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Parasympathetic influences on emmetropization in chicks: Evidence for different mechanisms in form deprivation versus negative lens-induced myopia

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Abstract

Ciliary ganglionectomy inhibits the development of myopia in chicks (Schmid et al., 1999), but has no effect on the compensatory responses to spectacle lenses (Schmid and Wildsoet, 1996). This study was done to assess the potential influence of the other parasympathetic input to the choroid, the pterygopalatine ganglia, on the choroidal and axial responses to retinal defocus, and to form deprivation.

4–5 week-old chicks had one of the following surgeries to one eye: (1) Section (X) of the autonomic part of cranial N VII (input to the pterygopalatine ganglia) (PPGX, n=16), (2) PPGX plus ciliary ganglionectomy (PPG/CGX, n=23) or (3) PPGX plus superior cervical ganglionectomy (PPG/SCGX, n=10). Experimental eyes were fitted with positive or negative lenses, or diffusers, several days after surgery. In one group of PPG/CGX, eyes did not wear any devices (n=8). Intact (no surgery) controls were done for all visual manipulations (lenses or diffusers). Sham surgeries were done for the PPG/CGX condition (n=4). Ocular dimensions were measured using A-scan ultrasonography prior to the surgery, 5 days later when visual devices were placed on the eyes, at the end of lens- or diffuser- wear, and in the case of diffusers, 4 days after diffuser removal to look at “recovery”. Refractive errors were measured using a Hartinger’s refractometer. IOP was measured in 7 PPG/CGX birds 7d after surgery.

PPGX/CGX resulted in choroidal thickening (125 μm) and a decrease in IOP over one week post-surgery. It also prevented the development of myopia in response to form deprivation (X vs intact: 0.2 D vs -4.1 D; $p<0.005$), by preventing the increase in axial elongation (250 μm vs 670 $\mu\text{m}/5\text{d}$; $p<0.005$). In fact, growth rate slowed below normal (X vs fellow eyes: 250 μm vs 489 $\mu\text{m}/5\text{d}$; $p=0.002$). By contrast, there were no effects of this lesion on the development of myopia in response to negative lenses (X vs intact: -5.4 D vs -5.3 D). All three lesions inhibited the compensatory choroidal thickening in response to myopic defocus (ANOVA, $p=0.0008$), but had no effect on the thinning response to hyperopic defocus. These results argue for different underlying mechanisms for the growth responses to form deprivation versus negative lens wear. They also imply that choroidal thickening and thinning are not opposing elements of a single mechanism.

Keywords

pterygopalatine ganglia; ciliary ganglion; form deprivation; myopic defocus; choroid; parasympathectomy

Introduction

Experiments on animal models have shown that the eye uses visual input to modulate its growth, resulting in a match between the optics (cornea and lens) and the length of the eye, so that the image plane falls on the retina, a process called emmetropization (review: Wallman and Winawer, 2004). This is a dynamic process of continual adjustment of the growth of the back of the eye, by means of alterations in scleral growth in response to changes in the plane of focus of the image (Rada et al., 1992). In chickens, another component of emmetropization is the choroid, which can increase its thickness by hundreds of microns in response to myopic defocus (image in front of the retina) and can thin by about 100 μm in response to hyperopic defocus (image behind the retina) (Wallman et al., 1995; Wildsoet and Wallman, 1995). These choroidal thickness changes are seen in mammals as well, although they are smaller (Hung et al., 2000; Troilo et al., 2000; Howlett and McFadden, 2009). While this phenomenon is of interest because of its functional role in emmetropization, acting as a focusing mechanism intermediate in speed between accommodation and the changes in the length of the eye, arguably more important is the consistent association of changes in choroidal thickness with changes in eye growth, which may indicate a role for the physiological state of the choroid in eye growth regulation (Nickla, 2007; Nickla and Wallman, 2010). Specifically, increases in choroidal thickness are associated with ocular growth inhibition, while decreases in thickness are associated with growth stimulation. If these two systems are indeed mechanistically linked, it is of crucial importance to determine the mechanism(s) underlying the choroidal changes in thickness, because it would give us insight into the mechanisms regulating the visual control of ocular growth, the critical determinant of refractive error development.

It is well known that ocular growth is largely locally regulated: optic nerve section (disconnecting the retina from the brain) has little effect on the responses to form deprivation (Troilo et al., 1987; Wildsoet and Pettigrew, 1988) or lens-induced myopic defocus, however, both the axial and choroidal responses to lens-induced hyperopic defocus are attenuated (Wildsoet and Wallman, 1995). Furthermore, sectioning of the ciliary nerves, which comprise the main parasympathetic input to the choroid in birds (Reiner et al., 1983; Fitzgerald et al., 1990; Fitzgerald et al., 1996), reduces the ocular growth stimulation in response to form deprivation (Schmid et al., 1999), but has no effect on the compensatory responses to either negative lens-induced hyperopic defocus or positive lens-induced myopic defocus (Schmid and Wildsoet, 1996). Together these findings suggest a role for the ciliary ganglion in emmetropization. We speculate that it may also influence the choroidal responses as well.

