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Colchicine attenuates compensation to negative but not to positive lenses in young chicks

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Abstract

Optic nerve-sectioned (ONS) chick eyes are capable of emmetropisation, but these eyes also exhibit increased hyperopia without any visual manipulations, which suggests altered eye growth regulation. These altered growth changes may be related to the loss of retinal ganglion cells that follows nerve lesioning. Colchicine, which also destroys retinal ganglion cells in chicks, was used to further examine the effects of retinal ganglion cell loss on emmetropisation. Growth responses of +10 D and –10 D lens-wearing colchicine-injected eyes were compared to those of +10 D and –10 D lens-wearing saline-injected eyes, respectively. Changes after removal of lenses were also analysed. Prior to lens-wear, colchicine-injected eyes exhibited longer optical axial lengths (OL; distance from cornea to retina; $p=0.0185$) but no differences in refractive error (RE; $p=0.6588$). Although myopic shifts were not significant for –10 D lens-wearing colchicine-injected eyes ($p=0.5913$), but were for the saline-injected eyes ($p=0.0034$), these changes were not different ($p=0.1646$). However, –10 D lens-induced OL changes in colchicine-injected eyes showed insignificant ($p=0.2214$) and reduced ($p=0.0102$) changes compared to those of saline-injected eyes. +10 D lens-treated colchicine-injected eyes showed significant hyperopic shifts ($p<0.0001$) and significant reductions in OL ($p<0.0001$) that were similar to those of saline-injected eyes ($p=0.7990$ and $p=0.1495$, respectively). Growth responses in eyes recovering from –10 D lenses were minimal, with REs unaffected ($p=0.3325$), but OL reductions affected ($p=0.0199$) by colchicine. colchicine-injected eyes recovering from +10 D lenses showed significant myopic shifts ($p=0.0003$) and OL elongations ($p<0.0001$) that were similar to those of saline-injected eyes ($p=0.3999$ and $p=0.4731$, respectively). The results showing that colchicine suppresses the ability to respond to negative lenses but leaves compensation to positive lenses relatively unchanged, are opposite to those of optic nerve sectioned eyes. We speculate that the differences are probably related to the way retinal cells are lost.

Keywords

colchicine; emmetropisation; retinal ganglion cell loss; chicks; optic nerve section; axial length; refractive error; vitreous chamber depth

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INTRODUCTION

In a variety of young animals, ocular growth is modulated by the visual environment (Hung et al., 1995; Irving et al., 1992; Schaeffel et al., 1988; Siegwart and Norton, 1993; Wallman et al., 1978). This modulation process, called emmetropisation, has been studied experimentally with induced refractive errors; myopia can be induced by covering young eyes with diffusers (form deprivation) or negative lenses (imposed hyperopic defocus), and hyperopia can be induced by the addition of positive lenses (imposed myopic defocus). Several studies show that eye growth is locally regulated, presumably by the retina (Troilo et al., 1987; Wallman et al., 1987; Wildsoet and Wallman, 1995). The most direct evidence comes from studies involving optic nerve section (ONS), used to sever the input from the retina to the brain. Even after ONS, eyes show characteristic growth responses to visual manipulations, such as form deprivation (Troilo et al., 1987; Wildsoet and Wallman, 1995). Nonetheless, there are subtle changes in eye growth after ONS, such as the observation that otherwise untreated ONS eyes show a choroidal thickening that is coupled to increased hyperopia (Wildsoet, 2003).

The ONS-induced changes to eye growth may either suggest a central influence on eye growth or be secondary to associated retinal changes, or both. In rats and birds, ONS leads to the loss of retinal ganglion cells (RGCs), leaving behind a small population of displaced amacrine cells and glia (Ehrlich, 1981; Lee et al., 2003; Muchnick and Hibbard, 1980; Perry et al., 1984). This loss of RGCs is likely to lead to some rewiring in the inner retina (Lee et al., 2005), possibly explaining the eye growth changes observed with ONS. However, in birds, ONS also severs the centrifugal fibres that project from the isthmo-optic nucleus (ION) to a subset of retinal amacrine cells (Cowan and Powell, 1963), changes to which have possible implications for eye growth (Fischer et al., 1999a).

