998; Troilo, Nickla, & Wildsoet, 2000; Wallman et al., 1995; Wildsoet & Wallman, 1995).

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Because the eye cannot elongate without the sclera changing, we consider the changes in the elongation rate to be a scleral response, and, in fact, altered elongation rates are accompanied by changes in growth of the posterior sclera, including protein content, DNA content, and proteoglycan synthesis (Christensen & Wallman, 1991; Marzani & Wallman, 1997; Nickla, Wildsoet, & Wallman, 1997; Rada, Thoft, & Hassell, 1991).

These findings showing that myopia can be caused by the hyperopia imposed by wearing negative lenses have given new strength to the view, dating back at least to Kepler (Duke-Elder & Abrams, 1970), that reading may lead to nearsightedness. According to one version of this view, long periods of nearwork cause hyperopic defocus, for which the eye compensates by becoming myopic. Unlike most experiments with defocusing lenses or diffusers, in which the device is worn continuously, daily life subjects the eye to continuous fluctuations in the magnitude and sign of defocus. This occurs despite ocular accommodation, as there is a tendency to underaccommodate for near objects and overaccommodate for distant ones. Furthermore, most visual scenes will include objects both nearer and farther than the accommodative plane. Thus under normal circumstances the eye experiences periods of hyperopic and myopic defocus. To understand how emmetropization deals with these complexities, we examined spectacle lens compensation to brief, frequent episodes of defocus, and we simulated the alternation of myopic and hyperopic defocus by alternating negative and positive lens-wear. Such experiments may provide knowledge useful in considering prophylactic visual interventions to minimize the progression of myopia in schoolchildren.

Epidemiological studies have repeatedly shown an association between degree of education and prevalence of myopia (for reviews, see Ong & Ciuffreda, 1997; Rosenfield & Gilmartin, 1998), but have been less successful in showing correlations between the amount of time spent reading and the degree of myopic progression in individual children (Saw, Katz, Schein, Chew, & Chan, 1996; Saw et al., 2000, 2001, 2002; Zadnik, 1997). Such studies often assume that the periods of near vision (and therefore of hyperopic defocus or the accommodation that results) are averaged together linearly, as is evident in the use of statistics such as the average daily “diopter hours,” calculated for an individual by multiplying the reciprocal of the distance of various nearwork activities with the amount of time spent in such activities (e.g., Saw et al., 2002; Zadnik, 1997). The assumption that blur is integrated linearly has been explicitly postulated in one mathematical model of the development of refractive errors (Flitcroft, 1998).

Animal studies have suggested that this assumption may be incorrect. Experiments in which defocusing lenses are fitted on animals for only part of the day show

that the amount of compensation is not proportional to the time lenses were worn, especially for negative lenses. For example, a few hours of daily vision without any ocular device eliminates the compensation for minus lenses in chicks (Schmid & Wildsoet, 1996) and tree shrews (Shaikh, Siegart, & Norton, 1999), and eliminates the response to diffusers in chicks (Napper et al., 1995) and monkeys (Smith, Hung, Kee, & Qiao, 2002). On the other hand, much more vision per day without lenses was required to prevent positive lens compensation in chicks (Schmid & Wildsoet, 1996). These studies suggest that the effects of positive and negative lenses differ in time course and that the total hours of daily lens-wear may not be the only important variable determining lens compensation.

The main aim of this paper is to investigate how the emmetropization controller integrates blur signals presented in short episodes. To this end, we conducted several experiments in which chicks wore lenses for brief periods each day. In the first experiment, we compared the responses to brief, infrequent periods of lens-wear with the responses to brief, frequent periods (30 min either twice a day or seven times a day), with the animals kept in the dark between episodes of lens-wear. In three more sets of experiments (Experiments 2–4), we kept the total duration of lens-wear constant within each experiment, but varied the frequency and duration of the episodes. In another set of experiments (Experiment 5), we fitted lenses of opposite signs successively on the same eye for brief periods. Our studies differ from the interrupted lens- or diffuser-wear studies discussed above in two ways: (1) by using multiple, repeated episodes of lens-wear, we could make inferences about the integration of episodes of defocus, and (2) by explicitly imposing blur of opposite signs in the same eye instead of interrupting lens-treatments with unobstructed vision, we could see how signals from opposite signs of blur interact. Some of these results have previously been presented in a preliminary form (Winawer, Wallman, & Kee, 1999).

2. Methods

White Leghorn chicks were acquired from either Truslow Farms (Hyline-W98-strain; Chestertown, MD; Experiments 1, 2, 4, 5) or Cornell University (Cornell K-strain; Ithaca, New York; Experiment 3), either one day after hatching, or as eggs. Upon arrival or hatching, chicks were raised on a 14:10 h light:dark cycle, with food and water ad libitum. On day 6 or 7 post-hatching, refractive error was measured (except in Experiment 1 and the “infrequent” subgroups of Experiment 2) and ultrasound biometry was performed (all experiments). Measurements were made under 1% halothane, without

cycloplegic agents, although the halothane produces mydriasis, and presumably somewhat cycloplegic refractions as muscle fibers of the iris play a prominent role in accommodation in the chick eye (Glasser, Murphy, Troilo, & Howland, 1995). Refractive error was measured with a modified Hartinger refractometer (Wallman & Adams, 1987), and ocular dimensions with A-scan ultrasonography, using separate sound velocities for each ocular component (Nickla, Wildsoet, & Wallman, 1998; Wallman & Adams, 1987). In contrast to the usual clinical practice of measuring axial length from cornea to retina, we calculated the total ocular length as the sum of all components from the front of the cornea to the back of the sclera. It is important to distinguish between the total ocular length (which is not affected by choroid thickness) and vitreous chamber depth (which is) because some experiments showed a change in ocular length but not in choroid thickness, and others showed the reverse.

The measurements at the end of each experiment were always made at the same time of day as the measurements at the start (usually between 10 am and 2 pm) to avoid the confounding effects of the daily rhythms in eye length and in choroid thickness (Nickla et al., 1998). All experiments lasted three days except for those in two of the subgroups in Experiment 3, which lasted four days (see Section 2.3 below). Thus if the birds were measured from 10 am to 12 pm initially, and the lens-wear was 2 min every hour, the first episode was at 12 pm on day 1, and the last was at 9 am on day 3, so that the final measurement could also start at 10 am. In these experiments the treated eyes wore lenses whenever the lights were on. Thus we use the terms “visual episodes” and “periods of lens-wear” interchangeably.

In each experiment, a +6 or –6 D lens, mounted on a Velcro ring, was fitted to one eye, leaving the contralateral eye as a control. Curved PMMA contact lenses as described in Wildsoet and Wallman (1995) were used, except for Experiment 3, which used +6.7 D flat, glass lenses. Lenses were inspected and cleaned twice per day and food and water was replaced at each cleaning. During the experiments, chicks were housed in a sound- and light-proof chamber (61 × 81 cm), under fluorescent lighting. The lights were controlled by an automatic timer and activated by an instant-start ballast to ensure precise control of the timing, which was important for experiments with very short visual episodes. Because many of the experiments involved keeping the chicks in the dark most of the time, the chambers contained large trays of food, and chicks’ crops were checked twice daily to ensure that birds were eating adequately.

Thus the general experimental design was that chicks wore a lens over one eye for brief periods, with the fellow eye serving as a control, and were kept in the dark between periods of lens-wear.

2.1. Experiment 1: Frequent and infrequent episodes, each episode 30 min

Two factors, sign of lens and frequency of lens-wear, were varied to produce four groups: frequent plus, frequent minus, infrequent plus, and infrequent minus (Table 1). “Frequent” lens-wear was 30 min seven times a day, every 2 h starting at 8 am. “Infrequent” lens-wear was 30 min twice a day, at 8 am and 8 pm.

2.2. Experiment 2: Frequent and infrequent episodes, 28 min of daily lens-wear

As a complement to Experiment 1, we kept the amount of lens-wear per day fixed and let the duration of each episode vary (Table 1). The frequent group had 2 min episodes every hour (14 times per day) starting at 8 am, whereas the infrequent group had 7 min episodes every 4 h (four times per day), also starting at 8 am.

2.3. Experiment 3: Frequent episodes versus once per day (plus lenses only)

Because even the infrequent episodes of plus lens-wear in Experiments 1 and 2 produced as much compensatory inhibition of ocular elongation as continuous lens-wear, for plus lenses only we decreased the frequency still further to once per day (28 min) in one group and compared this to a group with the same total amount of vision, but divided into shorter, more frequent episodes (2 min/h, Table 1). Plus 6.7 D glass lenses were worn in both groups.

To address the concern that the results of the once-a-day group depend on the time of day the lenses were worn, we used four patterns of lens-wear. A schematic of the visual episodes is included in Fig. 5. One group had the episode each day at 5 pm. For the other groups, the “average” time of day that lenses were worn was 1 pm, close to the 2:30 pm average of the frequent group (first episode at 8 am, last at 9 pm).

2.4. Experiment 4: Extremely brief episodes, with equal amounts of total vision

To see whether compensation for frequent episodes of lens-wear is constrained by a requirement for a minimum duration of each episode of lens-wear, we compared a range of episodes of lens-wear from 2 s every 2 min to 2 min every 2 h (Table 1). The total amount of vision was 14 min/day (half that of Experiments 2 and 3).