The other parasympathetic input to the choroid is the pterygopalatine ganglion (PPG), which, in birds, is a series of microganglia within the orbit (Baumel, 1975; Nickel et al., 1977; Gienc and Kuder, 1985). The axon terminals in the choroid from both the ciliary and the pterygopalatine ganglia contain nitric oxide synthase (Uddman et al., 1980; Stone et al., 1987; Yamamoto et al., 1993; Alm et al., 1995; Stubinger et al., 2010; Cuthbertson et al., 1997), and synapse in close apposition to non-vascular smooth muscle (NVSM) (Poukens et al., 1998; Stubinger et al., 2010; Schroedl et al., 2003). Because NO is a smooth muscle relaxant, it might play a role in changing choroidal thickness by regulating the tonus of the

choroidal NVSM (Wallman et al., 1995; Poukens et al., 1998; Schmid et al., 1999; Nickla and Wallman, 2010).

To further elucidate the role(s) of the parasympathetic system in emmetropization, we sectioned the autonomic part of N VII (Schroedl et al., 2006), which comprises the pre-ganglionic input to the PPG. We also combined this deafferentation (hereafter referred to as PPGX) with ciliary ganglionectomy (double parasympathectomy; PPG/CGX). Finally, because the effects of sympathectomy on emmetropization have not been tested, we combined PPGX with superior cervical ganglionectomy (PPG/SCGX). Superior cervical ganglionectomy would remove adrenergic input to the choroid. We examined the ocular responses to lens-induced myopic and hyperopic defocus, and to form deprivation and recovery from form deprivation-induced myopia, by measuring the changes in axial length and choroidal thickness using A-scan ultrasonography; we also measured end refractive errors. Some of these results have been presented in abstract form (Nickla and Schroedl, 2008; Nickla & Schroedl, 2011).

Methods

Subjects and general design

Subjects were White Leghorn chickens (*Gallus gallus domesticus*; Cornell-K strain; Cornell University, Ithaca, NY), hatched in an on-site incubator and raised in temperature-controlled brooders. The light cycle was 12L/12D (8:00 am to 8:00 pm). Food and water were supplied ad libitum. In all experiments, the right eye was the experimental one (surgery and/or visual manipulation) and the left eye served as the untreated fellow control. There are no known left/right eye differences. Care and use of the animals conformed to the ARVO Resolution for the Care and Use of Animals in Ophthalmic and Vision Research.

Surgical Procedures

On the right side of birds, aged 4–5 weeks, we performed one of three surgical procedures: lesions (X) of the autonomic facial nerve (here--within referred to as N VII) pre-synaptic to the pterygopalatine ganglia (PPGX) (Schroedl et al., 2006), double lesions of N VII and the superior cervical ganglion (PPG/SCGX), or double lesions of N VII and the ciliary ganglion (PPG/CGX). Surgery was done under general anesthesia, using an intramuscular injection of a mixture of ketamine and xylazine (100 mg/kg, 5 mg/kg respectively; Phoenix Pharmaceutical, St. Joseph, MO). Experimental eyes were subjected to myopic defocus (positive lenses or recovery from deprivation myopia), hyperopic defocus (negative lenses) or form deprivation, as described below. Numbers of animals in each surgery group for each visual manipulation are given in Figure 1 and Tables 2 & 3.

PPGX: N. VII Section—After disinfecting the skin around the auditory canal and removing the feathers, a C-shaped incision was made 3–5 mm dorsal to the opening of the external auditory canal. Bleeding of superficial veins was stopped by cauterization. The external auditory canal was then opened from the dorsal aspect with a vertical cut. The resulting flap was brought into an anterior position, resulting in direct access to the tympanic membrane, which was incised with a 23G needle in the cranio-caudal direction just anterior to the insertion of the columella (the bird's single auditory ossicle). The underlying bony canal containing the autonomic part of the facial nerve (i.e. the palatine nerve) was thus exposed (see Fig. 1 in Schroedl et al., 2006), opened with a 23G needle, and the autonomic part of the facial nerve transected. Following erythromycin ointment treatment (Fougera, Melville, NY), the external auditory canal and superficial tissue layers were sutured, and Erythromycin ointment mixed 1:1 with a 2% Lidocaine hydrochloride jelly (Akorn, Buffalo Grove, IL) was applied. We recognize that this procedure does not technically “lesion” the

pterygopalatine microganglia, but rather de-afferents it, for the sake of simplicity, we will use “PPGX” to refer to this surgery.

PPG/SCGX and PPG/CGX—For both double-lesion surgeries, N. VII was sectioned first. The superior cervical ganglion was accessed via an incision into the neck musculature, and exposed in between cranial nerve IX and X, according to Lauber et al., (1972). To ensure the elimination of all of the post-ganglionics, the ganglion was excised, because these nerves form a dense plexus as opposed to a solid nerve, and so transection would likely be less successful. Successful removal of the ganglion resulted in a slight ipsilateral ptosis (Lauber et al., 1972; Isomura, 1973), which could be observed in all SCG lesioned animals. The ptosis was minor, and did not cover the pupil.

The ciliary ganglion was accessed from the inferior temporal aspect, to minimize damage to the orbital structures and lids. The post-ganglionic nerve fibers were cut immediately rostral to the ganglion. Details follow those in Lin et al., (1996). Successful trans-section resulted in pupillary dilation that persisted over the experimental period. Loss of accommodative function is assumed (Schmid and Wildsoet, 1996). Sham surgeries were done (n=4) for the PPG/CGX, in which both the ciliary ganglion and cranial N. VII (pre-ganglionic to the pterygopalatine ganglia) were accessed as described above, but the pathways were not lesioned. These serve as operated controls for both the PPGX alone, as well as the PPG/CGX, for the effects prior to the visual manipulation, and for positive lens-wear. Sham surgeries were deemed unnecessary for the PPG/SCGX lesion, as there were no significant effects of this lesion.