To understand more about the effect of the loss of RGCs in the ocular growth abnormalities, we took advantage of the observation that colchicine, a cytotoxin, also destroys retinal ganglion cells in chicks when injected intravitreally within 24 hr of hatching (Fischer et al., 1999b; Morgan, 1981). Thus intravitreal colchicine injection represents an alternative way of disconnecting the brain from the eye. In contrast to the effects of ONS, however, colchicine-treated eyes show increased rather than reduced axial elongation, as well as corneal flattening (Fischer et al., 1999b). These differences suggest that colchicine affects eye growth in a different manner than ONS, which, in turn suggests that the emmetropisation process may also be different in these eyes. This study sought to characterise the effect of colchicine on emmetropisation in young chicks.

METHODS AND MATERIALS

White leghorn (*Gallus gallus domesticus*) chicks less than 1-day old received a 20 µl intravitreal injection of 25 mM colchicine in phosphate buffered saline (PBS) in one eye and PBS in the fellow contralateral eye (n=19). After 10 days, a subset of the colchicine-injected eyes were fitted with a +10 dioptre (D) lens (n=7) or a -10 D (n=6) lens. The fellow saline-injected non-lens-treated eyes served as controls. An additional set of control animals received bilateral injections of PBS within 1 day of hatching and one eye was subsequently fitted with either a +10 D (n=5) or a -10 D lens (n=5) 10 days later. The fellow non-lens-treated eyes again served as controls. The groups are summarised in Table 1.

Streak retinoscopy was used to measure refractive errors, which were expressed in spherical equivalent terms. High frequency A-scan ultrasonography was used to measure the axial dimensions of the various ocular components (anterior chamber, crystalline lens, vitreous chamber, retina, choroid and sclera). All measurements were made just prior to lens-wear, and 3, 5, 8, 13 and 15 days thereafter. Lenses were removed after 15 days and eyes were allowed

to recover for another 8 days before repeating refractive error and axial measurements (23 days after the start of the experiment).

To determine how colchicine affected lens compensation, the changes in lens-treated colchicine-injected eyes were compared to those of lens-treated saline-injected eyes. Negative lens- and positive lens-treatments were examined separately. Two-way repeated measures ANOVAs were used, with an alpha value of $p < 0.05$. Prior to comparison, values for colchicine-injected birds (groups I to III, Table 1) were adjusted so that for each eye, the baseline mean for each bird group matched the overall baseline mean (grand mean of groups I to III). Since this method involves subtracting the difference between the group mean and grand mean from each value, intragroup variability (standard deviation) remains unchanged as do the relative changes over time. For saline only-injected birds (groups IV and V, Table 1), values were also adjusted so that the baseline means for each eye of each lens group matched the overall baseline mean for all eyes. To account for any pre-existing differences between the treated and their fellow eyes prior to lens-wear, interocular differences, calculated as treated eye minus fellow eye changes, were compared as a function of time and lens group. Post-hoc multiple comparisons of interocular differences were limited to differences with respect to the initial (day 0) values (Dunnett's test at $p < 0.05$). Changes to the components that were affected by lens-wear, colchicine or both lens-wear and colchicine are reported.

To assess whether colchicine affected eye growth during recovery from lens-induced changes, differences between day 23 and day 15 were analysed as a function of lens group or drug injection. Only components that were significantly affected by lens-wear and/or colchicine are reported.

All means, unless otherwise noted, are reported with standard deviations.

RESULTS

Colchicine effects on eye growth prior to lens-wear

Qualitative examination of cryosections of retinas from a subset of the non lens-wearing colchicine-injected birds (group III, $n=6$) confirmed that colchicine had deleterious effects on the retinal ganglion cells compared to saline, with reductions in both the number of RGCs as well as the thickness of the nerve fibre layers (Fig. 1). Cells that were left in colchicine-exposed retinas are presumed to be displaced amacrine cells.

Prior to wearing the defocussing lenses, colchicine-injected eyes ($n=19$; groups I to III) exhibited thinner crystalline lenses ($p < 0.0001$; mean interocular difference $\pm$ SD: -0.056 ± 0.037 mm), longer vitreous chamber depths ($p=0.0012$; $+0.183 \pm 0.206$ mm) and thinner retinas ($p < 0.0001$; -0.039 ± 0.012 mm) than their fellow saline-injected eyes (Fig. 2). The optical axial lengths (OL; equal to anterior chamber depth + lens thickness + vitreous chamber depth) of colchicine-injected eyes were also significantly longer ($p=0.0185$; $+0.089 \pm 0.149$ mm), but their refractive errors were not significantly different from those of their fellow saline-injected eyes ($p=0.6588$; mean refractive error $\pm$ SD: $+3.80 \pm 1.19$ D vs. $+3.66 \pm 0.87$ D respectively).