2.5. Experiment 5: Plus and minus lenses worn in succession

Monocular lenses were worn for brief periods, with lenses switched from plus to minus (+6 to –6 D) or

Table 1
Experimental parameters

Lens-wear condition	Episodes per day	Episode duration	Total daily lens-wear	Lens (D)	Number of birds
Continuous	1	14 h	14 h	+7 –6	8 9
<i>Experiment 1: Frequent and infrequent episodes, each episode of equal duration</i>					
Frequent	7	30 min	3.5 h	+6 –6	6 21
Infrequent	2	30 min	1 h	+6 –6	12 16
<i>Experiment 2: Frequent and infrequent episodes, with equal amounts of total lens-wear</i>					
Frequent	14	2 min	28 min	+6 –6	30 28
Infrequent	4	7 min	28 min	+6 –6	12 12
<i>Experiment 3: Frequent episodes versus once per day</i>					
Frequent	14	2 min	28 min	+6.7	14
Infrequent	1	28 min	28 min	+6.7	20
<i>Experiment 4: Extremely brief episodes, with equal amounts of total vision</i>					
Longest episodes	7	2 min	14 min	+6 –6	8 8
	42	20 s	14 min	+6	10
	168	5 s	14 min	+6 –6	10 9
Shortest episodes	420	2 s	14 min	+6 –6	5 4
<i>Experiment 5: Plus and minus lenses worn in succession</i>					
A. Equal plus and minus lens-wear (in cage)	4	15 min 15 min	2 h	+6 –6	12
B. Equal plus and minus (in drum)	4	30 min 30 min	4 h	+6 –6	9
C. Five times more minus (in cage)	4	5 min 25 min	2 h	+6 –6	11
D. Five times more minus (in cage)	1 14	20 min 7 min	1 h 58 min	+6 –6	8

minus to plus midway between each episode (Table 1). The lights were momentarily turned out to switch the lenses, so that there was no unrestricted vision between plus and minus lens-wear.

For one group of birds (Group A), there were four 30 min episodes of lens-wear consisting of equal periods (15 min) of plus and minus lens-wear each day. To assess whether the lens sequence was important, half the birds wore plus lenses followed by minus lenses and half wore minus followed by plus.

A second group of birds (Group B) differed from the first in that they had their lens-wearing episodes while restrained in the center of a 60 cm diameter drum (see Schaeffel & Diether, 1999; Winawer, Zhu, Park, & Wallman, 2000), instead of unrestrained in a large cage. This ensured that the blur experienced with the positive lens was myopic blur because the drum wall was beyond the eye's far point when viewing through the plus lens. In contrast, an unrestrained chick with a plus lens could look at near objects and experience sharp vision or even hyperopic blur. The chicks were rotated in the drum at 30°/s, and the direction of rotation was reversed every 30 s. The purpose of the rotation was to induce the chick to use its optokinetic response to assist its vestibulo-

ocular reflex in stabilizing the visual scene, thereby keeping it awake and looking at the wall of the drum. The walls were lined with color images from magazines to ensure a broad range of spatial frequencies. We periodically checked to see that the chicks were awake by lifting the lid and looking for head stabilization or by listening for chirping. The chicks were generally awake at least three-quarters of the time in the drum.

Two other groups wore minus lenses five times longer than plus lenses (in their cages). Group C had 30 min of lens-wear—25 min of minus and 5 min of plus—four times a day (every 4 h). Again, half of these had the minus first every time and half had the plus first every time. Group D also wore minus lenses five times longer than plus lenses, but wore plus lenses only once per day for 20 min (some at 10:30 am and others at 4:30 pm) and minus lenses for 7 min every hour (14 times). Thus their total lens-wear per day was 20 min of plus and 98 min of minus.

2.6. Data presentation and statistics

The parameters we report are refractive error, total ocular length (cornea to posterior sclera), vitreous

chamber depth and choroid thickness. Because our experimental manipulations inevitably involve differences among groups in the amount and timing of light, as well as of lens-wear, we present our data in terms of the “relative change” in each parameter, that is the change in the lens-wearing eye minus the change in the fellow eye. This reduces the unwanted effects of the different patterns of illumination, which also affect the fellow eye, as well as reducing the effects of individual differences in growth rate, refractive status, etc. among chickens. This manner of data presentation has the ancillary advantage of yielding a regression line that crosses zero simultaneously for change in refractive error and change in vitreous chamber depth, because if the change in vitreous depth in the two eyes is the same (“relative change” of 0), then the change in refractive error will also generally be the same. In contrast, if we consider only the treated eyes, no change in vitreous depth over three days leads to about 1.7 D of hyperopia, presumably because of the normal developmental increases in the eye’s focal length over this period (Fig. 1). Separate changes in treated and fellow eyes are summarized for all experiments in Table 2.

To determine the effect of treatments within groups, paired *t*-tests between treated and untreated eyes were used. For Experiments 1–4, which all involved only a single sign of lens per animal, 1-tailed *t*-tests were used; for Experiment 5, in which animals wore plus and minus lenses successively over the same eye, 2-tailed *t*-tests were used. For comparisons between groups, we compared the relative change by 2-tailed, unpaired *t*-tests for two groups, or, for three or more groups, by analysis of variance with the “condition” as the only factor (see

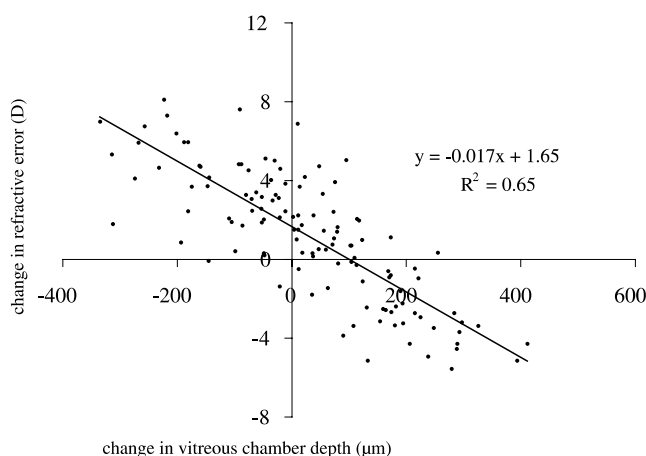


Fig. 1. Changes in refractive error as a function of changes in vitreous chamber depth over three days, pooling all lens-wearing eyes (except Experiment 1 and the infrequent groups from Experiment 2, for which refractive error was not measured). The strong correlation suggests that refractive changes were largely axial (as opposed to corneal or lenticular). The *y*-intercept of 1.65 D indicates that no ocular growth over three days results in hyperopic shift, because as the lens and cornea continue to grow and flatten the optical power decreases.

Table 1), using Bonferroni post hoc tests to determine which comparisons were significant.

3. Results

3.1. Brief episodes of vision produce robust refractive compensation

Very good lens compensation was seen with very brief periods of lens-wear. As an example taken from Experiment 2, 2 min of lens-wear per hour over three days produced at least half as much refractive change as continuous -6 D lens-wear over the same period (Fig. 2; see Section 3.3 for more details). For plus lenses, the treated eyes shifted 3.5 D more than the fellow eyes towards hyperopia ($P < 0.05$), compared to 6.9 D for continuous $+7$ D lens-wear (greater response for continuous lens-wear, $P < 0.05$). A comparable response in the opposite direction was seen with minus lenses: lens-wearing eyes shifted 2.8 D more than the fellow eyes towards myopia ($P < 0.05$), compared to 3.6 D for continuous lens-wear (continuous versus brief periods, $P > 0.05$).

Although there was substantial refractive compensation for brief episodes of vision, the degree of compensation, as well as the relative contribution of changes in elongation rates and changes in choroidal thickness, depended on the distribution of lens-wear throughout the day. Generally, better lens compensation was seen with frequent, brief episodes than with less frequent, longer ones, even if the total amount of vision was equal. If the episodes were too short, however, even if very frequent, there was little or no compensation. Thus, compensation for brief periods of lens-wear was most effective with episodes of a few minutes every hour or two throughout the day. Furthermore, when plus and minus lenses were worn successively over the same eye (for brief periods), the effects did not cancel; rather, the plus lenses had the dominant effect, regardless of which lens was worn first. These issues are explored in Experiments 1–5 below.

To assess whether the refractive changes in these experiments are due to changes in lens-to-retina distance, we plotted the change in refractive error against the changes in vitreous chamber depth across experiments (Fig. 1). We found these variables to be well-correlated, with a slope consonant with the expected refractive effect of the change in length, implying that the refractive changes we study are attributable to these axial variables, and not to changes in lens or corneal power.

3.2. Experiment 1: Frequent and infrequent episodes, each episode 30 min

Brief, frequent episodes of monocular $+6$ or -6 D lens-wear (30 min, seven times per day) caused greater ocular changes than infrequent lens-wear episodes