Visual Manipulations and Ocular Measurements

Ocular dimensions were measured using high frequency A-scan ultrasonography, as described in detail in Nickla et al. (1998); Figure 1 is a schematic of the time line of the measurements (arrows and arrowheads). Within each lesion group, a subset of birds (PPGX: n=7; PPG/SCGX: n=5; PPG/CGX: n=13) were measured immediately before the surgery (D0), and 5 days afterwards, to determine the effect of the lesions on “normal” eyes (“Pre” in Figure 1). In a subset of PPG/CGX birds (n=7), IOP was measured on day 1 and day 7 following the surgery, using a Tono-Pen XL tonometer (Mentor); birds were unanesthetized for this procedure.

Because there were no contralateral effects (Table 1), paired t-tests between experimental and fellow eyes were done. For the PPG/CGX only, an additional group of birds (n=8) did not wear visual devices, and were measured over the course of 3 weeks.

Lenses or diffusers were fitted to the experimental eyes (n's in Figure 1, right) between 5–7 days post-surgery. +10 D lenses (myopic defocus) were worn for 3 days, –10 D lenses (hyperopic defocus) were worn for 5 days; these intervals allow the maximal effects on ocular growth (Nickla, 2006). Sham PPG/CGX- operated controls wore +10 D lenses for 3 days. Another two groups of birds with PPGX or PPG/CGX, were fitted with translucent diffusers, to impose form deprivation (FD) for 5 days. The diffusers were then removed, and the eyes exposed to unrestricted vision for 4 days, to allow for “recovery” from the myopia. For all visual manipulations, age-matched birds served as intact (un-operated) controls (“Intact” in Tables and Figures).

Ultrasonography was done at the end of lens- or diffuser-wear, and at the end of the 4 day recovery period (Figure 1). Axial length is defined as the distance between the front of the cornea and the front of the sclera; all data for axial elongation is given as the change in length over time (rate). The same is true for the changes in choroidal thickness and anterior chamber depth (front of cornea to front of lens). Refractive errors were measured using a

Hartinger's refractometer at the end of device-wear ("off") and recovery (Figure 1). Measurements were done under isoflurane inhalation anesthesia; ultrasound measurements typically took less than 5 minutes per animal.

Statistics

The Shapiro-Wilk normality test showed borderline significance in two data sets, so we used the more conservative non-parametric version of one-way ANOVA (Kruskal-Wallis) with post-hoc comparisons, for each parameter across all groups (Tables 2 & 3). Because ANOVA showed borderline significant differences between the fellow eyes for "change in axial length" in some groups (Table 1), non-parametric paired t-tests (Wilcoxon matched pair) were done comparing experimental to fellow eyes, within each surgical condition, to test for the effect of the visual manipulations on axial elongation. Where noted, two-sample t-tests were done between lesion and "combined" fellow eyes (see below).

Results

I. Contralateral eyes

There were no significant effects of the surgeries on any parameter in the fellow (contralateral) eyes (Table 1), either prior to device-wear, or during it, with the exception of axial elongation prior to device-wear ("Pre-ax" $p=0.0177$), which is a consequence of both eyes in the PPG/CGX group growing less than the other groups (see Figure 2D). We assume that this reflects inter-experimental variability, and not a contralateral effect of the surgery, as none of the other data sets show any effect on the contralateral eyes in this PPG/CGX group. Therefore, for refractive error and choroidal thickness changes, the fellow eye data from all surgery groups, and intact controls, were combined within each visual manipulation, and are denoted "Fellow" in all graphs.

II. Effects prior to device-wear

There were two significant effects on ocular dimensions in the 5 days post-surgery, both resulting from PPG/CGX (Figure 2). First, the choroids in experimental eyes increased in thickness, compared to choroids of both fellow eyes (Figure 2A, left: $77\text{ }\mu\text{m}$ vs $1\text{ }\mu\text{m}$, paired t-test, $p<0.0005$) and sham controls ($77\text{ }\mu\text{m}$ vs $20\text{ }\mu\text{m}/5\text{d}$; two-sample t-test, $p<0.05$). Neither PPGX alone nor PPG/SCGX had any effect on choroidal thickness (all p 's >0.1). In the PPG/CGX group followed for 3 weeks (Figure 2B, top), choroids increased in thickness by $123\text{ }\mu\text{m}$ within a week, and thinned back to normal by 3 weeks (start vs end: $217\text{ }\mu\text{m}$ vs $263\text{ }\mu\text{m}$, $p=0.2$). Second, the growth of the anterior chamber was inhibited compared to that of both fellow eyes (Figure 2C: $68\text{ }\mu\text{m}$ vs $182\text{ }\mu\text{m}$; paired t-test, $p<0.005$) and sham controls ($68\text{ }\mu\text{m}$ vs $144\text{ }\mu\text{m}/5\text{d}$; two sample t-test, $p<0.05$). Neither PPGX alone nor PPG/SCGX had any effect on anterior chamber depth (Figure 2C). There was no effect on axial elongation in any of the lesion conditions (Figure 2D; all paired t-tests $p>0.1$).