Lens-induced changes not affected by colchicine

Of the -10 D lens-wearing birds, significant changes to refractive error (RE) were observed only for the saline-injected birds, which showed significantly more myopia than their fellow eyes after 5 days of lens-wear (mean difference: -4.45 ± 1.62 D; $p=0.0010$). In contrast, REs in colchicine-injected -10 D lens eyes did not differ from those of their respective fellow eyes as a function of time ($p=0.5913$). Although interocular RE differences appeared to be attenuated in -10 D colchicine-treated birds compared to -10 D saline-injected birds (Fig. 3A), these differences were not statistically significant ($p=0.1646$) indicating that colchicine had no effect

on negative lens compensation. Colchicine also had no effect on positive lens-induced RE changes (Fig. 3B). RE changes in both saline- and colchicine-injected birds were similar ($p=0.7990$), with REs in the treated eyes of both groups showing significantly more hyperopia than their fellow eyes after 3 days of lens treatment (mean differences: $+8.95 \pm 1.15$ D; $p<0.0001$ and $+8.80 \pm 0.62$ D; $p<0.0001$, respectively). In both cases, the relative hyperopia was sustained throughout the duration of the lens treatment period ($p<0.0001$ for all other time points for both groups; Fig. 3). Note that although colchicine did not affect lens compensation, as measured optically (*i.e.*, by measuring REs), the potential for changes in the individual ocular components to all together neutralise the refractive error does not preclude the possibility of colchicine effects on ocular growth.

Lens-induced changes similarly affected by colchicine

Both anterior segment components, the anterior chamber and crystalline lens, were similarly affected by colchicine. Anterior chamber (AC) depths in saline-injected -10 D lens-treated birds changed significantly over time ($p<0.0001$), with AC depths of treated eyes significantly longer than those of their fellow eyes by 5 days of lens-wear (mean difference: $+0.129 \pm 0.066$ mm; $p=0.0014$). In contrast, interocular AC depth differences in colchicine-injected -10 D lens-wearing birds did not change as a function of time ($p=0.4625$). The AC depth changes in the two sets of -10 D lens-wearing birds were significantly different ($p=0.0285$), indicating that colchicine had suppressive effects on the AC depth expansion normally observed negative lens-wearing birds (Fig. 4A). Colchicine had similar suppressive effects on AC expansion in $+10$ D lens-treated birds (Fig. 4A). In colchicine-injected $+10$ D lens-wearing birds, AC depths in lens-treated eyes became significantly shorter than those in their fellow eyes ($p=0.0205$), showing significant relative changes after 13 days of lens treatment (mean difference: -0.129 ± 0.113 mm; $p=0.0006$). In contrast, interocular AC depth changes in saline-injected $+10$ D lens-treated birds did not significantly change as a function of time ($p=0.5564$). Note that for both negative and positive lens compensation, the AC expansion of lens-treated eyes were relatively slower than those of their fellow eyes in colchicine-injected birds, while AC depth changes were opposite in saline-injected birds, showing relatively faster expansion in the treated eyes (Fig. 4A).

A general colchicine effect was also observed for crystalline lens (LN) thickness changes (Fig. 4B), with colchicine suppressing the lens thinning that is normally observed for both negative ($p=0.0040$) and positive lenses ($p<0.0001$). -10 D lens-wearing saline-injected birds showed significant interocular LN thickness changes as a function of time ($p<0.0001$), with lenses of the treated eyes significantly thinner by 5 days of lens-wear (mean difference: -0.059 ± 0.016 mm; $p=0.0045$); -10 D lens-wearing birds injected with colchicine did not change over time ($p=0.9224$). Both sets of $+10$ D lens-wearing birds showed significant LN thickness changes over time (saline: $p<0.0001$; colchicine: $p=0.0056$), however, the patterns of LN thinning differed (Fig. 4B), with significant interocular LN thickness changes occurring at 3 days of lens-wear for saline-injected birds (mean difference: -0.083 ± 0.031 mm; $p<0.0001$) but after 15 days of lens-wear for colchicine-injected birds (mean difference: -0.065 ± 0.046 mm; $p=0.0030$).