Table 2
Results for all experiments for lens-wearing and fellow eyes

Daily lens-wear	Refractive error (D)		Ocular length (μm)		Vitreous depth (μm)		Choroid thickness (μm)	
	Treated	Fellow	Treated	Fellow	Treated	Fellow	Treated	Fellow
<i>Continuous</i>								
14 h, 1 \times (+7 D)	5.6 \pm 0.8	-1.3 \pm 0.9	-13 \pm 44	143 \pm 42	-159 \pm 48	51 \pm 34	107 \pm 25	-5 \pm 24
14 h, 1 \times (-6 D)	-3.3 \pm 0.7	0.3 \pm 0.6	290 \pm 53	179 \pm 27	273 \pm 24	58 \pm 27	-75 \pm 35	-7 \pm 21
<i>Experiment 1</i>								
30 min, 7 \times (+6 D)	NM	NM	10 \pm 40	242 \pm 43	-192 \pm 23	63 \pm 32	81 \pm 15	10 \pm 24
30 min, 7 \times (-6 D)	NM	NM	347 \pm 36	258 \pm 17	263 \pm 29	163 \pm 19	-57 \pm 11	-41 \pm 10
30 min, 2 \times (+6 D)	NM	NM	127 \pm 32	308 \pm 24	23 \pm 28	190 \pm 28	-13 \pm 14	-4 \pm 14
30 min, 2 \times (-6 D)	NM	NM	220 \pm 28	240 \pm 22	165 \pm 22	140 \pm 18	-56 \pm 15	-22 \pm 14
<i>Experiment 2</i>								
2 min, 14 \times (+6 D)	3.8 \pm 1.1	0.4 \pm 0.2	90 \pm 12	208 \pm 21	4 \pm 16	175 \pm 15	-7 \pm 18	-92 \pm 15
2 min, 14 \times (-6 D)	-3.8 \pm 0.9	-1.0 \pm 0.3	312 \pm 16	211 \pm 20	278 \pm 14	184 \pm 18	-99 \pm 16	-82 \pm 15
7 min, 4 \times (+6 D)	NM	NM	63 \pm 22	232 \pm 29	-10 \pm 23	179 \pm 22	-14 \pm 16	-39 \pm 13
7 min, 4 \times (-6 D)	NM	NM	206 \pm 23	191 \pm 19	225 \pm 20	193 \pm 18	-106 \pm 20	-90 \pm 19
<i>Experiment 3</i>								
2 min, 14 \times (+6.7 D)	2.4 \pm 0.7	-1.5 \pm 0.5	82 \pm 21	301 \pm 26	-11 \pm 25	219 \pm 19	-13 \pm 19	-33 \pm 14
28 min, 1 \times (+6.7 D)	1.2 \pm 0.5	-0.9 \pm 0.4	137 \pm 17	276 \pm 21	49 \pm 22	199 \pm 16	-18 \pm 13	-37 \pm 9
<i>Experiment 4</i>								
2 min, 7 \times (+6 D)	4.0 \pm 0.7	0.3 \pm 0.4	115 \pm 52	281 \pm 36	10 \pm 41	202 \pm 16	-27 \pm 8	-18 \pm 17
2 min, 7 \times (-6 D)	-3.8 \pm 0.3	0.3 \pm 0.7	389 \pm 24	294 \pm 32	280 \pm 29	178 \pm 33	-34 \pm 14	1 \pm 12
20 s, 42 \times (+6 D)	0.2 \pm 0.8	0.1 \pm 0.6	71 \pm 46	136 \pm 22	7 \pm 44	106 \pm 22	-50 \pm 21	-86 \pm 31
5 s, 168 \times (+6 D)	1.0 \pm 0.8	0 \pm 0.4	41 \pm 23	82 \pm 12	42 \pm 30	125 \pm 29	-101 \pm 19	-150 \pm 20
5 s, 168 \times (-6 D)	-2.5 \pm 0.6	-0.8 \pm 0.5	112 \pm 34	99 \pm 39	191 \pm 30	155 \pm 29	-175 \pm 23	-142 \pm 24
2 s, 420 \times (+6 D)	-0.9 \pm 1.2	-0.8 \pm 0.6	224 \pm 43	214 \pm 18	118 \pm 22	100 \pm 18	-43 \pm 15	-21 \pm 20
2 s, 420 \times (-6 D)	-2.7 \pm 1.1	-1.1 \pm 1	217 \pm 26	168 \pm 42	244 \pm 21	134 \pm 18	-158 \pm 33	-92 \pm 48
<i>Experiment 5</i>								
A. 15 min, 4 \times (+6 D)	3.6 \pm 1.0	-0.5 \pm 0.8	11 \pm 17	316 \pm 28	-108 \pm 36	168 \pm 38	14 \pm 29	3 \pm 17
15 min, 4 \times (-6 D)	3.8 \pm 0.9	-1.0 \pm 0.6	-1 \pm 32	268 \pm 28	-165 \pm 32	125 \pm 22	32 \pm 20	0 \pm 11
B. 30 min, 4 \times (+6 D)	5.3 \pm 0.5	-0.1 \pm 0.7	88 \pm 34	273 \pm 30	-244 \pm 17	123 \pm 35	153 \pm 10	-18 \pm 10
30 min, 4 \times (-6 D)	4.1 \pm 1.4	-1.0 \pm 0.5	156 \pm 70	299 \pm 51	-211 \pm 69	130 \pm 49	193 \pm 40	18 \pm 16
C. 5 min, 4 \times (+6 D)	0.8 \pm 1.1	0.1 \pm 0.6	148 \pm 34	293 \pm 59	16 \pm 30	171 \pm 22	6 \pm 24	-9 \pm 37
25 min, 4 \times (-6 D)	2.6 \pm 1.2	-0.5 \pm 0.3	205 \pm 43	335 \pm 51	44 \pm 21	230 \pm 29	39 \pm 19	-23 \pm 19
D. 20 min 1 \times (+6 D)	NM	NM	176 \pm 43	169 \pm 41	148 \pm 19	168 \pm 30	-62 \pm 28	-76 \pm 30
7 min, 14 \times (-6 D)								

Expressed as mean changes \pm 1 standard error. NM: not measured.

(30 min, two times per day). With plus lenses in the frequent condition, the changes were about the same as with continuous lens-wear (Fig. 3A and B). For minus lenses in the frequent condition, the changes in ocular length and choroid thickness (relative to the changes in fellow eyes) were about half as much as with continuous lens-wear.

3.2.1. Minus lenses

Frequent episodes of lens-wear produced increased ocular elongation, with 90 μm (35%) more elongation in

the lens-wearing eyes than the fellow eyes ($P < 0.05$, Fig. 3A). In 14 out of 21 individuals the lens-wearing eye elongated more than the untreated eye. Infrequent episodes, on the other hand, had no effect on ocular elongation (7 of 16 treated eyes grew more than the fellow eyes). There was thus a greater increase in elongation in the frequent than in the infrequent group, relative to fellow eyes ($P < 0.05$, unpaired t -test). In contrast, there was no difference between the amount of choroidal thinning relative to the fellow eyes in the frequent versus infrequent groups ($P > 0.05$, unpaired t -test). The

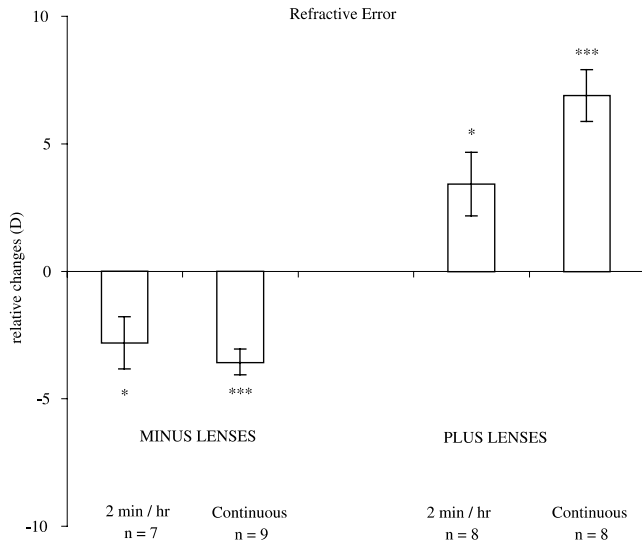


Fig. 2. Refractive compensation for brief episodes of lens-wear and continuous lens-wear over three days. Bars show relative changes (change in treated eyes minus change in fellow eyes) in refraction over three days ± 1 standard error of the mean. Episodes of 2 min/h produced substantial refractive compensation in the appropriate direction for both plus and minus lenses. The data plotted are taken from the frequent condition of Experiment 2, for which ocular elongation and changes in choroid thickness are also plotted in Fig. 4. One asterisk indicates $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

choroid thinned in response to both frequent ($P < 0.05$) and infrequent ($P < 0.01$) lens-wear (Fig. 3B). In 13 out of 21 birds (frequent) and 13 out of 16 (infrequent) the choroids thinned more in the lens-wearing eyes than in the fellow eyes.

3.2.2. Plus lenses

Plus lenses showed the opposite pattern: both the frequent and infrequent groups had robust compensatory inhibition of ocular elongation, but only the frequent group showed a choroidal response (Fig. 3A and B). The inhibition of elongation was dramatic in both groups (frequent, 232 μm less growth than fellow eyes; infrequent, about 181 μm less growth than fellow eyes). There was no significant difference in the amount of inhibition between groups (unpaired t -test, $P > 0.05$). Thus only two periods of 30 min each per day were almost as effective in inhibiting ocular elongation, as were seven periods per day of the same duration. In every animal in both the frequent and infrequent groups, the elongation in the lens-wearing eye was less than in the fellow eye. In contrast, the frequency of lens-wear did have an effect on the choroidal response: Infrequent lens-wear did not cause the choroids to thicken ($P > 0.05$), whereas the frequent lens-wear induced the choroids to thicken by an average of almost 100 μm ($P < 0.01$; difference between groups: $P < 0.01$, unpaired t -test).

