

# In a Matter of Minutes, the Eye Can Know Which Way to Grow

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**PURPOSE.** The fitting of chick eyes with positive or negative lenses causes eye growth to decelerate or accelerate, respectively, thereby minimizing the imposed blur. This study was conducted to determine whether the eye can initially assess the correct direction of growth or whether it relies on trial and error, reversing its direction if the magnitude of blur increases. The rapid changes in choroidal thickness in response to brief periods of defocus were measured.

**METHODS.** After their eyes were measured by ultrasound biometry, chicks wore either a +10-D lens over one eye for 10 minutes while restrained in the center of a 60-cm drum (to ensure myopic blur), or a negative lens (−7 or −8.6 D) over one eye for 10 minutes or 1 hour in a normal cage environment. They were then kept in darkness until they were remeasured 2 hours, 1 day, or 2 days after the first measurement. Other chicks wore +10 or −8.6-D lenses briefly and were measured several times over the next 7 hours in darkness.

**RESULTS.** Wearing positive or negative lenses for only 10 minutes produced significantly different effects on choroidal thickness measured 2 hours later. Wearing positive lenses for 10 minutes caused an increase in choroidal thickness (in 28 of 32 eyes) and a concomitant decrease in vitreous chamber depth, relative to the amount of change in the untreated fellow eye over the same period. Wearing negative lenses for 1 hour caused significant changes in the opposite direction. Wearing lenses for 2 hours resulted in choroidal changes that persisted in darkness for up to 6 hours after positive lens wear, but returned to normal after negative lens wear. Finally, 1 hour of positive lens wear caused significant inhibition of ocular elongation over the next 2 days.

**CONCLUSIONS.** The eyes of chicks require only a brief period of lens wear to initiate compensation in the appropriate direction. Because the refractive status changes little during the period of lens wear, the authors conclude that eyes can rapidly determine the sign of the imposed blur without resorting to a trial-and-error method. (*Invest Ophthalmol Vis Sci.* 2005;46:2238–2241) DOI:10.1167/iovs.04-0956

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Supported by Grants EY02727 from the National Eye Institute and RR03060 from the National Institutes of Health.

Submitted for publication August 6, 2004; revised December 6, 2004, and February 15, 2005; accepted February 28, 2005.

Disclosure: **X. Zhu**, None; **T.W. Park**, None; **J. Winawer**, None; **J. Wallman**, None

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One of the astonishing aspects of eye growth is that eyes appear able to detect whether the blur they experience is myopic (image focused in front of the photoreceptors) or hyperopic (image focused behind the photoreceptors) and to accelerate or decelerate growth in such a way as to reduce the blur.<sup>1</sup> Accommodation accomplishes a similar feat. There is considerable controversy about the extent to which accommodation is guided by trial and error, as opposed to its responding at the outset in the correct direction for the sign of the defocus.<sup>2–4</sup> This uncertainty looms even larger for emmetropization. On the one hand, it is difficult to imagine the eye's determining the sign of defocus by trial and error—that is, by keeping track of whether its growth is increasing or decreasing the amount of blur being experienced—since this would seem to require a memory of what the blur was days or months ago, depending on the species. On the other hand, the optical signals used by the eye to determine the sign of defocus have not been identified.

The evidence to date suggests that the mechanism of compensation for imposed defocus is highly conserved across primates, rodents, birds, and fish,<sup>5–10</sup> whereas the eyes of these species differ widely in acuity, in the presence or absence of accommodation or of a fovea, and in the degree of color and binocular vision. This diversity makes it attractive to consider an uncomplicated mechanism that determines the direction of compensatory growth, such as a trial-and-error strategy, which would make it unnecessary to distinguish between myopic and hyperopic defocus.