PPG/CGX significantly reduced IOP by the first day after surgery (Figure 2B, bottom: 12.5 vs 16 mm Hg ; ANOVA, $p=0.004$; post-hoc Bonferroni test, $p=0.01$). IOP remained lower than normal a week later (12.7 mm Hg ; $p=0.01$).

III. Effects on responses to form deprivation and "recovery"

Form Deprivation—PPG/CGX prevented eyes from developing myopia in response to form deprivation (Table 2, Figure 3A, left, ANOVA, $p=0.0063$; PPG/CGX vs Intact: 0.2 D vs -4.1 D ; post-hoc comparison $p=0.0034$); the refractions of all 5 lesioned eyes were similar to those of fellow eyes (stacked histogram, Figure 3B, black and white bars). This effect was due to an inhibition of axial elongation (Figure 3C, ANOVA, $p=0.006$; PPG/

CGX vs Intact: 246 vs 630 μm ; post-hoc comparison $p=0.0034$). Lesioned eyes grew at a rate even slower than their fellow eyes (Figure 3C and D: 246 vs 489 μm ; paired student t-test, $p=0.0019$; Wilcoxon signed rank test, $p=0.0625$), and were significantly smaller after 5 days of deprivation (10.56 μm vs 10.757; paired t-test, $p<0.005$). There was no effect on the choroidal thinning response (Table 2, ANOVA, $p=0.641$; Figure 3E), nor was there any effect on anterior chamber growth (Table 2, ANOVA, $p=0.149$; data not shown).

PPGX had no effect on the development of myopia (Figure 3A, Table 2: $p=0.609$), nor on axial elongation (Figure 3C; Table 2: $p=0.47$). Experimental eyes grew significantly faster than fellow eyes (Figure 3C: 714 vs 489 μm ; paired t-test $p<0.05$). There was no effect on the choroidal thinning response (Figure 3E: ANOVA, $p=0.641$), nor on anterior chamber elongation (Table 2, $p=0.149$).

Recovery—Normally, eyes recover from deprivation-induced myopia after removal of the diffusers by reducing their rate of axial elongation (Wallman and Adams, 1987). In eyes with PPG/CGX, however, despite the lack of a refractive error (see Figure 3A), eyes responded to the restoration of vision by *increasing* their rate of elongation above that of intact controls (Figure 4A: ANOVA, $p=0.0329$, 602 vs 391; post-hoc comparison $p=0.009$) and fellow eyes (Figure 4B, left; paired t-test; $p=0.005$). This resulted in experimental and fellow eyes elongating by the same amount over the 9 days (848 μm vs 897 $\mu\text{m}/9\text{D}$; data not shown), ending in similar lengths (Figure 4B, right: 11.162 vs 11.164 mm; paired t-test, $p=0.98$). Furthermore, the choroids that had thinned during form deprivation (Figure 3E) now thickened by 119 μm over the “recovery” period (Figure 4C), returning to normal. There were no significant effects on the choroidal thickening response to the myopic defocus (ANOVA $p=0.0698$). All experimental choroids thickened compared to fellow eyes (two-sample t-test $p<0.05$ for all).

PPGX had no effect on the recovery response: myopic eyes returned to emmetropia (lesion vs Intact controls: -0.1 vs 1.2 ; $p=0.9$; data not shown) as the result of a decrease in growth rate (Figure 4A: lesion vs intact: 352 vs 391 μm ; $p=1.0$) and an increase in choroidal thickness (Figure 4C: 363 vs 224 μm ; $p=0.2$). There was no effect on the anterior chamber (Table 2, $p=0.248$).

IV. Effects on responses to negative lenses

In contrast to the robust effects of PPG/CGX on the development of myopia in form deprived eyes, there were no effects of this lesion on myopia development in eyes wearing negative lenses: eyes became as myopic as intact controls (Table 3; Figure 5A: ANOVA, $p=0.0502$; -5.4 D vs -5.3 D; $p=0.8075$) and grew just as rapidly (Figure 5B: ANOVA, $p=0.735$; 640 μm vs 628 μm ; $p=0.92$). The same was true for the PPGX (Figure 5A: -4 D vs -5.3 , $p=0.41$; Figure 5B: 634 vs 628 μm ; $p=0.93$). All lesioned eyes wearing negative lenses grew faster than fellow controls (2-tailed paired t-tests: $p=0.001$, 0.1, 0.03, respectively).

PPG/SCGX resulted in significantly less myopia than that of intact controls (Figure 5A: ANOVA, $p=0.0502$; -0.8 D vs -5.3 D; $p=0.0105$). This was not the result of ocular growth inhibition, because there were no significant differences in axial elongation (Figure 5B: ANOVA, $p=0.735$; 672 vs 628 μm ; $p=0.89$). We speculate that the reduced hyperopia may reflect corneal flattening.

There were no effects of any of the lesions on the choroidal thinning response to hyperopic defocus (Table 3, Figure 5C: ANOVA, $p=0.335$). (Note that the data for the PPG/CGX reflects the change that occurred *after* the initial thickening that occurred prior to lens-placement.)