Lens-induced changes differentially affected by colchicine

Since refractive errors (REs) are an optical measure of the mismatch between the refractive power of the eye and the optical path length from cornea to retina, changes in optical axial (or path) length (OL; distance from retina to cornea) were included in the analyses. Linear regressions showed that interocular OL differences were negatively correlated with interocular RE differences (data not shown), for both lens-treated colchicine-injected birds ($r^2=0.7720$) and lens-treated saline-injected birds ($r^2=0.8285$). Like the RE changes, significant OL changes in -10 D lens-treated birds were only observed for those that had been injected with saline

($p < 0.0001$); OLs in the treated eyes of these birds became significantly longer compared to their fellow eyes after 3 days of lens treatment (mean difference: $+0.232 \pm 0.104$ mm; $p = 0.0290$). In contrast, interocular OL differences in -10 D lens-treated birds did not change as function of time ($p = 0.2144$; Fig. 5A), indicating that the initial colchicine-induced interocular OL difference between the eyes was maintained throughout the duration of lens-wear. Colchicine significantly suppressed the increase in OL typically seen with negative lenses ($p = 0.0102$; Fig. 5A) but had no effect on the positive lens-induced reductions in OL ($p = 0.1495$; Fig. 5B). Both groups of $+10$ D lens-treated birds showed significant reductions in interocular OL differences ($p < 0.0001$ for both) with significant interocular OL differences occurring after 3 days of lens treatment for both groups (-0.511 ± 0.087 mm; $p < 0.0001$ and -0.501 ± 0.075 mm; $p < 0.0001$, respectively).

The changes in optical axial length were predominantly attributable to vitreous chamber (VC) depth changes and thus the pattern of VC depth changes was generally similar to that of OL changes (Fig. 5C). Like the OL changes, VC expansion in saline-injected -10 D lens-treated eyes was greater relative to that in their fellow eyes ($p = 0.0002$), with significant interocular VC depth differences occurring after 5 days of lens treatment ($+0.325 \pm 0.113$ mm; $p < 0.0031$). However, unlike the changes for the OL, interocular VC depth differences in -10 D lens-treated colchicine birds also significantly increased as a function of time ($p = 0.0001$), with significant increases occurring after 8 days of lens wear ($+0.109 \pm 0.132$ mm; $p = 0.0397$; Fig. 5C). Despite both -10 D lens groups showing significant VC depth differences, VC depth changes of the colchicine-injected birds were significantly smaller than those of the saline-injected birds ($p = 0.0286$), indicating that colchicine had suppressive effects on negative lens compensation (Fig. 5C). In contrast, and like the OL results, positive lens compensation in this ocular component was not affected by colchicine ($p = 0.5698$; Fig. 5D). For the $+10$ D lens-treated groups, the interocular differences in VC depth changed were significantly reduced by 3 days of lens-wear for both saline- and colchicine-injected birds (mean differences: -0.465 ± 0.069 ; $p < 0.0001$ and -0.466 ± 0.063 mm; $p < 0.0001$, respectively).

Changes in interocular choroidal thickness were only observed in the two $+10$ D lens bird groups (both $p < 0.0001$; Fig. 6). For both saline- and colchicine-injected birds, the choroidal thickness differences between treated and fellow eyes peaked after 3 days of lens treatment (mean difference: $+0.414 \pm 0.049$; $p < 0.0001$ and $+0.266 \pm 0.069$; $p < 0.0001$, respectively) before returning to the pre-lens value at day 13 ($p = 0.0771$) and day 8 ($p = 0.1651$), respectively. While both groups of $+10$ D lens-treated birds showed similar patterns of choroidal changes (Fig. 6B, colchicine significantly attenuated this choroidal thickness response ($p = 0.0021$)).

Effects of lens-wear and colchicine on axial lengths at the end of the lens treatment period

The overall changes to the lens-treated eyes at the end of the lens treatment period (and prior to the recovery period) are summarised in Figure 7. The length or depth of each axial component of the treated eyes was expressed as a percentage of the total length of its fellow eye. Thus, it can be noted that although elongation was not as great for -10 D lens-treated colchicine-injected eyes, it was still relatively longer than its fellow eye. Note too that for $+10$ D lens-treated eyes, the overall total lengths were similar.

Changes during recovery unaffected by colchicine

Both saline- and colchicine-injected birds recovering from -10 D lens-wear showed minimal changes in interocular refractive error (RE) after 8 days of recovery (mean difference: $+2.950 \pm 3.143$ D; $p = 0.1038$ and $+1.000 \pm 3.145$ D; $p = 0.4714$, respectively; Fig. 8A). These insignificant hyperopic shifts were similar ($p = 0.3325$), indicating that colchicine had no effect on refractive error changes during the recovery period. For birds recovering from $+10$ D lens-wear, both saline- and colchicine-injected birds showed significant myopic shifts (mean

difference -9.500 ± 1.428 D; $p < 0.0001$ and -8.232 ± 2.958 D; $p = 0.0003$, respectively) but as was the case for those recovering from -10 D lens-wear, colchicine did not affect refractive error changes ($p = 0.3999$; Fig. 8B).