We tested the plausibility of such a strategy by giving chicks a single episode of lens wear, too brief for any refractive compensation to occur, and then placing them in the dark and determining the direction of compensation in vitreous chamber depth and choroidal thickness over the next 2 hours and the direction of change in the rate of ocular elongation over the next 1 to 2 days. If a trial-and-error strategy were being used, one might expect the imposition of defocus to cause either half the eyes to grow initially toward myopia and half toward hyperopia or all the eyes to grow in the same direction. In either case, however, one would expect there to be no initial difference between the effects of briefly imposing myopic and hyperopic defocus. We also measured the time-course of the changes resulting from single episodes of lens wear. These experiments differ from previous work involving brief periods of lens wear,<sup>11–14</sup> in that in our study the animals wore a lens only once, rather than daily.

## METHODS

White Leghorn chicks (*Gallus gallus domesticus*, Cornell K strain), 6 to 10 days old, had their choroidal thickness and other axial dimensions measured by high-frequency ultrasound biometry (for details see Ref. 15). They then wore a lens over one eye briefly and were remeasured 2 hours to 2 days later. We use the term ocular elongation to refer to the increase in the length of the globe—that is, from the anterior surface of the cornea to the posterior surface of the sclera—not the axial length of clinical practice, which is the distance from cornea to retina. The procedures used were approved by the Institutional Animal Care and Use Committee and are in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

## Experiment 1

To evaluate the effects of a single brief episode of lens wear, chicks wore either positive (+10 D) lenses for 10 minutes ( $n = 25$ ) or negative (-7 or -8.6 D) lenses for either 10 minutes ( $n = 10$ ) or 1 hour ( $n = 16$ ) and were then placed in darkness until measured again 2 hours after the first measurement. Some of these birds were remeasured after 1 or 2 days in darkness ( $n = 7$  and 9, respectively). The positive lenses were worn while the chicks were confined to the center of a 60-cm diameter drum (details in Ref. 16). Because the walls of the drum were beyond the far point of the lens-wearing eye, the restraint of the chicks ensured that the eye experienced continuous defocus and that the sign of the defocus was myopic. Had the chicks been free in a cage, some retinal regions would have experienced hyperopic defocus at some times and myopic defocus at other times, depending on where the eye was focused and the three-dimensional geometry of the surroundings. The negative lenses were worn while the chicks were unrestrained in their home cages, because, whether the chick was in a cage or a drum, the hyperopic defocus imposed by the lenses could be cleared by accommodation. Some of the chicks in this experiment had worn +2- or +3-D lenses for 2 days while in their cages before the experiment began, a manipulation without effect on the results reported herein.

## Experiment 2

To better understand the time course of the responses in experiment 1, we had to make repeated measures on the same eyes. Therefore we gave chicks, in their cages, 10 minutes ( $n = 14$ ) or 2 hours ( $n = 8$ ) of +10-D lens wear or 2 hours of -8.6-D lens wear ( $n = 10$ ), followed by darkness. During this time, their eyes were periodically measured by ultrasound, with the only illumination being 30 seconds of exposure to the alignment light of the ultrasound apparatus (10 foot-candles). We did not control the sign of the blur experienced by these chicks.

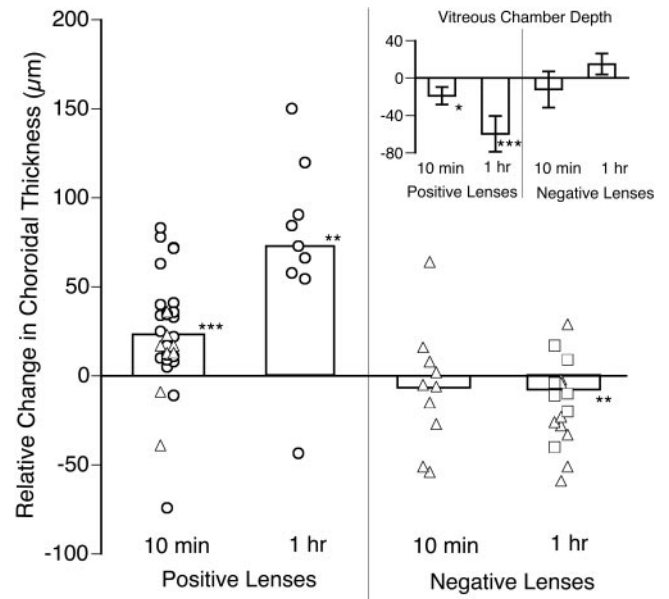
Because darkness by itself increases ocular length and thins the choroid over days,<sup>17,18</sup> in experiment 1, we expressed the changes in choroidal thickness, vitreous chamber depth, and ocular length over the course of the experiment (i.e., 2 hours, 1 day, or 2 days) as the "relative changes," that is, the changes in the lens-wearing eye minus the equivalent changes in the untreated fellow eye. In experiment 2, we reported the data as the interocular differences at the time of measurement. Because we saw no significant effects on the choroids of the fellow eyes over hours, we infer that the short period of darkness did not distort our results.