V. Effects on responses to positive lenses

PPG/CGX caused two significant effects on eyes wearing positive lenses. First, there was a reduction in the refractive compensation: lesioned eyes became less hyperopic than intact controls (Figure 6A: ANOVA, $p=0.0086$; $+1.6$ vs $+6.7$ D; $p=0.003$). This was not the result of a change in axial elongation (Figure 6B; ANOVA, $p=0.563$), but rather was likely due to a reduction in the choroidal thickening compared to intact controls (Figure 6C: ANOVA, $p=0.0008$; $66\text{ }\mu\text{m}$ vs 376 ; $p=0.0038$). Note that this increase reflects that occurring *after* the pre-lens increase of $77\text{ }\mu\text{m}$, for an absolute change of $143\text{ }\mu\text{m}$). Because a similar amount of thickening occurred in birds that did not wear lenses (see Figure 2B), it is likely that the additional $66\text{ }\mu\text{m}$ was not in response to the myopic defocus, but rather was a continuation of the lesion-induced effect. If we assume that 1 mm change in axial length accounts for 17 D (Kee, et al. 2001), the difference in choroidal thickness between groups ($376-143=233\text{ }\mu\text{m}$) would account for approximately 4 D difference in refractive error, in good agreement with our 5 D difference ($6.7\text{ D} - 1.6\text{ D}$).

Neither PPGX nor PPG/SCGX had any effect on the refractive compensation to lens-induced myopic defocus (Figure 6A: $+5.3\text{ D}$ and $+4.6$ vs $+6.7\text{ D}$; $p=0.182$), nor axial elongation (Figure 6B: $140\text{ }\mu\text{m}$ and $150\text{ }\mu\text{m}/3\text{d}$ vs $202\text{ }\mu\text{m}/3\text{d}$). However, for axial elongation (Figure 6B), it should be noted that for all surgery conditions, paired t-tests between experimental and fellow eyes indicated no differences, in contrast to the significant interocular difference in intact controls ($p=0.01$), hinting at a possible effect of the surgeries on eye growth that may be masked by the underpowered statistics due to small sample sizes. Both PPGX and PPG/SCGX inhibited the choroidal thickening response (Figure 6C; ANOVA, $p=0.0008$; $300\text{ }\mu\text{m}$ and $274\text{ }\mu\text{m}$ vs $376\text{ }\mu\text{m}$; $p=0.0176$ and $p=0.0104$ respectively), but to a lesser extent than did the PPG/CGX. (Note that the effect for PPG/SCGX is borderline significant with the Bonferroni criterion.)

VI. Effects on anterior chamber elongation in lens-wearing eyes

There is a trend towards a reduction in growth of the anterior chamber in both double-lesion groups for the negative lens-wearing birds (Figure 7B; ANOVA, $p=0.0177$), similar to that seen for PPG/CGX prior to lens-wear (see Figure 2C). The ANOVA for the positive lens-wearing groups (Figure 7A) did not reach significance (Table 3, ANOVA. $p=0.1172$).

Discussion

Our main findings are that double parasympathectomy prevented the development of form-deprivation myopia; eyes grew more slowly than normal. It did not, however, alter the axial or refractive compensation to negative lenses. Double parasympathectomy also caused significant choroidal thickening unrelated to visual input. All three lesions prevented the full compensatory choroidal thickening response to positive lenses, but had no effect on the choroidal thinning response to negative lenses.

Axial Length

Double parasympathectomy prevented the axial elongation and myopia development in response to form deprivation. To our knowledge, this is the first instance in which form deprivation led to a decrease rather than an increase in growth rate. These findings are in accord with reports by others that ciliary ganglionectomy reduced the axial and refractive responses to form deprivation (Shih et al., 1994; Schmid et al., 1999), (but see also (Lin et al., 1996)), however, the effect of double parasympathectomy is much stronger, as neither of the above groups reported a reversal of the growth response. Together these results imply that lesioning both parasympathetic inputs has a synergistic effect on the eye growth controller, as PPGX alone does not affect the response. By contrast, this lesion has no effect

on the myopia development in eyes responding to hyperopic defocus (nor does ciliary ganglionectomy, (Schmid & Wildsoet, 1996)), adding to the increasing evidence that the mechanisms underlying the responses to form deprivation versus negative lens-wear are fundamentally different (Schaeffel et al., 1994; Schmid and Wildsoet, 2004; Bartmann et al., 1994; Wildsoet and Wallman, 1995; Kee et al., 2001; Nickla and Totonelly, 2011).

Restoration of normal vision upon diffuser removal normally causes a rapid cessation of ocular growth in response to the myopia, until the optics “catch up” to the eye length to produce emmetropia (Wallman and Adams, 1987). In eyes with double parasympathectomy, the eyes were emmetropic at the time of diffuser removal, and so the ensuing large increase in growth rate was not a visually-induced response, suggesting that eyes “sensed” they were too small and grew accordingly, while remaining emmetropic. This result supports the existence of a “shape factor” (Schaeffel and Howland, 1991; Nickla et al., 2005), as first hypothesized by van Alphen (1961). Furthermore, this increase in growth rate shows that the lesion did not compromise the ability for ocular growth stimulation, but argues instead that the “default” growth pattern to form deprivation had been altered.

There were no significant effects of any of the lesions on ocular growth in response to positive or negative lenses, in accord with the results from ciliary ganglionectomy (Schmid and Wildsoet, 1996). Together these findings suggest that the retina can distinguish the defocus, and respond appropriately, and that the signal cascade to the sclera was intact.