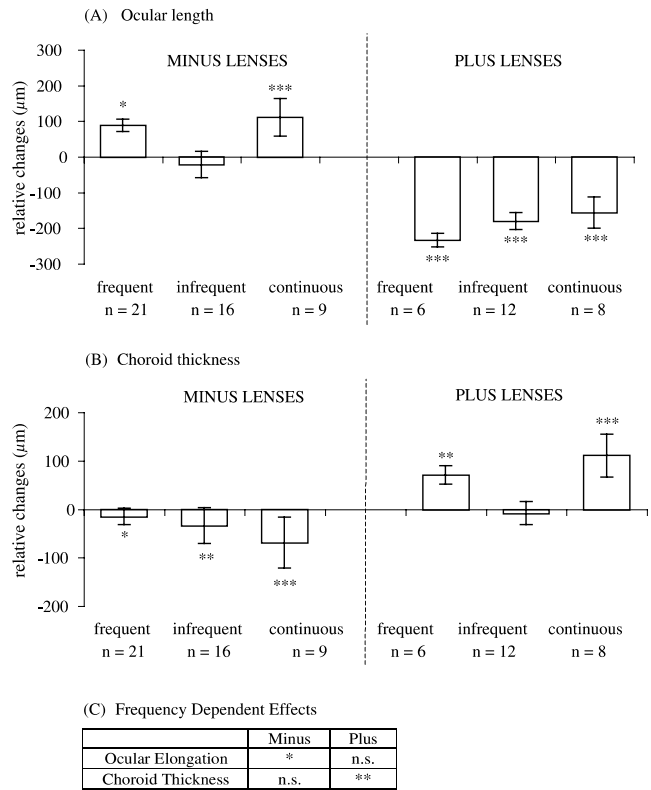


Fig. 3. Effects of different frequencies of +6 or -6 D lens-wear, each episode lasting 30 min. (A) Relative changes ± 1 SEM in ocular length over three days (change in treated eyes minus change in fellow eyes). Control experiments with continuous plus or minus lens-wear are shown for comparison. For minus lenses, the treated eyes elongated more than their fellow eyes only if the lens-wear was frequent. For plus lenses, the treated eyes elongated less than the fellow eyes whether the lens-wear was frequent or infrequent. (B) Relative changes in choroid thickness over three days. The pattern of choroidal compensation was reversed from the pattern of compensatory changes in elongation: with minus lenses, the choroids thinned more in the treated eyes whether the lens-wear was frequent or infrequent, but for plus lenses, the choroids expanded only with frequent lens-wear. Thus, the infrequent conditions caused a reduced choroid response for plus lenses, and a reduced elongation response for minus lenses. (C) A summary of significant differences based on frequent versus infrequent lens-wear. The increase in ocular elongation in response to minus lens-wear was significantly greater for the frequent than for the infrequent condition (greater change relative to fellow eyes), whereas the inhibition of ocular elongation in response to positive lens-wear did not differ between the frequent and infrequent groups. The reverse was true for the choroidal response: a frequency-dependant difference was seen for plus lens-wear but not for minus lens-wear. One asterisk indicates $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

3.2.3. Summary of Experiment 1

In summary, the frequency of lens-wear had a strong effect, though the effect was not limited to a particular sign of lens or a particular tissue. Instead, the choroid only thickened with frequent plus-lens-wear, although it thinned with any negative lens-wear; and the ocular elongation was accelerated only by frequent negative lens-wear, although it was decelerated by any positive lens-wear (Fig. 3C).

3.3. Experiment 2: Frequent and infrequent episodes, 28 min of daily lens-wear

In the results just presented the greater compensation in the frequent than in the infrequent lens-wear could have been due to either the greater frequency or the greater total duration of lens-wear in that group. In Experiment 2, in which only the frequency differed, we found the same general pattern of results as in Experiment 1 in that the more frequent episodes supported more robust compensation.

3.3.1. Minus lenses

Only the more frequent episodes produced a significant increase in the rate of ocular elongation (Fig. 4A), even though both groups had the same total duration of lens-wear. There was thus a significantly greater increase in ocular elongation in the frequent than in the infrequent group ($P < 0.01$, unpaired t -test; Fig. 4A). In the frequent group, the lens-wearing eyes grew an average of 101 μm more than the contralateral eyes ($P < 0.001$, Fig. 4A), with 26 of 28 individuals showing more growth in the lens-wearing eye. In the infrequent group, the two eyes did not differ ($P > 0.05$), with as many lens-wearing eyes growing faster (6 of 12) as slower compared to the fellow eye. Thus 28 min of lens-wear per day in 14 episodes of 2 min each was effective in inducing increased ocular elongation for minus lenses, but 28 min/day in 4 episodes of 7 min was ineffective.

Unlike the elongation response, compensatory choroidal thinning was produced to the same degree by the two timing patterns (Fig. 4B, $P > 0.05$, unpaired t -test). The degree of thinning relative to the fellow eyes was small, with a mean of 17 μm more thinning in the treated than the fellow eyes for the two groups ($P < 0.05$ for the two groups combined). This small difference between lens-wearing and fellow eyes may be due to a saturation effect, as even in the fellow eyes, the choroids thinned by about 30% of their initial thickness, presumably due to the darkness.

3.3.2. Plus lenses

As in Experiment 1, both the frequent and infrequent conditions induced significant compensatory inhibition of ocular elongation, with no significant difference between groups ($P > 0.05$, unpaired t -test; Fig. 4A). For each group, lens-wearing eyes elongated less than half as rapidly as the fellow eyes (both groups, $P < 0.001$). The distribution of changes was similar in the two groups, with 26 of 30 showing slowed growth in the frequent group and 12 of 12 in the infrequent group. Choroidal thickening, however, as in Experiment 1, occurred in the frequent lens-wear, but not in the infrequent (difference between groups: $P < 0.05$, unpaired t -test; Fig. 4B). Thus the difference in the choroid response seen in the first experiment, in which the two groups differed in

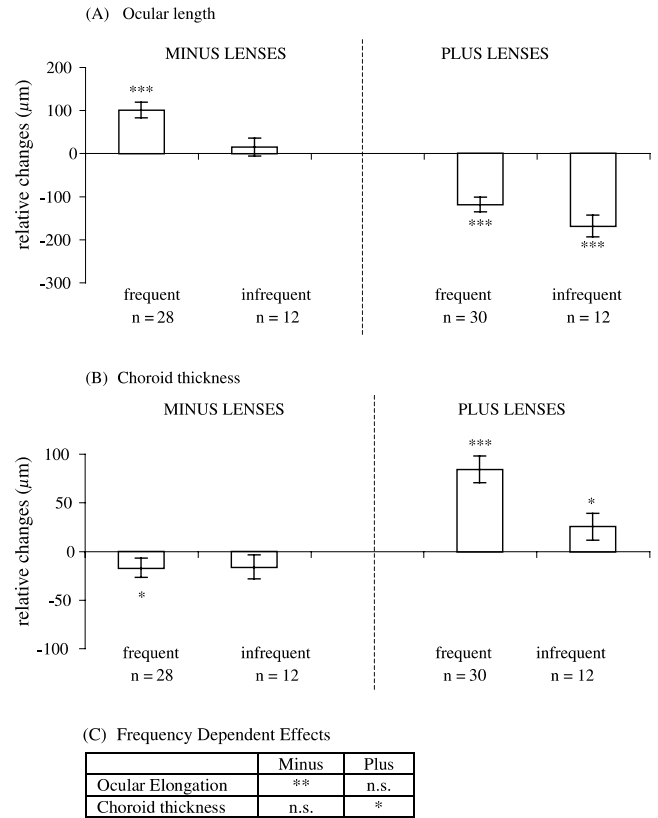


Fig. 4. Effect of different frequencies of lens-wear, with 28 min total per day. (A) Relative changes in ocular length over three days (change in treated minus change in fellow eye). As in Fig. 3, with minus lenses the treated eyes elongated more than their fellow eyes only if the lens-wear was frequent, and with plus lenses the treated eyes elongated less than the fellow eyes whether the lens-wear was frequent or infrequent. (B) Relative changes in choroid thickness over three days. The pattern was reversed for the choroid (as in Fig. 3): with minus lenses, the choroids thinned more in the treated eyes whether the lens-wear was frequent or infrequent, but for plus lenses, the choroids expanded only with frequent lens-wear. (For minus lenses, the thinning relative to fellow eyes was significant only when the two minus lens groups were pooled.) (C) A summary of significant differences based on frequent versus infrequent lens-wear. As in Fig. 3, the compensatory responses that showed significant frequency-related differences are ocular elongation in the case of minus lenses and choroidal thickness in the case of plus lenses. One asterisk indicates $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

both frequency of lens-wear and total amount of lens-wear, was also seen when only the frequency differed. In this experiment, however, the choroidal “thickening” in the frequent group was only relative to the fellow eyes, in that the choroids in the lens-wearing eyes were unchanged while the choroids in the fellow eyes thinned, probably because of the extensive darkness (Table 2).

3.3.3. Summary of Experiment 2

Similar to Experiment 1, we found that the frequency of lens-wear had selective effects on ocular responses to lens-wear: Infrequent compared to frequent lens-wear

caused a significantly smaller change in elongation in the case of minus lenses, but had no significant effect on the amount of ocular elongation in the case of plus lenses (Fig. 4C). Also, as with Experiment 1, infrequent compared to frequent lens-wear caused a weaker choroidal response to plus lenses, but not to minus lenses (Fig. 4C; cf. Fig. 3C).

3.4. Experiment 3: Frequent episodes versus once per day (plus lenses only)

When we reduced the number of episodes in the infrequent group to once per day (28 min), and the frequent group had plus lenses for 2 min every hour (28 min total), we found that the group with more frequent lens-wear showed about 60% more inhibition of ocular elongation than the once-per-day-group (Fig. 5A, $P < 0.01$). For the birds with one episode per day, the time of day did not seem to have an effect, as all subgroups (see Fig. 5C for subgroups) had about the same amount of inhibition of ocular elongation, regardless of the time of the episodes. The greater inhibition of ocular elongation in the group with repeated episodes was reflected in a greater shift towards hyperopic refractions (Fig. 5B). Thus, though Experiments 1 and 2 showed that the inhibition of ocular elongation in response to plus lens-wear is about the same in frequent and infrequent conditions, these results show that if the episodes are infrequent enough (once a day), the response does diminish, even though the total amount of lens-wear is the same in all groups.

Surprisingly, neither the once-a-day group nor the frequent group showed a choroid response (data not shown). This may be because in this experiment, unlike all the other experiments, the final measurement for most birds took place the day after the last visual episode; there was thus a delay of 2–26 h between the last visual episode and the last measurement (mean = 12 h) during which birds were kept in the dark. For both the frequent and the once-a-day condition, the second subgroup, which had the final measurement 2 h after the last visual episode less, showed a small choroid response (data not shown).

3.5. Experiment 4: Extremely brief episodes, with equal amounts of total vision

In the experiments described above, the frequency of the episodes of lens-wear was more important than their duration. With even briefer episodes, however, duration was more important than frequency. Robust compensation in these experiments only occurred in the groups with the longest episodes of lens-wear, 2 min every 2 h.