## RESULTS

### Experiment 1

A single brief episode of lens wear was sufficient to elicit significantly different responses to positive compared with negative lenses. Ten minutes of wearing positive lenses in the drum caused the choroids to become thicker than those of eyes wearing negative lenses for the same time (difference in means, 29  $\mu\text{m}$ ;  $P < 0.01$  by one-tailed  $t$ -test). The eyes that had worn positive lenses had thicker choroids than their untreated contralateral eyes in 28 of 32 cases ( $P < 0.001$ , paired  $t$ -test; Fig. 1). The vitreous chamber depth of these eyes was significantly reduced (-19  $\mu\text{m}$ ;  $P < 0.05$ ; Fig. 1, inset), presumably as a consequence of the choroidal thickening without a change in the external dimensions of the eye during this 2-hour period. In contrast, 10 minutes of wearing negative lenses did not cause changes in the dimensions of either the choroid or the vitreous chamber (Fig. 1). The choroidal thickness of the untreated fellow eyes did not change over the 2 hours in darkness (mean = 1  $\mu\text{m}$ ).

One hour of lens wear led to even greater differences in the effects of positive versus negative lenses on choroidal thickness (difference in means, 89  $\mu\text{m}$ ;  $P < 0.001$ ). With this



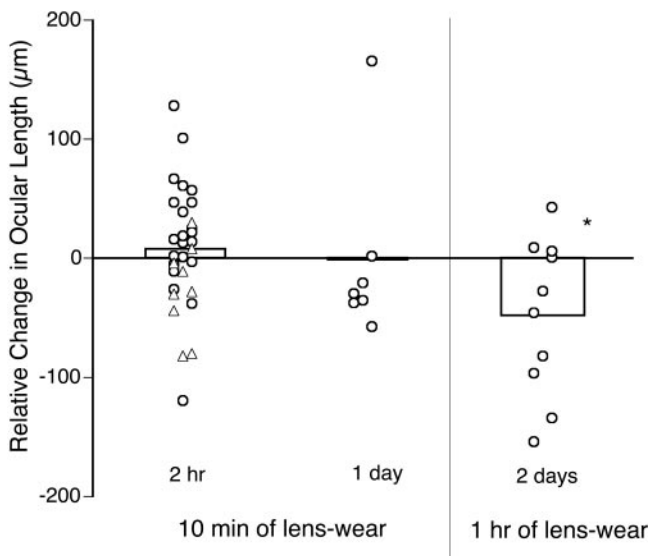
**FIGURE 1.** Changes in the chick eye's choroidal thickness and vitreous chamber depth 2 hours after wearing lenses for durations shown (experiment 1). Even 10 minutes of wearing positive lenses or 1 hour of wearing negative lenses caused significant changes. Data shown are changes over the course of the experiment in the lens-wearing eye minus the corresponding change in the untreated fellow eye. All positive lenses were +10 D; negative lenses were either -7 D ( $\Delta$ ) or -8.6 D ( $\square$ ). Some birds ( $\Delta$ ) had worn weak positive lenses before the experiment began. Error bars, SEM. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ , from paired  $t$ -tests, comparing the two eyes of each bird.