Changes during recovery affected by colchicine

Colchicine had differential effects depending on whether eyes were recovering from positive or negative lens-wear. Neither saline- nor colchicine-injected birds treated with -10 D lenses showed significant interocular changes in crystalline lens thickness during the recovery period ($+0.032 \pm 0.032$ mm; $p = 0.0874$ and -0.023 ± 0.048 mm; $p = 0.2977$, respectively) and although the interocular changes in the two groups were opposite, with treated eyes of the saline group showing more, rather than less crystalline lens thickening than their fellow eyes, the two groups were not significantly different ($p = 0.0578$), indicating that colchicine did not have effects on this compartment during the recovery period. Like the patterns observed for the two -10 D lens-treated groups, saline-injected $+10$ D lens-treated eyes showed more crystalline lens thickening relative to their fellow eyes ($+0.067 \pm .037$ mm; $p = 0.0157$) while colchicine-injected $+10$ D lens-treated eyes showed very little interocular lens thickness differences during the recovery period. (-0.005 ± 0.056 mm; $p = 0.8367$). However, for the $+10$ D lens-treated groups, these differences were significant ($p = 0.0330$), indicating that colchicine had suppressive effects on crystalline lens growth during recovery from $+10$ D lenses. Although colchicine did not affect crystalline lens changes during recovery from -10 D lens-wear, the trends of growth were similar for both -10 D and $+10$ D lenses.

While both saline- and colchicine-injected birds recorded insignificant OL changes during recovery from -10 D lenses (-0.277 ± 0.277 mm; $p = 0.0891$ and $+0.137 \pm 0.209$ mm; $p = 0.1710$, respectively), colchicine had a significant effect on OL changes ($p = 0.0199$) owing to the opposite trends in growth (Fig. 9A); colchicine, in effect, reversed the slower expansion of treated eyes relative to their fellow eyes that was observed in saline-treated birds, causing, instead, relatively more expansion of the OL in eyes recovering from -10 D lenses (Fig. 9A). Unlike the -10 D lens-treated groups, both $+10$ lens-treated birds showed significant interocular OL changes during the recovery period ($+0.772 \pm 0.154$ mm; $p = 0.0004$ and $+0.696 \pm 0.184$ mm; $p < 0.0001$, respectively) that were not different from each other ($p = 0.4800$; Fig. 9B). Thus, colchicine had no effect on OL changes during $+10$ D lens-wear, nor during recovery from $+10$ D lens-wear.

As with lens-wear, much of the OL changes over the recovery period were attributable to vitreous chamber (VC) depth changes and VC changes during recovery also mirrored those of OL changes during the same time period. Thus, once again, interocular vitreous chamber depth differences were not significant during the recovery period for -10 D lens-wearing birds (saline: -0.222 ± 0.278 mm; $p = 0.0891$ and colchicine: $+0.119 \pm 0.199$ mm; $p = 0.1710$) but their opposite trends resulted in a significant difference between the groups ($p = 0.0416$; Fig. 9C), with colchicine-treated eyes showing relatively more, rather than less, VC expansion relative to their fellow eyes. $+10$ D lens-treated eyes showed significantly greater VC expansion compared to their fellow eyes during recovery (saline: $+0.660 \pm 0.192$ mm; $p = 0.0015$ and colchicine: $+0.677 \pm 0.105$ mm; $p < 0.0001$) and again, no difference was observed between the two groups of $+10$ D lens-wearing birds ($p = 0.4731$; Fig. 9D).

The effects of colchicine on positive and negative lens compensation on the various ocular components, during lens-wear and recovery, are summarised in Table 2.