“Non-visual” choroidal thickening—Double parasympathectomy caused an increase in choroidal thickness over a week, that thinned back to normal by 3 weeks. This is in agreement with Lin et al. (1996), who found a thickening of 55 μm in eyes with ciliary ganglionectomy. Their smaller effect is likely the consequence of measuring choroidal dimensions in histological sections, but we cannot rule out the possibility that the two lesions had a synergistic effect. We interpret the small amount of thickening we saw after the imposition of myopic defocus as reflecting a continuation of the lesion-induced effect, because it plateaued at about the same thickness ($\sim 350 \mu\text{m}$) and about the same time (3 weeks) as in non-lens-wearing eyes (Figure 2B).

The mechanism whereby this occurs is open to speculation. In normal eyes, compensatory changes in choroidal thickness occur within hours of myopic defocus (Zhu et al., 2005; Nickla, 2007), and are associated with changes in the size of the lymphatic lacunae (Wallman et al., 1995; Junghans et al., 1999), suggesting that changes in fluid flux between the vasculature and the stroma might play a role (Wallman et al., 1995; Junghans et al., 1999; Fitzgerald et al., 2002). In birds, both the ciliary ganglion and the pterygopalatine ganglia innervate the choroidal vasculature and mediate vasodilation, using acetylcholine (ACH), nitric oxide (ciliary ganglion) (Cuthbertson et al., 1997; Schroedl et al., 2006) and vasoactive intestinal peptide (VIP) (PPG) (Walcott et al., 1989; Cuthbertson et al., 1997). If vasomotor responses play a role in the choroidal response, with vasodilation leading to extravasation and fluid flux into the stroma, then double parasympathectomy, which would *reduce* blood flow (as occurs when the Edinger-Westphal nucleus is lesioned; Fitzgerald et al., 1996), should *inhibit* choroidal thickening, not cause it, weakening a potential role for blood flow. The thickening is also unlikely to be due to loss of nitrergic input on the NVSM, as NO is a smooth muscle relaxant, hence its absence would inhibit, not cause, thickening.

We propose instead, that the non visual choroidal thickening reflects the loss of an excitatory input, probably cholinergic, to the choroidal NVSM (Meriney and Pilar, 1987), which may normally restrain thickening in opposition to osmotic forces that swell the lacunae (Krebs and Krebs, 1991; De Stefano and Mugnaini, 1997; Junghans et al., 1998) and

thicken the choroid (Schmid et al., 1999; Nickla and Wallman, 2010). Both the ciliary and pterygopalatine ganglia are cholinergic.

An alternative explanation may involve the decrease in IOP that occurs over the first week, concurrent with the choroidal expansion. Previous work has shown that paracentesis (removing aqueous from the anterior chamber) causes a large decrease in IOP, which is associated with a backflow of venous blood into the choroidal lymphatic lacunae and the engorgement of the suprachoroid (DeStefano and Mugnaini, 1997). Perhaps a similar mechanism is at work here.

Inhibition of choroidal thickening in response to myopic defocus—Both PPGX and PPG/SCGX inhibited, but did not prevent, the thickening of the choroid in response to myopic defocus; this was also true in the PPG/CGX, despite the initial “non-visual” thickening. That choroidal thickening was *reduced* but not *eliminated* suggests the loss of a fine-tuning mechanism as the retina nears the image plane, and the refractive error diminishes. Because lesions of the superior cervical ganglion alone did not have this effect (Schmid et al., 1999), and because the loss of the PPG was common to all three groups, a role for the pterygopalatine ganglia is supported. If this is true, then the “fine tuning” may be mediated by VIP, a potent smooth muscle relaxant (Zhang et al., 2010) the loss of which would be common to all three groups. Perhaps acetylcholine and VIP innervate different subsets of NVSM. Other as yet unknown neuropeptides might also be involved.

The choroid, form deprivation and recovery

Double parasympathectomy prevented the development of form deprivation-induced myopia, but the choroidal thinning was unaffected, evincing a dissociation between axial growth stimulation and choroidal thinning. A similar dissociation occurs when eyes wear negative lenses a few minutes several times a day (Winawer and Wallman, 2002). Together these results imply that the growth and choroidal responses to form deprivation are separate, in parallel pathways, and that choroidal thinning is not part of the signal cascade mediating ocular growth stimulation.

After the diffusers were removed, the choroids thickened back to normal, in the absence of a myopic refractive error, in concurrence with an increase in axial elongation that reversed the prior growth inhibition. This is the first report of choroidal thickening being associated with ocular growth stimulation, instead of inhibition. These findings support the hypothesis that in the case of form deprivation, the thinning choroid may reflect pathological alterations in fluid flux between the retina and choroid, that reverse after diffuser removal (Junghans et al., 1999; Liang et al., 2004). The implications are that the mechanisms underlying choroidal thinning in response to deprivation versus that in response to lens-induced hyperopic defocus are fundamentally different, which is further evidence for different mechanisms mediating the two myopigenic paradigms.

Anterior chamber depth and lens thickness

There is a trend for both double lesion conditions inhibiting the growth of the anterior chamber in eyes wearing negative lenses. Double parasympathectomy also inhibited anterior chamber growth in eyes prior to lens-wear. In general, these results are in accord with those of Schmid and Wildsoet (1996), who reported that ciliary ganglionectomy inhibited anterior chamber growth in positive lens-wearing eyes. Because both the ciliary and superior cervical nerves project to the anterior uvea, and the superior cervical ganglion influences aqueous dynamics (Lauber et al., 1972) and IOP (Schmid et al., 1999) in birds, it is not surprising that both double lesions might influence anterior chamber growth. We found no significant effects of the lesions on lens thickness, although others reported lens thinning in ciliary

ganglionectomized eyes (Lin et al., 1996; Schmid and Wildsoet, 1996), probably because our short experimental period precluded its manifestation.