amount of lens wear, both the eyes wearing positive and those wearing negative lenses differed significantly from their untreated fellow eyes (Fig. 1;  $P < 0.01$ ; paired  $t$ -tests;  $n = 9$  for positive lenses,  $n = 16$  for negative lenses), more so for the eyes that had worn positive lenses. Again the thickening of the choroid caused the vitreous chamber depth of the eyes that had worn positive lenses to become significantly shallower than that of the untreated fellow eyes (-60  $\mu\text{m}$ ;  $P < 0.01$ ; Fig. 1, inset). We saw no significant difference in choroidal thickening related to whether the chicks had worn weak positive lenses before the experiment ( $P > 0.05$ , unpaired  $t$ -test). As before, the choroidal thickness of the untreated fellow eyes did not change (mean = 1  $\mu\text{m}$ ).

Ocular elongation was also affected by brief lens wear. One hour of wearing a positive lens caused ocular elongation over the next 2 days to be significantly less than in the untreated fellow eye (Fig. 2; -48  $\mu\text{m}$ ;  $P < 0.05$ ;  $t$ -test; the untreated fellow eyes grew by 103  $\mu\text{m}$ ). After 10 minutes of lens wear, the amount of elongation of the two eyes did not differ, either 2 hours after lens wear or after a day in darkness.

### Experiment 2

In experiment 1, we measured the eyes after a period in darkness because we assumed that the choroidal thickening would continue in darkness. To test this assumption, we followed another group of birds with repeated measurements (Fig. 3a), either every 30 minutes (in one group) or every hour (in another group). After the chicks had worn positive lenses for 10 minutes, the choroids continued to thicken in the dark for 1 to 2 hours and then slowly returned toward normal thickness, but remained thicker than normal throughout the measurement period. At 30 minutes, seven of nine choroids in lens-wearing eyes were thicker than those in untreated fellow eyes (mean difference,  $27 \pm 12 \mu\text{m}$ ). Even 6 hours after lens



**FIGURE 2.** Changes in the amount of ocular elongation at times shown after wearing of +10-D lenses. The effects of a single brief episode of lens wear were evident 2 days later. Conventions as in Figure 1.

wear, choroids were still thicker than those in fellow eyes by an average of 14  $\mu\text{m}$ .

To compare the time course of the changes in eyes that wore positive and negative lenses, we had birds wear lenses for 2 hours. As expected, during the period of lens wear, choroidal thickness increased in eyes wearing positive lenses and decreased in those wearing negative lenses (Fig. 3b). After lens wear, the choroids of eyes that had worn positive lenses remained thick in the dark, but those that had worn negative lenses returned toward their original thickness. Thus, in the eyes wearing negative lenses, the slope of the changes in choroidal thickness during the 2-hour lens-wearing period was significantly more negative than during the subsequent 2 hours (Wilcoxon signed rank test,  $P < 0.01$ , with 7 of the 10 eyes having negative slopes during the lens wear and 9 of the 10 having positive slopes afterward). In the eyes wearing positive lenses, the slopes tended to be less positive after the lens wear than during it, but not significantly so.