DISCUSSION

Our results confirm that colchicine perturbs normal eye growth, but in a manner different from retinal ganglion cell axotomy (ONS). As reported by others, colchicine by itself (without any

lenses) was associated with expansion of the vitreous chamber (Fischer et al., 1999b). In contrast, retinal ganglion cell axotomy, which initiates choroidal thickening, is generally coupled to slowed eye elongation. In our study, colchicine eliminated most of the ganglion cells but its effects on other cells, such as the amacrine cells were not tested. A previous study by Fischer et al. (1999b) indicates that in addition to retinal ganglion cells, colchicine eliminates specific subsets of amacrine cells, some of which have been implicated to be involved in ocular growth. Of note was their finding that the tyrosine hydroxylase-immunoreactive amacrine cells, a source of dopamine in the retina, were eliminated by intravitreal colchicine. The evidence linking retinal dopamine and lens-induced myopia is indirect; intravitreal injection of apomorphine, a dopamine agonist, inhibits lens-induced and form deprivation myopia in young chicks (Schmid and Wildsoet, 2004). While we did not test for changes in retinal dopamine levels, the current result for negative lenses would predict reduced retinal dopamine levels in colchicine-injected eyes. Finally, colchicine has effects that are not cell-specific, such as disruption of microtubular function in all cells, and thus may have exerted its effects by altering the physiology of the cells that remained in the retina.

The findings that prior to lens-wear, colchicine-injected eyes exhibited longer vitreous chamber depths and optical axial lengths than their fellow saline-injected eyes, but were not more myopic might be attributable to reduced corneal power (Padmanabhan et al., 2007) or reduced crystalline lens power, or both. Colchicine-injected eyes showed thinner lenses than their fellow eyes, so it is likely that their surfaces were flatter and therefore power was reduced. In a similar vein, colchicine-treated eyes had shallower than normal anterior chambers, which is typically coupled with flatter corneas, although corneal curvature was not measured in the current study. Moreover, our finding of slightly shallower anterior chamber depths in colchicine-injected eyes is consistent with the results of Fischer and colleagues for 14 day-old colchicine-injected birds (1999b). Notably, in our study, anterior chamber depth also had a greater influence on the optical axial lengths of colchicine-injected eyes compared to saline-injected eyes. Thus, while vitreous chamber changes were well-correlated with OLs in saline-injected eyes ($r^2 = 0.9627$; Fig. 10B, inset), these parameters were not as strongly correlated in colchicine-injected eyes ($r^2 = 0.6730$; Fig. 10A, inset). On the other hand, adding anterior chamber data to vitreous chamber data improved the correlation with OLs for colchicine-injected eyes ($r^2 = 0.9822$; Fig. 10A) but did not have much effect for saline-injected eyes ($r^2 = 0.9768$; Fig. 10B).

In our experiment, colchicine had less effect on refractions than previously reported by Fischer and colleagues (1999b). While in both their and our studies, colchicine-injected eyes were significantly longer than their fellow eyes, in the current study, colchicine-injected eyes were slightly, though not significantly, more hyperopic (see Results above), with Fischer and colleagues (1999b) reporting an interocular refractive error difference of approximately -5.25 ± 1.65 D at 14 days of age (compared to $+0.606 \pm 0.674$ D at 13 days of age in our experiments). The similarity in refractive errors between treated and fellow eyes was not related to the time after exposure to colchicine since the refractive errors were relatively stable as a function of age over the pre-lens treatment period (data not shown). The attenuated effects of colchicine are also apparent for axial length data of our study; colchicine eyes were significantly longer than their fellow eyes by 0.020 ± 0.110 mm at 13 days of age, but this difference was about 4 to 5 times smaller than the difference reported by Fischer and colleagues (approximately 0.95 ± 0.26 mm). The origins of the differences between these two studies might be related to differences in the strain or breed of the birds used or in the effectiveness of the colchicine in eliminating certain amacrine cells, such as the dopaminergic subset. However, since the effects of colchicine on amacrine cells were not examined in this study, this thought remains speculative.

Given that compensation to negative lenses requires eyes to increase their growth, the finding that negative lens-treated colchicine-injected eyes failed to elongate indicates that colchicine

suppresses the ability to respond to negative lenses. These results are consistent with a report showing a reduced ability of colchicine-injected eyes to elongate in response to form deprivation (diffusers) compared to saline-injected eyes (Fischer et al., 1999b). In contrast, the responses of colchicine-treated eyes to positive lenses was nearly normal in this study, with the exception of a slightly suppressed choroidal response.

The results of the current study show many parallels with results of a closely related study by Padmanabhan and colleagues (2007) involving birds reared in constant light (CL). Without defocusing lenses, these eyes also showed increased elongation of their vitreous chambers and reduced elongation of their anterior chambers. They also showed nearly normal responses to positive lenses but impaired compensation to negative lenses. However, there were subtle differences in the way their eyes responded to some of the visual cues. One was that CL-reared birds showed an enhanced response to positive lenses, as indicated by an increased expansion of the choroid, while colchicine was associated with a reduction in the amount of choroidal expansion. However, because colchicine-injected eyes showed similar refractive error changes to saline-injected eyes, the reduced choroidal response of colchicine-injected eyes likely reflects an increased compensatory response from one or more other ocular components. Another difference was in the ability of eyes to recover from the effects of negative lens-induced changes; this ability was retained in CL, but was suppressed in colchicine-injected eyes. Part of this apparent difference in behavior may be due to the magnitude of the changes from which eyes were recovering; these changes were very small in the case of the colchicine-injected eyes and thus the impetus to elongate during recovery may have been attenuated.