3.5.1. Plus lenses

When plus lenses were worn for very brief episodes, there was a complex pattern of effects on the various ocular components. For the group with the longest episodes (also the fewest episodes), 2 min every 2 h, there was a significant hyperopic refractive shift ($P < 0.01$; Fig. 6A), inhibition of vitreous chamber expansion ($P < 0.001$; Fig. 6B), inhibition of ocular elongation

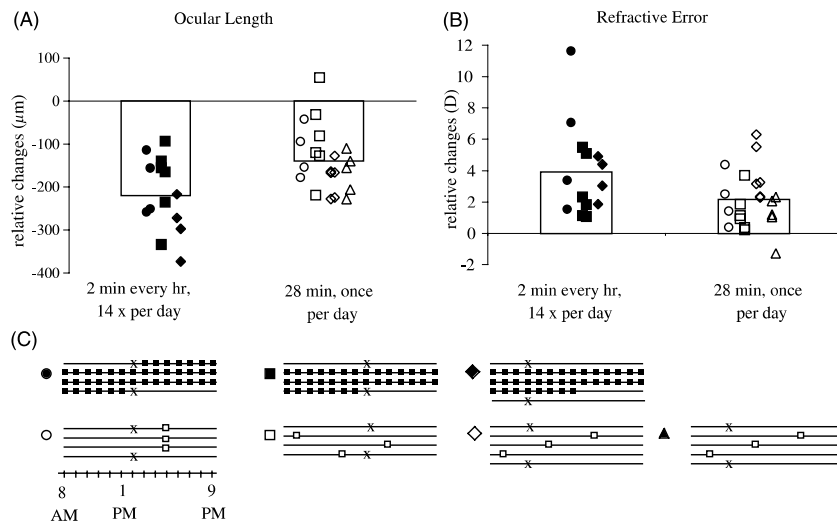


Fig. 5. Relative change in (A) ocular length and (B) refractive error over three or four days (change in lens-wearing eye minus change in fellow eye) for brief periods of plus lens-wear, either 2 min every hour (totaling 28 min/day, filled symbols), or in one daily episode of 28 min (unfilled symbols). The bars show the means across subgroups for the frequent condition and once-per-day condition. (C) Lens-wearing schedules for the various subgroups. Each line represents one day. Ocular measurements ("X"s) and lens-wearing episodes (filled squares for 2 min episodes, top row; unfilled squares for 28 min episodes, bottom row) are indicated at the appropriate times. Note that for the three rightmost subgroups, measurements were made four days apart instead of three. There was a significantly greater inhibition of ocular elongation in the frequent than the once-a-day group ($P < 0.01$) and a greater shift toward hyperopia ($P < 0.05$).

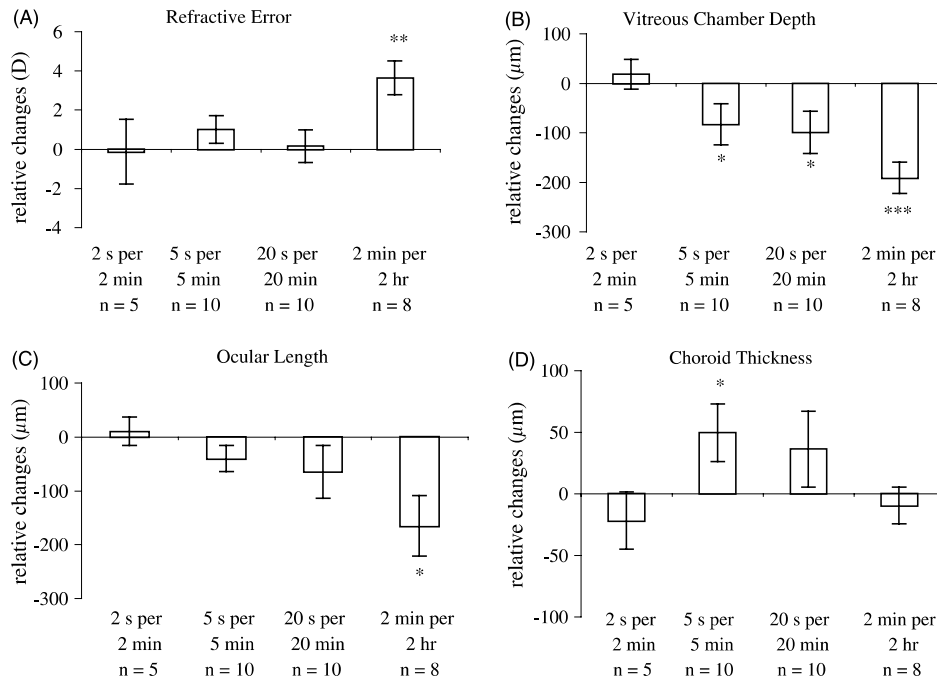


Fig. 6. Extremely brief periods of vision with plus lenses, totaling 14 min/day. Plots show relative changes over three days in experimental and fellow eyes in terms of (A) refractive error, (B) vitreous depth, (C) ocular length, and (D) choroid thickness. Bars show mean relative changes. The only group showing consistent changes was the group with the longest episodes, 2 min every 2 h, for which there was significant hyperopia and inhibition of ocular elongation and vitreous expansion. One asterisk indicates $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

($P < 0.05$; Fig. 6C), but no significant choroidal response (Fig. 6D). Among the other plus lens groups, the next two longest duration (least frequent) groups, 20 s every 20 min, and 5 s every 5 min, also showed a slowing of vitreous chamber expansion (relative to fellow eyes, $P < 0.05$, both groups; Fig. 6B), but without a significant reduction in ocular elongation or a significant shift towards hyperopia. Thus in contrast to Experiment 2, which showed that more frequent plus lens-wear tended to produce greater choroidal thickening, but a similar slowing of growth, compared to less frequent lens-wear, here we see a stronger slowing of growth in the less frequent (but longer duration) groups, and little to no choroid responses in any groups (perhaps because the total amount of vision per day was not enough). ANOVAs showed that there were significant differences in responses among groups for both refractive error (2 min/2 h was different from 20 s/20 min, Bonferroni post hoc test, $P < 0.05$) and vitreous chamber depth (2 min/2 h was different from 2 s/2 min, Bonferroni post hoc test, $P < 0.05$). Thus these results imply that episodes of plus lens-wear that are a few seconds each do not support emmetropization as well as episodes that are a few minutes each, even when the total lens-wear is the same.

3.5.2. Minus lenses

In the minus lens groups, only the longest duration episodes of lens-wear (2 min every 2 h) caused the typ-

ical pattern of minus lens-compensation (Fig. 7): increased rate of vitreous chamber expansion ($P < 0.01$) and ocular elongation ($P < 0.01$), thinning of the choroid ($P < 0.01$), and negative refractive errors ($P < 0.01$). Among the two other groups with very short episodes of lens-wear (2 s/2 min and 5 s/5 min), the only significant changes were an elongation of the vitreous chamber depth and a shift towards myopia in the 2 s/2 min group ($P < 0.05$, both measures). Thus, as with plus lenses, the longest duration/least frequent group seemed to have the strongest response. However, despite these trends, ANOVAs did not show any significant differences among groups for any variable among the minus lens groups (ocular length, vitreous depth, choroid thickness, and refractive error).

3.5.3. Summary of Experiment 4

The implication of these findings is that it is not sufficient to have frequent lens-wear to get full responses; each episode of lens-wear must also be at least several minutes in duration. With shorter duration, frequent lens-wear we observed a response attenuation as we had observed with long duration, infrequent lens-wear. The fact that there were significant differences among the plus lens groups but not the minus may be due to the fact that the groups with minus lenses were slightly smaller than those with plus, and there was one fewer group with minus lenses.

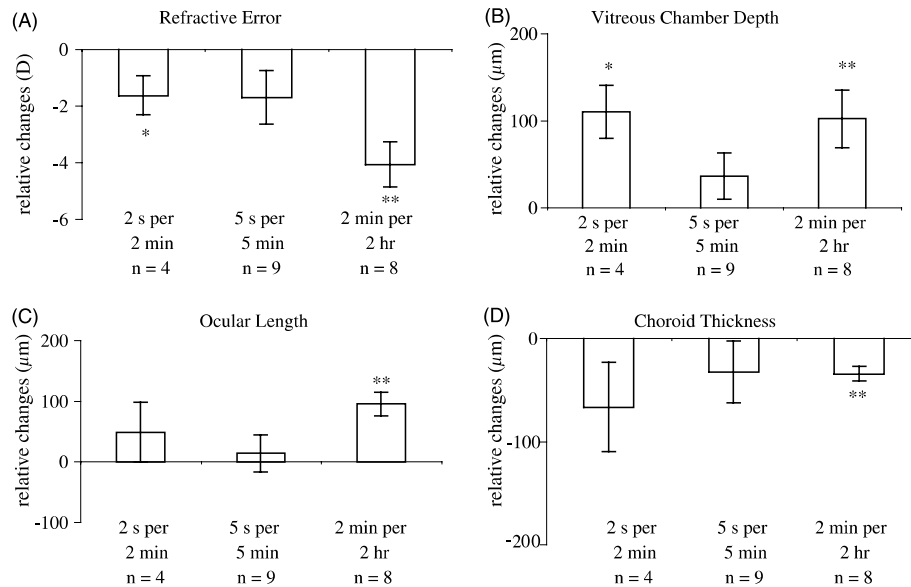


Fig. 7. Extremely brief periods of vision with minus lenses, totaling 14 min/day. Plots show relative changes in experimental and fellow eyes in terms of (A) refractive error, (B) vitreous depth, (C) ocular length, and (D) choroid thickness. Bars show mean relative changes. As with plus lenses, the only group showing consistent changes was 2 min every 2 h, for which there was significant ocular length and vitreous depth elongation, choroidal thinning, and myopia. ANOVAs, however, comparing differences between the changes in paired eyes, did not show any significant differences between the groups in terms of any of the components. One asterisk indicates $P < 0.05$; two asterisks, $P < 0.01$; three asterisks, $P < 0.001$.