## DISCUSSION

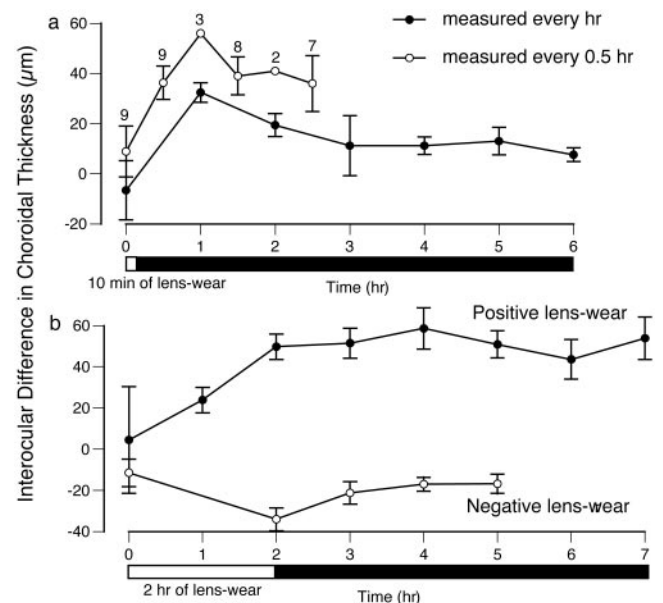
We report three principal results: First, even 10 minutes of wearing positive, but not negative, lenses resulted in a thickening of the choroids over the subsequent 1 to 2 hours. Second, wearing negative lenses for 1 hour caused choroidal thinning. Third, an hour of wearing positive lenses reduced the amount of ocular elongation over the next 2 days.

For an eye to infer the sign of the defocus it experiences by a trial-and-error procedure, its refractive error must change by an amount greater than its depth of focus during the visual episode, so that it can judge whether its current direction of growth (toward myopia or toward hyperopia) is the correct one to reduce the defocus. We found that a 10-minute period of wearing positive lenses led to the vitreous chamber's becoming shallower by 18  $\mu\text{m}$  at 30 minutes after the start of lens wear and by 32  $\mu\text{m}$  at its peak change at 1 hour (data from experiment 2). The first of these would correspond to 0.3 D of hyperopic shift and the second to 0.5 D, on the basis that 1 mm of vitreous chamber depth corresponds to 17.5 D, according to the formula in Wallman et al.,<sup>19</sup> using the mean ocular length of 9.0 mm measured in these experiments. The depth of focus is estimated as at least 0.7 D in chickens of the age used in the present study, calculated

from the retinal ganglion cell spacing, eye size, and pupil diameter.<sup>20</sup> This seems to be the lowest estimate of the depth of focus, in that it includes all the ganglion cells, whereas any particular retinal output would use a subset of the ganglion cells, which would therefore have a greater depth of focus. However, if the retinal circuitry used by the emmetropization mechanism constituted a subpopulation of bipolar or amacrine cells, the depth of focus could not be estimated without knowing which cells are involved, but it might well be less than 0.7 D. Finally, if we roughly estimate the "acuity" of the emmetropization process by averaging the spatial frequencies of stimuli that prevent form-deprivation myopia and those that do not, a greater depth of focus of 1.4 D is predicted.<sup>20,21</sup>

It seems from our measurements that the amount of change in refractive status during 10 minutes of lens wear would probably be substantially less than 0.3 D and thus would be unlikely to be detectable by the chick eye. The amount of change during 1 hour of lens wear may be 0.5 D, which is still below the lowest estimate of the depth of focus. Finally, our finding of significantly increased choroidal thickness in 88% of eyes wearing positive lenses for 10 minutes must not simply reflect a bias toward choroidal thickening occurring whenever blur increases, because it did not occur in those eyes wearing negative lenses for 10 minutes. Thus, it seems highly unlikely that the chick eye can use the miniscule changes in retinal position during 10 minutes of lens wear to infer whether it is growing in the appropriate direction.

It has been argued that the eye could emmetropize by trial and error, even without remembering the degree of blur if it had access to the rate of change of blur or of image-degradation to guide eye growth.<sup>22</sup> We are skeptical of the applicability of this hypothesis to our results for two reasons. First, it would seem to require an unreasonably high sensitivity to the rate of



**FIGURE 3.** Time course of changes in choroidal thickness after a single episode of lens wear (experiment 2). (a) Ten minutes of wearing a +10-D lens and (b) 2 hours of wearing either a +10-D lens or a -8.6-D lens. In both panels, each bird had its overall mean subtracted from each data point, and so the variability of the changes is not confounded by the variability of initial eye size. The mean of all the data points in the group was then added back to each point so that the y-axis scale is correct. The numbers by the symbols in one group in (a) is the number of eyes averaged. In the other group, 5 eyes were averaged for each point; in (b), 8 eyes were averaged for the positive-lens group and 10 for the negative-lens group. Error bars, SEM.