Like the constant light-exposed birds examined by Padmanabhan and colleagues (2007), there was an inherent asymmetry in the responses of eyes to lens-imposed defocus and their recovery from induced refractive errors, a separate source of retinal defocus. Specifically, colchicine-injected eyes were able to increase their growth to recover from the hyperopia induced by positive lenses, but were unable to compensate to negative lens-induced hyperopic defocus. These results add support to the idea that the mechanisms mediating these two types of responses are different (Padmanabhan et al., 2007; Schaeffel and Howland, 1991). We speculate that the recovery responses in the current study are associated with a non-visual mechanism that is highly sensitive to departures from normal eye shapes (Schaeffel and Howland, 1991). Eyes may have shape memories, which allow them to return to their “natural” shape when the inducing stimuli are removed, *i.e.*, during recovery. Since recovery need not be visually regulated, it would be less dependent on the functional state of the retina. In contrast, normal-sized eyes fitted with defocussing lenses must first decode the sign of the defocus, and then are required to grow away from their natural or “default” shape. Note that the results for both CL and colchicine-injected eyes suggest that the “default” shapes can be reset by the environmental conditions; removal of the lenses resulted in a return of eye lengths to non-lens CL conditions and colchicine-injected conditions, respectively. Moreover, in the case of eyes exposed to CL, the “CL condition” is not permanent, as eyes were shown to recover from the effect of CL when birds were returned to normal diurnal lighting. Circulating growth modulatory substances presumably contribute to this shape regulation. Alternative explanations, including the different states of the retinal circuitry, have been suggested by others (Ashby et al., 2007).

One of the motivations for the current study was to gain some insight into the role of retinal ganglion cells and/or higher centres in early eye growth regulation. The responses to defocussing lenses of colchicine-injected eyes are opposite to those of eyes that underwent ONS and were later fitted with defocussing lenses (Wildsoet, 2001); in the latter case, responses to negative lenses are nearly normal, but those to positive lenses are impaired. Note too, that in eyes left without lenses, the growth responses are also opposite, *i.e.*, ONS-eyes exhibited decreased elongation while colchicine-injected eyes showed increased elongation. These

results may suggest that the pre-existing rate of eye growth limits further growth changes in the same direction, as hinted at in a previous study by Wildsoet and Wallman (1997). Thus, in the current study, colchicine-injected eyes, which were all ready myopic, could not be made more myopic with negative lenses.

In summary, the results of this study show that colchicine-induced loss of retinal ganglion cells results in increased eye growth and impaired emmetropisation to negative lenses. Compensation to positive lenses remains mostly unchanged. These altered growth responses, both with and without lenses, are opposite to those of eyes for which retinal ganglion cell loss was induced by axotomy (optic nerve section). Clearly, the differences in retinal cell loss that are specific to the two methods play a role in how eye growth is altered. More detailed investigations of the cellular and molecular changes to the retina in these eyes are warranted.

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Figure 1.

Representative micrographs of toluidine-stained retinas from (A) a colchicine-injected and (B) a saline-injected eye. Note loss of cells in the ganglion cell layer (GCL) and loss of the nerve fibre layer (NFL) in the colchicine-exposed retina. Bar = 50 μ m.

Figure 2.

Bar graphs of mean total axial lengths of colchicine-treated and their fellow eyes prior to lens-wear. Colchicine-treated eyes (top bar) exhibited thinner lenses (red; $p=0.0014$), longer vitreous chamber depths (green; $p=0.0006$) and thinner retinas (yellow; $p<0.0001$). Vitreous chamber effects contributed to longer optical axial lengths ($p=0.0025$; front of cornea to retina) in these eyes. Legend: black - cornea; red - crystalline lens; green - vitreous chamber; yellow - retina; blue - choroid; purple - sclera

Figure 3. Interocular refractive error differences $\pm$ s.e.m. of (A) -10 D lens-treated colchicine-injected (red squares) and saline-injected (purple up-triangles) birds and (B) +10 D lens-treated colchicine-injected (green diamonds) and saline-injected (teal down-triangles) birds. Colchicine had no effect on RE changes during the lens treatment period.