3.6. Experiment 5: Plus and minus lenses worn in succession

When plus and minus lenses were worn successively on the same eye for brief periods, the response of the eye was dominated by the plus lens in most conditions. This was surprising because when a lens of only one sign, either +6 or -6 D, was worn for 2 min every hour (Fig. 4) we found responses of roughly comparable magnitude (but in opposite directions) to the plus alone and the minus alone in terms of ocular elongation (absolute value of change relative to fellow eyes not significantly different, $P > 0.05$). To our surprise, it made no difference whether the plus or minus lens was worn first (Fig. 8). Therefore, for statistical purposes, we pooled these subgroups.

3.6.1. Equal amount of plus and minus lens-wear

In the present experiment, Group A had equal episodes of +6 and -6 D lens-wear four times per day. In 11 of 12 birds in this group, refractions shifted toward hyperopia (Fig. 8A, $P < 0.001$). In all 12 birds, the vitreous chamber (Fig. 8B) and the ocular length (Fig. 8C) shortened relative to the fellow eyes ($P < 0.001$, both measures), and 8 of 12 choroids thickened relative to the fellow eyes, though this was not significant (Fig. 8D). Thus, though plus and minus lenses were worn for equal durations, the eyes responded as if they had only worn plus lenses. This is true whether the plus lenses were worn first or second during each episode (Fig. 8A–D; MANOVA, no effect of the order of lens-wear on any ocular parameter).

In Group B, the animals were restrained in the center of a 60 cm diameter drum (see Section 2.5) to ensure that the plus lenses imposed myopic blur, as unrestrained birds looking at close objects can have sharp vision or even hyperopic blur with plus lenses. The visual episodes were 60 min, four times per day (30 min plus lenses and 30 min minus lenses). The results were similar to those of the unrestrained group, in that the plus lenses had the dominant effect regardless of the order of lens-wear: compared to the fellow eyes, the refractions shifted towards hyperopia (9 of 9, Fig. 8A, $P < 0.001$), the vitreous chambers got shorter (9 of 9, Fig. 8B, $P < 0.001$), the ocular elongation slowed (8 of 9, Fig. 8C, $P < 0.001$), and the choroids thickened (9 of 9, Fig. 8D, $P < 0.001$). Again, the order of lens-wear had no effect on any parameter (MANOVA, $P > 0.05$). There were some differences between the drum group and the unrestrained group: the drum group showed more choroidal thickening than the unrestrained group ($P < 0.001$, unpaired t -test), perhaps because the visual episodes were longer (1 h compared to 30 min), and showed less inhibition of ocular elongation ($P < 0.01$), perhaps because of the greater choroid thickening. Because both a slowed rate of ocular elongation and a thickened choroid shorten the vitreous chamber, the changes in vitreous depth and in refractive error were about the same in both groups.

3.6.2. Five times more minus than plus lens-wear

Even Group C, with 25 min of minus and 5 min of plus lens-wear every 4 h, responded predominantly to the

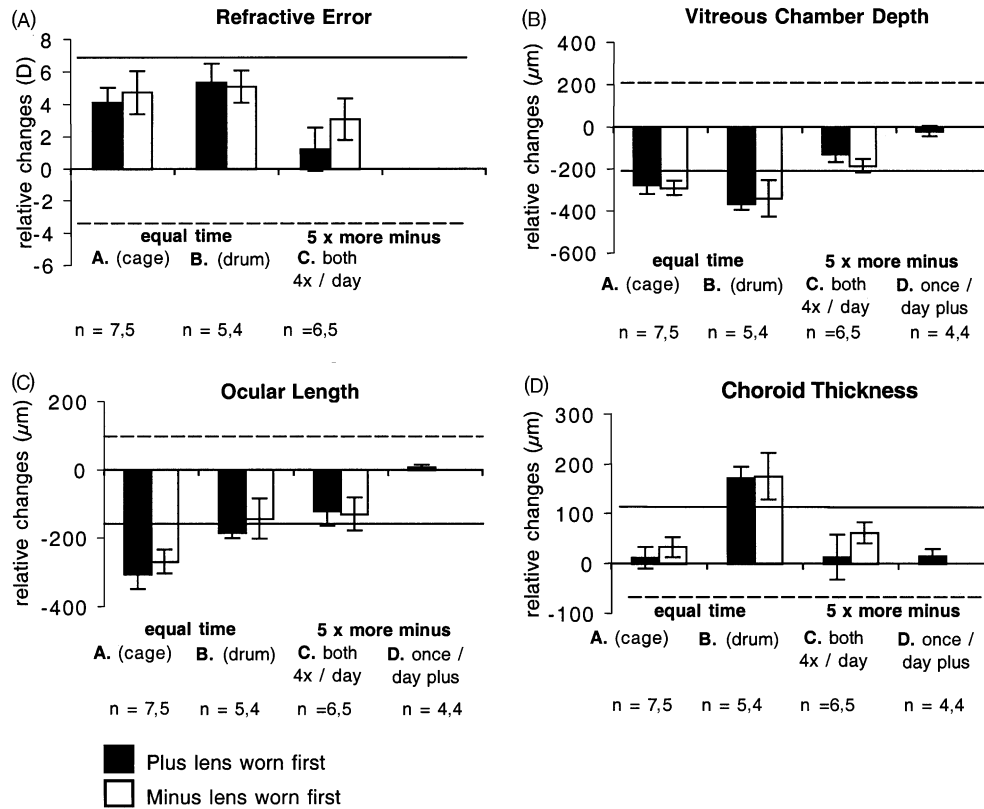


Fig. 8. Plus and minus lenses worn in succession. Each plot shows relative changes for each group, with groups A–C subdivided into those with plus lenses (filled bars) or minus lenses (unfilled bars) worn first in each episode. Additionally, changes are shown in each plot for birds with continuous positive (solid line) or negative (dashed line) lens-wear over the same period. Group A had 15 min each of plus and minus lens-wear every 4 h. Group B had 30 min each of plus and minus lens-wear every 4 h, while restrained in the center of a 60 cm diameter drum. Group C had 5 min of plus and 25 min of minus lens-wear every 4 h, and Group D had 20 min of plus lens-wear once per day, and 7 min of minus every hour. In both of the groups with equal durations of plus and minus lenses, the refractions generally went toward hyperopia (A), the vitreous expansion was slowed (B), as was the ocular elongation (C), and the choroids tended to thicken (D), especially in the group that had lens-wear in the drum. Thus the trend in these two groups was towards the direction of plus lens-wear alone. For eyes with five times more minus than plus lens-wear, the plus lenses still had the dominant effect, with the refractions becoming hyperopic (A), the vitreous depths (B) and ocular lengths (C) decreasing, and the choroids thickening (D), all relative to fellow eyes. Only with the combination of the most frequent minus lens-wear and the least frequent plus lens-wear did the plus and minus lens effects cancel each other.

plus lenses, with a significant inhibition of vitreous expansion ($P < 0.001$) and ocular elongation ($P < 0.001$), as well as significant refractive change towards hyperopia ($P < 0.05$), again, with the order of lens-wear having no effect on these parameters (MANOVA, $P > 0.05$).

Only Group D, which not only had five times more total minus than plus lens-wear, but also had the minus lens-wear 14 times more frequently than the plus, did the lenses tend to cancel each other's effects. For this group, no component was significantly different from that of the fellow eyes. Unpaired *t*-tests of the relative changes (change in treated minus change in untreated) showed Group D to have less inhibition of vitreous expansion ($P < 0.001$) and ocular elongation ($P < 0.05$) than Group C, which had 5 min of plus and 25 min of minus lens-wear four times per day. Neither Group C nor Group D showed significant choroid changes, though the trend in Group C was toward thicker choroids. (Refractive errors were not measured for Group D.)

Thus with five times more minus than plus lens-wear, the plus lens had the dominant effect in Group C but not in Group D.

3.6.3. Summary of Experiment 5

With equal amounts of periodic plus and minus lens-wear (Groups A and B), or five times more minus than plus lens-wear (Group C), the responses were dominated by the plus lens in terms of refractive error, vitreous depth, ocular elongation, and choroidal thickness. Only with the combination of five times more minus lens-wear and infrequent plus lens-wear (Group D), did we see the effects of plus and minus lens-wear cancel. In none of the groups did it make any difference whether the plus lens was worn first or the minus was worn first (this possibility does not apply to Group D, with plus lens-wear once a day, and minus lens-wear every hour). A multivariate analysis of variance showed that the order of lens-wear (plus first or minus first) had no effect on

vitreous chamber depth, ocular elongation, choroid thickness, or refractive error (in all cases, $P > 0.05$).

4. Discussion

By fitting defocusing lenses on chicks, we have found that the visual feedback system regulating eye growth is exquisitely sensitive to brief periods of defocus. Even minutes of daily lens-wear can produce quite robust compensatory eye growth in either the myopic or hyperopic direction. For example, 2 min/2 h produced about two-thirds of full compensation with either plus or minus lenses (4 D of change with ± 6 D lenses; Figs. 6 and 7). Furthermore, we have found that the amount of compensational myopia is not predicted by the total amount of negative lens-wear, nor is the amount of compensational hyperopia predicted by the amount of positive lens-wear. That is to say, there is no evidence of a linear summation of the effect of periods of defocus that the chicks are subjected to. Rather, we find three strong non-linearities: First, numerous very brief episodes are more effective than fewer brief episodes, or a single daily episode of the same total amount of time. This implies that the strength of the effect of wearing lenses either declines during each episode or declines between episodes of lens-wear, even in darkness. Second, if the episodes are too brief (< 2 min or so) they are ineffective, even if repeated very often. This implies that the start of each episode of lens-wear is less effective than later times during the episode. Third, the effect of alternating plus and minus lenses, presumably imposing myopic and hyperopic blur, strongly favors the plus lens, an asymmetry which cannot be predicted from the effects of wearing either lens alone.