change of blur to respond to the change in blur caused by eye growth or choroidal expansion—at most, 0.01 D/min—according to the calculations we have just described. Second, one would expect that imposing a spectacle lens would change the magnitude of defocus much more than anything the eye could do in the 10 minutes of lens wear. Thus, when we suddenly increased the amount of blur, either by fitting positive lenses to chicks restrained in the center of a drum (because the walls were beyond the far point of the lens-wearing eye) or by fitting negative lenses to birds in a cage with most objects nearby, we should have caused the initial response to be in the same direction; instead we got responses in opposite directions.

Beyond the question of whether eyes can discern the sign of the blur, our results point out two additional differences between wearing positive and negative lenses: The positive lenses are more potent than the negative ones, in that they require less lens wear to cause changes in choroidal thickness (Fig. 1), and the effect of one episode of wearing positive lenses is more enduring than one of wearing negative lenses (Fig. 3b). These results are consistent with the findings reported in three recent papers. Winawer and Wallman<sup>13</sup> found that if birds wear positive and negative lenses alternately for 30 minutes each, four times a day, the choroids thicken, showing that the myopic defocus of the positive lenses dominates. This thickening occurs irrespective of whether the sign of defocus alternates every 6 seconds, 75 seconds, or 15 minutes.<sup>23</sup> Furthermore, Zhu et al.<sup>14</sup> found that 2 minutes of wearing positive lenses four times a day while wearing negative lenses the rest of the time causes a significant increase in choroidal thickness relative to that of the untreated fellow eye. All of these differences between the effects of positive and negative lens wear may be part of a conservative growth strategy, in that an excessive ocular elongation in response to hyperopic defocus could leave the eye permanently too long, whereas excessive inhibition in response to myopic defocus could be subsequently corrected.

We found that wearing a positive lens for 1 hour, but not 10 minutes, caused a significant change in ocular elongation when measured 2 days later. Might one argue that the scleral response, which determines the elongation rate of the eye, still operates by trial and error, even though the choroidal response does not? We cannot rule this possibility out entirely, because the vitreous chamber depth changed by the equivalent of 0.5 D during the 1 hour of lens wear. However, there was no change in ocular elongation during this period. Therefore, even if the scleral response relies on visual feedback, the feedback cannot come from changes in ocular elongation; rather, a scleral trial-and-error mechanism would have to rely on the visual consequences of the choroidal response, a mechanism that itself apparently does not operate by trial and error. Moreover, we find, on the one hand, that among those eyes that elongated less than their fellow eyes over the 2 days after wearing positive lenses for 1 hour, there is a modest correlation ( $r = 0.6$ ) between the degrees of ocular elongation and choroidal thickening at 2 hours, suggesting that both responses may be coupled. However, there is substantial evidence of a dissociation between visual effects on these two ocular parameters.<sup>13,16</sup> It thus seems parsimonious to conclude tentatively that neither of these two components of lens compensation operates by a trial-and-error mechanism. In lens compensation, and presumably also in emmetropization, the eye appears to know which way to grow.

This conclusion adds weight to previous evidence that it is not the blurring of vision, per se, that is important in guiding eye growth, but whether blur is myopic or hyperopic. Furthermore, short periods of myopic blur are as effective as longer periods of hyperopic blur.<sup>13,14,23</sup> Given that the temporal properties of the emmetropizing mechanism appear similar across very different species,<sup>24</sup> it seems prudent to suppose

that the progression of myopia in children may be influenced by the temporal characteristics of the child's visual experiences. If long periods of mild hyperopic defocus during reading increase myopia, brief doses of clear vision or myopic defocus when one looks up from reading may signal the eye to reduce the progression of myopia.

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