Figure 4.

Interocular anterior chamber (AC) depth (**A**) and crystalline lens (LN) thickness (**B**) differences $\pm$ s.e.m. in colchicine-injected (green diamonds, red squares) and saline-injected (teal down-triangles, purple up-triangles) birds wearing +10 D and -10 D lenses, respectively. Compared to saline-injected eyes (both triangles), colchicine eyes (diamonds, squares) showed relatively negative interocular AC depth differences (**A**), an opposite finding to that in saline-injected eyes and (**B**) suppressed lens thickening.

Figure 5.

Interocular (A,B) optical axial length (OL) and (C,D) vitreous chamber (VC) depth differences differences $\pm$ s.e.m. in colchicine-injected (green diamonds, red squares) and saline-injected (teal down-triangles, purple up-triangles) birds wearing +10 D and -10 D lenses, respectively. Colchicine suppressed negative lens compensation in these two components (A,C: red vs. purple; OL: $p=0.0102$, VC: $p=0.0286$).

Figure 6. Interocular choroidal thickness differences $\pm$ s.e.m. of (A) -10 D lens-treated colchicine-injected (red squares) and saline-injected (purple up-triangles) birds and (B) +10 D lens-treated colchicine-injected (green diamonds) and saline-injected (teal down-triangles) birds. Colchicine suppressed positive lens compensation in this ocular component (B: green vs. teal; $p=0.0021$).

Figure 7.

Axial lengths of (A,A') -10 D lens- and (B,B') +10 D lens-treated eyes, expressed as a percentage of the total length of the fellow eye, at the end of the lens treatment period. Note that although changes in colchicine-injected eyes (A) were not as great as for saline-injected eyes (A'), both -10 D lens-treated eyes were longer than their fellow eyes. Note also, the similar lengths of the +10 D lens-treated eyes (B,B'). Legend: black - cornea; red - crystalline lens; green - vitreous chamber; yellow - retina; blue - choroid; purple - sclera.

Figure 8. Interocular refractive error (RE) differences $\pm$ s.e.m. during recovery from (A) -10 D lens-wear and (B) $+10$ D lens-wear in colchicine- (red squares, green diamonds) and saline-injected (purple down- and teal up-triangles) birds, respectively. Colchicine had no effect on RE changes during the recovery period.

Figure 9.

Interocular (A,B) optical axial length (OL) and (C,D) vitreous chamber (VC) depth differences $\pm$ s.e.m. during recovery from -10 D (A,C) and +10 D (B,D) lens-wear in colchicine-injected (green diamonds, red squares) and saline-injected (teal down-triangles, purple up-triangles) birds, respectively. While both -10 D lens-treated groups showed no significant OL (A) or VC (B) changes during the recovery period, their trends in growth were opposite, indicating that colchicine had effects on this ocular component during recovery (OL: $p=0.0199$; VC: $p=0.0416$).

Figure 10.

Plots showing the contribution of the anterior chamber and vitreous chamber depths to optical axial lengths for (A) colchicine-injected and (B) saline-injected eyes. Correlations of interocular optical axial length differences with interocular vitreous chamber depth differences only were poorer for colchicine-injected eyes (A: inset) but were relatively unchanged for saline-injected eyes (B: inset).

Table 1
Summary of the treatment details for the five groups of birds used in this study.

Bird group	Number	Treated Eye		Fellow Eye	
		Injection	Lens	Injection	Lens
I	6	colchicine	-10 D	saline	none
II	7	colchicine	+10 D	saline	none
III	6	colchicine	none	saline	none
IV	5	saline	-10 D	saline	none
V	5	saline	+10 D	saline	none

Table 2

Summary of colchicine effects on lens compensation and recovery from lens-wear in the various ocular components. Differences are noted relative to interocular (treated eyes - fellow eye) changes.

Ocular component	Effects on lens-wear		Effects on recovery from lenses	
	-10 D lens	+10 D	-10 D lens	+10 D
Refractive error	none	none	none	none
Optical axial length	↓ relative expansion	none	opposite effect (relative expansion)	none
Vitreous chamber	↓ relative expansion	none	opposite effect (relative expansion)	none
Choroid	none	↓ relative thickening	none	none
Anterior chamber	↓ relative elongation	relative shortening	none	none
Crystalline lens	↓ relative thinning	↓ relative thinning	none	↓ relative thickening