Furthermore, we have found two other unexpected effects. First, the two predominant components of refractive change, change in rates of ocular elongation and in choroid thickness, do not always occur in tandem: If the episodes of lens-wear are infrequent and brief, they have little effect on the ocular elongation if negative lenses are worn, and have little effect on the choroid thickness if positive lenses are worn. Second, when plus and minus lenses are worn in succession for short periods, it makes no difference which lens is worn first; that is, wearing one sign of lens does not cancel the effect of the immediately preceding lens.

4.1. Small amounts of spectacle lens-wear can have large effects on ocular development

Previous studies have shown that the chick eye accurately compensates for a range of defocus when lenses are worn continuously (e.g., Irving et al., 1992). We found that even 2 min of plus or minus lens-wear every hour produced significant compensatory changes both

in the rate of ocular elongation and in choroid thickness (Experiment 2). The magnitude of these changes is comparable to what we find for continuous lens-wear over the same number of days (Fig. 3), even though the total duration of lens-wear in our experiment was 30 times less than that of the continuous lens-wear experiments (28 min versus 14 h daily).

These results can be compared with two previous reports of intermittent minus lens-wear. Removing minus lenses for 1 h/day out of a 12 h daily light period in tree shrews (Shaikh et al., 1999) or chicks (Schmid & Wildsoet, 1996) eliminated more than half of the response to the lenses, and 3 h of vision without lenses eliminated the response completely. Thus, wearing minus lenses for 9 h with 3 h of vision without lenses produced no lens compensation, but in our study, only 28 min/day of lens-wear (Experiment 2, “frequent” group) produced a robust response when (1) the lens-wear was divided into frequent episodes, and (2) the animals were kept in the dark between episodes. Thus, the weak response to minus lenses reported by Shaikh et al. (1999) and Schmid and Wildsoet (1996) must be due to the visual consequences of viewing without lenses, rather than because compensation for minus lenses requires long periods of hyperopic blur each day.

With plus lenses too, we found that short periods of lens-wear produced a strong response. The one previous report that looked at intermittent plus lens-wear found that plus lens compensation was much more resistant than minus lens compensation to periods of unrestricted viewing. Schmid and Wildsoet (1996) found that 3 h of plus lens-wear, with 9 h of viewing without lenses, produced about 25% of the magnitude of response that continuous lens-wear did. In contrast, when we gave only 28 min of plus lens-wear broken up into frequent episodes, with darkness the rest of the time (Experiment 2), we got half as much compensation as with continuous lens-wear, and with 3.5 h of lens-wear (Experiment 1) we got about as much axial length inhibition and almost as much choroidal thickening as we found with continuous lens-wear.

4.2. Short, repeated episodes of lens-wear are more effective than longer, more spaced episodes

The first non-linearity we find in the way that blur is integrated over time is that several daily brief episodes of lens-wear support more effective emmetropization than fewer daily episodes, even if the total amount of vision is the same. This implies that the blur-integrating mechanism is more complicated than a simple pure integrator, in which the output (for example, the concentration of a growth factor) rises linearly in proportion to the input and holds the accumulated value in the dark. Instead our results suggest that the accumulated “blur signal,” whether stored in a neural circuit or in the level

of growth-related molecules, decays with time in the dark. Evidence for this is that when the total amount of lens-wear is constant but the episodes are infrequent, the effect on ocular elongation weakens: with positive lenses, one 28 min episode of lens-viewing per day was 60% less effective at inhibiting ocular elongation than fourteen 2 min episodes (Fig. 5A), or with negative lenses, seven 4 min episodes per day had no effect at all on ocular elongation, but fourteen 2 min episodes were effective (Fig. 4). The most parsimonious explanation would seem to be that over the hours in the dark, the effect of the previous blur signal declines. In control theory this would be described as a leaky integrator.

Alternatively, if there were no decay in the dark, our first non-linearity might arise from the blur having an immediate effect on some neural or chemical process which rapidly saturates. In such a system, the first 5 min of a 30 min episode of blur would have a greater effect than the last 5 min, because there would be a limit to how much the output can be changed within a short time. Evidence for such a process is that the change in ocular elongation is about the same (about 100 μm more than the fellow eyes for minus lenses, and 120–220 μm less than the fellow eye for plus lenses), whether the duration of each episode of lens-wear is 30 min (7 per day, totaling 210 min) or 2 min (14 per day, totaling 28 min). This can be seen by comparing the amount of ocular elongation among the frequent groups of Experiments 1, 2, and 3. Because our interest here was to test whether periods of blur add linearly, we kept the total lens-wear constant. This required changing both the duration and frequency of the lens-wear episodes. Thus our data to date do not permit definitively characterizing the strength or identifying the existence of both effects, the decline of the signal in the dark and the saturation of the signal during lens-wear. However, we can say that the lens-wear episodes do not sum linearly.

4.3. *Lens-wear that is too brief, even if repeated frequently, does not support effective emmetropization*

The second non-linearity in our results—that very brief lens-viewing episodes are disproportionately ineffective—cannot be accounted for by a rapidly saturating input stage, as suggested above, which would predict that the briefer, more frequent episodes would be at least as effective as longer episodes. Instead, either a delay or an accelerating efficacy of blur must be postulated. This non-linearity is shown, for example, by the fact that when chicks were given 14 min/day of plus-lens-viewing in various episode durations, those durations shorter than 2 min did not show refractive compensation (Fig. 6). We propose that a second process intervenes, either to ignore very brief periods of defocus, thereby avoiding having the eye growth machinery fired up by every momentary blur, or to require

some priming before it acts most effectively. Biological examples of the latter abound: enzymes like calcium-calmodulin kinase II or tyrosine kinase receptors require themselves to be phosphorylated to a certain degree before they act to phosphorylate other proteins. (We raise these examples only to suggest the prevalence of biochemical processes with sigmoidal input/output functions, not to suggest that one of these reactions accounts for the temporal characteristics of lens-wear.)

A caveat to our finding that very brief, very frequent episodes of lens-wear have little effect on lens compensation is that in our briefest episodes, the effects of defocus may be confounded with the luminance transients. The animals may, for example, close their eyes when the lights are flashed on briefly, or their accommodation and behavioral patterns may be different during these very brief flashes than during more extended periods. This caveat presumably is most applicable to the shortest flashes we used (2 s each), and less so to the 5 and 20 s conditions.

4.4. *When worn alternately, plus lenses exert a stronger effect than minus lenses*

The third prominent non-linearity in our results is that alternation of plus and minus lenses gave results strongly dominated by the plus lenses, even though brief episodes of either lens alone were sufficient to induce compensation in the appropriate direction. This bias did not depend on which lens was worn first, so it cannot be argued either that the experience of one lens cancels that of the following one, or that the second lens experience determines how the eye grows in the subsequent period of darkness. Instead, we propose that the eye integrates the blur it experiences over these intervals, with added weight for myopic blur (imposed by plus lenses), and then continues to respond according to this integration in the ensuing dark period when there is no visual feedback.

The bias to respond to the plus lens could have arisen in two ways. (1) With the particular frequencies and durations of episodes used, plus lenses alone might tend to produce a stronger response than minus lenses alone, and the combined effects were summed linearly when the lenses were worn in succession. (2) Alternatively, the responses to single lenses alone might have been equal (but in opposite directions), but did not cancel when lenses were worn successively. The latter could arise if the signal generated from plus lens-wear weakened or degraded the signal generated from minus lens-wear.

Our results do not allow us to eliminate either explanation decisively, as our single-lens experiments did not exactly match the conditions of the successive lens-wear experiments, but both explanations are supported. If the episodes of plus lens-wear are brief, they generally resulted in a greater magnitude of response than brief

episodes of minus lens-wear (Experiments 1 and 2). This suggests that the non-linearity (a “plus lens response” even when the duration of minus lens-wear is equal to or greater than the duration of plus lens-wear) arises at least in part from the greater response to briefly worn plus lenses in the single-lens experiments. Other (unpublished) experiments from our lab, in which chicks wore plus lenses for 2 min four times per day, but otherwise wore minus lenses, also showed a dominant effect of the plus lenses, although, in this case, the amount of plus lens-wear alone would give a weaker response than would near-continuous minus lens-wear alone. It thus seems that the non-linearity found in the successive lens-wear experiments may have arisen both from asymmetric responses to brief episodes of plus lenses alone and minus lenses alone, and from a tendency of the plus lens signal to suppress that of the minus lens.

4.5. Implications of lens-switching results for discrimination of sign of blur

The differences between the effects of wearing plus and minus lenses raise the question of how the eye can distinguish myopic from hyperopic defocus. Some have argued that bi-directional compensation could be achieved if image quality were consistently increased by one sign of lens and decreased by the other sign (Norton & Siegwart, 1995). In Experiment 5 (Group B) we found that having lenses worn only when chicks were in drums that prevented sharp vision for plus lenses did not affect the powerful effects of plus lens-wear, when alternated with minus lens-wear. Because both the plus and minus lenses increased the image blur, this result contributes to evidence that the sign of blur can be discriminated. Other evidence pointing in the same direction is (a) compensation for plus lenses worn alone in drums (Schaeffel & Diether, 1999; Winawer et al., 2000); (b) compensation for weak spherical lenses of either sign in the presence of massive blur from crossed cylindrical lenses $+5/-5$ D Jackson crossed cylinders (McLean & Wallman, 2002); (c) no difference in the initial refractive, choroidal or elongation response to different powers of lenses of the same sign (Wildsoet & Wallman, 1997). Thus the results presented here together with these studies argue that to predict the degree of myopia resulting from lens-wear, what is important is not the amount of blur experienced, but the sign of the blur and the separate temporal distributions of the myopic and hyperopic blurs.