In conclusion, we present evidence that both the ciliary and the pterygopalatine ganglia influence the choroidal responses to myopic defocus, but not to hyperopic defocus, implying different underlying mechanisms for compensatory choroidal thickening versus thinning. The choroidal responses to myopic defocus are attenuated, but not abolished, which argues for a loss of fine-tuning control, presumably by the pterygopalatine ganglia. Finally, double parasympathectomy abolished the ability of the eye to respond to form deprivation, while not affecting the responses to negative lens-wear. None of the lesions affected the choroidal thinning response, dissociating choroidal thinning from ocular growth stimulation and implicating separate pathways for the choroidal and axial responses.

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Parasympathectomy prevents the development of deprivation myopia by inhibiting eye growth.

Parasympathectomy has no effect on the axial compensation to lens-induced defocus.

Parasympathectomy inhibits the choroidal thickening response to myopic defocus.

Figure 1. Time line for ultrasound measurements (arrows and arrowheads) for each visual manipulation (left), and numbers for each surgical condition (right). The first measurement was at day 0 for all groups; this was immediately prior to the surgery. 5 days later, devices were affixed to one eye (“on”) after ultrasound was done. This period is referred to as “pre” in the Tables, and reflects effects on eyes prior to the visual manipulation. “Off” (arrowheads) is when the devices were removed, and the eyes remeasured. In the case of the form deprivation group (FD), eyes were allowed to “recover” for 4 days following diffuser removal, at which time they were re-measured.

Figure 2.

Effects of lesions on eyes over the 5 days prior to the wearing of visual devices; black bars are experimental eyes, white bars are fellow controls. “Sham” are data for PPG/CGX sham-operated controls. A. Mean change in choroid thickness for each group over 5 days after surgery. B. Top: Effect of PPG/CGX as a function of time on choroids in eyes without lenses ($n=8$). Bottom. Effect of PPG/CGX on IOP following the surgery. Day 0 is immediately prior to surgery. C. Mean change in anterior chamber depth; same conventions as in A. D. Mean change in axial elongation; same conventions as in A. Error bars are standard errors of the mean in all graphs. * $p<0.05$, ** $p<0.005$, *** $p<0.0005$

Figure 3.

Effects of lesions on eyes wearing diffusers (form deprivation) over the course of 5 days. “Intact” refers to form deprived eyes without surgery. A. End refractive errors for all groups in diopters (D). “Fellow” are the combined fellow eye data from all groups, in all graphs. B. Histogram showing numbers of eyes for all refractive errors (D) for the PPG/CGX birds for lesioned eyes (black bars), fellow controls (white bars), and intact controls (striped bars). Note that the refractions for the lesioned eyes completely overlap with those of the fellow eyes. C. Change in axial length (μm/5d) in paired experimental (black bars) and fellow (white bars) eyes. D. Change in axial length for individual experimental (black bars) and fellow (white bars) eyes for all 5 birds in the PPG/CGX condition. E. Change in choroid thickness. *p<0.05, **p<0.005

Figure 4.

Effects of lesions on eyes “recovering” from form deprivation myopia over the course of 4 days. “Intact” refers to “recovering” eyes without surgery. A. Change in axial length ($\mu\text{m}/4\text{d}$). B Change in axial length (left) and final eye size (right) for individual experimental (black bars) and fellow (white bars) eyes for all 5 birds in the PPG/CGX condition. C. Change in choroidal thickness. ** $p < 0.005$

Figure 5. Effects of lesions on eyes wearing negative lenses. Intact” refers to negative lens-wearing eyes without surgery. A. End refractive error for all groups (D). B. Change in axial length ($\mu\text{m}/5\text{d}$) for paired experimental (black bars) and fellow control (white bars) eyes. C. Change in choroidal thickness. * $p < 0.05$

Figure 6.

Effects of lesions on eyes wearing positive lenses. “Intact” refers to positive lens-wearing eyes without surgery. A. End refractive error for all groups (D). B. Change in axial length ($\mu\text{m}/3\text{d}$) for paired experimental (black bars) and fellow control (white bars) eyes. Note that only the intact and sham groups have significant differences between experimental and fellow eyes. C. Change in choroid thickness. Black diamond: sham-operated controls. * $p < 0.05$, ** $p < 0.005$

Figure 7.

Effects of lesions on anterior chamber depth for eyes responding to positive (A) and negative (B) lenses. Black bars: experimental eyes, white bars: fellow controls. “Sham” is the sham-operated PPG/CGX group. There is a trend towards an inhibition in anterior chamber growth for both of the double-lesion conditions in negative lens-wearing eyes. * $p < 0.05$, ** $p < 0.005$

Table 1

Means, standard deviations (sd), n's and Kruskal-Wallis ANOVA p-values for fellow eye data in all groups. All refer to changes in dimension, except for refractions. Intact=non-surgical controls. Refractive error units are diopters, all others are in μm .