4.6. With brief, infrequent lens-wear, there is a dissociation between the ocular elongation and choroidal responses

In general, choroidal expansion occurs in the same situation as the inhibition of ocular elongation, that is,

in response to plus lens-wear or during recovery from form-deprivation myopia. Conversely, choroidal thinning usually occurs in tandem with increased ocular elongation, either in response to minus lens-wear or to form-deprivation myopia. Consequently, we found it surprising that infrequent plus lens-wear was sufficient to inhibit ocular elongation but not sufficient to thicken the choroid (Figs. 3 and 4). Furthermore infrequent minus lens-wear was sufficient to thin the choroid but not sufficient to cause increased ocular lengthening (Figs. 3 and 4). Thus the difference is neither one of lens type (positive versus negative) nor of the tissue responding (sclera versus choroid), but of a more complicated interaction.

One can take two views of the relation between changes in choroid thickness and ocular elongation. One can view this pattern as suggesting that the retina puts out a signal (or signals) directing the eye either towards myopia or hyperopia, and that the asymmetry arises because a lower level of this signal is required downstream to inhibit ocular elongation than to stimulate choroidal thickening (in the case of plus lenses) or to induce choroidal thinning than to increase ocular elongation (in the case of minus lenses). Alternatively, one can view this asymmetry as evidence that there are several distinct retinal signals, each controlling a different output pathway: one for thickening the choroid, one for thinning it, one for accelerating ocular elongation, and one for inhibiting the normal ocular elongation.

In support of the first hypothesis, that the elongation rate and the choroidal thickness responses are regulated by the same retinal signal, but require different levels of the signal, we note that we cannot achieve all possible dissociations. That is, we have found no conditions that cause the choroids to expand, but have no effect on ocular elongation, or conversely, that cause the eye to increase its elongation without causing choroidal thinning. If there were truly independent signal cascades regulating the choroid thickness and eye length, we might expect that by combining frequent minus lens-wear, which caused choroidal thinning and increased elongation, with infrequent plus lens-wear, which caused slowed elongation without choroidal expansion, we would see a myopic phenotype in the choroid (minus lens wins) and an emmetropic phenotype in the elongation rate (plus and minus cancel). We did not find this (see Section 4.4). If there is a single signaling mechanism for both the choroid and scleral responses, we can imagine a substance which when more abundant than usual causes the eye to grow towards hyperopia and when less abundant than normal causes the eye to grow towards myopia. If this substance is slightly increased it stops ocular elongation, and if slightly decreased it causes choroidal thinning. Only with larger changes in either direction would both the choroid thickness and elongation rate be altered.

Support for the second hypothesis, that there are separate retinal signals regulating choroid thickness and ocular elongation, comes from experiments in which a weak, image-degrading diffuser was worn over a plus lens (Park, Winawer, & Wallman, submitted for publication). These experiments showed that the diffuser reduced the choroid thickening normally seen with plus lens-wear, but enhanced the inhibition of ocular elongation, perhaps because of the lack of choroid response. As the one response was reduced (choroidal thickening) while the other was enhanced (inhibition of elongation), it seems plausible that the two responses are regulated by separate retinal signaling mechanisms, although other explanations also exist. Our finding that eyes with infrequent plus-lens-viewing show a compensatory inhibition of elongation, but not a compensatory choroidal thickening, could be explained similarly. Furthermore, two visual manipulations have been shown to interfere with experimentally induced myopia by blocking ocular elongation, while having little effect on choroidal thinning: (1) brief periods of strobe light at dawn and dusk in eyes wearing translucent diffusers (Kee, 1998; Nickla, 1996), and (2) brief periods of light during the night in eyes wearing minus lenses (Kee, 1998).

Regardless of whether the choroidal and ocular elongation responses are driven by one or more signals, having choroid thickness be more sensitive to negative lenses and ocular elongation more sensitive to positive lenses would be a developmentally conservative strategy. It is less dangerous to halt ocular elongation than to increase it, in that if the eye mistakenly gets beyond its adult length it probably is stuck there, but errors in the opposite direction can be corrected later. Thus with small amounts of myopic defocus, as from plus lens-wear, it is “safer” for the eye to halt ocular elongation without a change in choroid thickness rather than to expand the choroid while elongating at a normal rate and risk overshooting the appropriate eye length. Similarly, with small amounts of hyperopic defocus, it is safer to thin the choroid and elongate normally than to maintain normal choroid thickness while increasing the rate of elongation, again to prevent overshooting the eye length. We note, however, that the asymmetry being discussed is found after several days of brief periods of lens-wear; it is not reflected in the order of response with continuous lens-wear. Rather, the normal response with continuous lens-wear of either sign is an early change in choroid thickness in the appropriate direction followed by a slower change in ocular elongation (Hung et al., 2000; Kee, Marzani, & Wallman, 2001).

The dissociation between choroidal and ocular elongation responses shows that a choroidal thickening is not necessary for slowed ocular elongation. It has been suggested that the thickening of the choroid may inhibit scleral growth (and therefore ocular elongation) by

acting as a physical barrier to growth-related molecules coming from the retina (Troilo et al., 2000; Wallman et al., 1995). Alternatively, because the choroid provides structural support for the eye, thereby reducing the effect of intraocular pressure on the sclera (Van Alphen, 1961, 1986), a thickened choroid may produce even more support and thus result in reduced growth. Because infrequent plus lens-wear causes an inhibition of eye growth but little to no expansion of the choroid (Figs. 3 and 4), the choroidal effects are clearly not necessary to stop eye growth. However, it is still possible that when the choroid does expand it has chemical or physical effects that slow scleral growth.

4.7. *Relevance to emmetropization*

We believe the rules resulting from the experiments presented here have a generality beyond chicks kept in the dark between episodes of lens-wear. We found that if chicks wore plus lenses for brief episodes, and for the rest of the day either had unrestricted vision or wore minus lenses, the plus lens-wear still showed strongly protective effects, similar to the results of the lens-switching experiments reported here (Winawer et al., 2000). In the course of daily life we experience a continuous sequence of episodes of myopic and hyperopic blur arising from objects beyond and in front of the point of regard. The three non-linearities that we have discussed would combine in such a way as to make the refractive error reached extremely dependent on the precise distribution of the timing of the episodes of myopic and hyperopic defocus. Consider a myopic child reading or engaged in other nearwork for most of the day, thereby experiencing hyperopic defocus much as chicks wearing negative lenses do. When the child looks up she would experience myopic defocus, similar to a chick wearing a plus lens. These periods might have protective effects if they were not too brief or too infrequent.

How likely is it that similarly non-linear processes exist in other species, in particular primates? Although we know of no lens-switching experiments in primates (some are underway), Smith et al. (2002) reviewed four studies in which negative lenses or diffusers were removed for part of each day. These animals had visual experiences somewhat similar to our chicks in that once they had developed some myopia; they experienced periods of hyperopic defocus while wearing the lenses alternating with periods of myopic defocus during the periods without lenses. Specifically, Smith showed that the relative myopia as a function of hours of unrestricted vision per day follows the same exponential-decay curve whether from form-deprived chicks (Napper et al., 1995), minus lens-wearing chicks (Schmid & Wildsoet, 1996), minus lens-wearing tree shrews (Shaikh et al., 1999), or form-deprived macaque monkeys (Smith et al.,

2002). In all four cases, the myopia was reduced by 50% with about 1 h of unrestricted viewing per day, with the data points from the four separate studies fitting a single curve quite well. This similarity is surprising, given the different rates at which each species compensates for lenses with continuous wear: about five days in chicks (–10 D lenses; Schmid & Wildsoet, 1996), 11 days in tree shrews (–5 D lenses; Siegwart & Norton, 1998), and about two months in macaques (–3 D lenses; Smith & Hung, 1999). Thus at least our third non-linearity seems likely to exist in primates.

In light of the three non-linearities we have documented here it is not astonishing that attempts to predict myopic progression in children by logging the number of hours per week that they spend at nearwork have failed. Many epidemiological studies have shown a strong association between amount of education and myopia (e.g., Goldschmidt, 1968; Sperduto, Seigel, Roberts, & Rowland, 1983; Zylbermann, Landau, & Berson, 1993; for reviews, see Ong & Ciuffreda, 1997; Rosenfield & Gilmartin, 1998; Zadnik & Mutti, 1998), presumably because of the hyperopic blur experienced while reading. On the other hand, the correlations between the amount of time individual children spend reading or doing other nearwork and the development of refractive errors have been very low (Saw et al., 2000, 2002; Zadnik, 1997; Zadnik & Mutti, 1998). Some of the complications we report here in the temporal integration of refractive error may bear on the magnitude of those correlations. For example, the frequency with which children look up while reading, or the duration of each pause, may be more important than the total amount of time spent reading. To resolve the discrepancy between the strong epidemiological associations of myopia with reading across groups and the weak associations of myopia with assessments of hours of reading may require examining the temporal patterns of nearwork and distance viewing.

We have shown that brief periods of myopic defocus (from positive lenses) prevents myopia (compensation for negative lenses). If this is true in humans, it would upset two theories about the development of myopia. First, it would imply that all blur is not necessarily myopiagenic; myopic blur being protective. Second, it would turn on its head the theory that the transient myopia resulting from long periods of accommodation leads to permanent myopia (Ciuffreda & Wallis, 1998; Ebenholtz, 1983). Our results suggest that, on the contrary, this transient myopia might protect the eye against the myopia resulting from the preceding nearwork. Our successive lens-wear experiments imply that short periods (but not too short) of myopic defocus outweigh longer periods of hyperopic defocus. If, however, the duration of transient myopia following nearwork is too brief (about 1 min for adult myopes (Ciuffreda & Wallis, 1998)), it might have little to no effect on emmetropization.

In daily life the strong compensatory effect of myopic defocus may protect the eye from developing myopia in response to viewing near objects because the hyperopic defocus from the objects viewed would be offset by myopic defocus from features behind these objects. Reading may be different from most other nearwork in that a page held close blocks out the world at a distance. Once we understand the details of the temporal integration of myopic and hyperopic defocus signals, we would be in a better position to predict, and perhaps prevent, myopic progression.

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