	Intact Fellow		PPGX Fellow		PPG/CGX Fellow		PPG/SCGX Fellow		ANOVA
	N	Mean(sd)	N	Mean(sd)	N	Mean(sd)	N	Mean(sd)	P-Value
Pre ax	0	--	7	317 (151)	13	236 (65)	6	338 (33)	0.0177
Pre cho	0	---	7	13 (108)	13	1.1 (28)	5	25 (56)	0.4903
Pre AC	0	----	5	148 (82)	6	182 (20)	5	178 (33)	0.6924
FD ref	8	0.8 (0.6)	4	1.0 (0.72)	5	0.28 (0.7)	-	---	0.2576
FD ax	8	480 (52)	4	486 (52)	5	489 (61)	-	---	0.9815
FD cho	4	40 (24)	4	17 (74)	5	33 (62)	-	---	0.8425
FD AC	4	196 (97)	4	166 (92)	5	129 (79)	-	---	0.3777
RE ax	5	335 (45)	4	321 (34)	5	408 (103)	-	---	0.3748
RE cho	5	3.8 (21)	4	-17 (48)	5	-18 (60)	-	---	0.685
RE AC	5	106 (84)	4	181 (115)	5	168 (62)	-	---	0.5220
+ ref	12	0.3 (1.4)	4	0.0 (0.8)	13	0.1 (0.9)	5	0.1 (0.5)	0.8218
+ axi	16	278 (76)	5	181 (95)	13	236 (65)	5	200 (86)	0.059
+ cho	6	14 (51)	4	28 (20)	13	3.6 (47)	5	-32 (66)	0.4603
+ AC	9	118 (59)	5	105 (38)	13	111 (30)	5	143 (27)	0.2592
- ref	7	0.89 (.66)	7	0.7 (1.1)	5	0.54 (1.2)	5	-0.4 (74)	0.1559
- axi	7	512 (59)	7	441 (46)	5	523 (33)	5	496 (72)	0.0551
- cho	7	6.7 (40)	7	26 (31)	5	43 (30)	4	1.3 (39)	0.1957
- AC	7	238 (24)	7	207 (35)	4	216 (34)	4	206 (32)	0.2039

Pre: prior to visual manipulation; FD: form deprivation; RE: recovery; +: positive lens; -: negative lens; ax: axial length; cho: choroids; ref: refractive error; AC: anterior chamber depth.

Table 2

Means, standard deviations (sd), n's and p-values for form-deprived (FD) and recovering (RE) eyes. All data refer to changes in dimension, except for refractions. Refractive error units are diopters, all others are in μm . C=Intact controls.

	Intact		PPGX		PPG/CGX		Kruskal-Wallis test	Post-hoc p values	
	N	Mean(sd)	N	Mean(sd)	N	Mean(sd)	P value	PPGX vs C	PPG/CGX vs C
FD ref	8	-4.1 (1.8)	4	-4.4 (2.1)	5	0.2 (1.0)	0.0063	0.609	0.0034
FD ax	8	670 (120)	4	714 (100)	5	250 (60)	0.006	0.47	0.0034
FD cho	8	-76 (11)	4	-83 (43)	5	-115 (32)	0.641		
FD AC	4	244 (26)	4	120 (140)	5	93 (120)	0.149		
RE ax	5	391 (30)	4	352 (230)	5	602 (90)	0.0329	1.0	0.009
RE cho	5	224 (70)	4	363 (160)	5	120 (110)	0.0698	0.0864	0.251
RE AC	4	209 (119)	4	128 (78)	5	245 (70)	0.248		

ax: axial length; cho: choroids; ref: refractive error; AC: anterior chamber depth.

* For post-hoc pair comparison tests, the significance level set at 0.025 due to Bonferroni correction.

Table 3

Means, standard deviations (sd), n's and p-values for lens-wearing (positive and negative) groups. All data refer to changes in dimension, except for refractions. Refractive error units are diopters, all others are in μm . C=intact controls.

	Intact			PPGX		PPG/CGX		PPG/SCGX		Kruskal-Wallis	Post-hoc p values			
	N	Mean(sd)	N	Mean(sd)	N	Mean(sd)	N	Mean sd	P Value		PGX vs C	PG/CG vs C	PG/SC vs C	
+ ref	6	6.7 (2.3)	5	5.3 (0.86)	13	1.6 (4.5)	5	4.6 (1.7)		0.0086	0.1819	0.003	0.0578	
+ axi	10	202 (30)	5	140 (15)	13	193 (31)	5	150 (100)		0.563	-----	-----	-----	
+ cho	6	376 (40)	5	300 (30)	13	70 (200)	5	274 (55)		0.0008	0.0176	0.0038	0.0104	
+ AC	10	101 (55)	5	25 (63)	13	62 (36)	5	66 (51)		0.1172	-----	-----	-----	
- ref	5	-5.3 (3.3)	7	-3.8 (2.5)	5	-5.4 (4.4)	5	-0.9 (1.2)		0.0502	0.4062	0.8075	0.0105	
- axi	5	628 (63)	7	634 (67)	5	640 (97)	5	672 (122)		0.735	-----	-----	-----	
- cho	5	-93 (26)	7	-42 (31)	5	-106 (85)	5	-82 (56)		0.335	-----	-----	-----	
- AC	7	217 (59)	7	179 (43)	4	115 (30)	4	126 (46)		0.0177	0.2774	0.0082	0.0376	

ax: axial length; cho: choroids; ref: refractive error; AC: anterior chamber depth.

* For post-hoc pair comparisons, the significance level set at 0.0167 due to Bonferroni correction.