Published in final edited form as:

J Comp Physiol A Neuroethol Sens Neural Behav Physiol. 2019 February ; 205(1): 139–149. doi: 10.1007/s00359-018-01310-4.

Effects of autonomic denervations on the rhythms in axial length and choroidal thickness in chicks

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Abstract

In chicks, axial length and choroidal thickness undergo circadian oscillations. The choroid is innervated by both branches of the autonomic nervous system, but their contribution(s) to these rhythms is unknown. We used two combination lesions to test this. For parasympathectomy, nerve VII was sectioned presynaptic to the pterygopalatine ganglia, and the ciliary post-ganglionics were cut (double lesion; n=8). Triple lesions excised the sympathetic superior cervical ganglion as well (n=8). Sham surgery controls were done (n=7). 8–14 d later, axial dimensions were measured with ultrasonography at 4-hr intervals over 24 hrs. Rhythm parameters were assessed using a “best fit” function, and growth rates measured. Both types of lesion resulted in ultradian (>1 cycle/24 hr) rhythms in choroidal thickness and axial length, and increased vitreous chamber growth (Exp-fellow: double: 69 μ m; triple: 104 μ m; p<0.05). For double lesions, the frequency was 1.5 cycles/day for both rhythms; for triples, the choroidal rhythm was 1.5 cycles/day, and the axial was 3 cycles/day. For double lesions, the amplitudes of both rhythms were larger than sham surgery controls (axial: 107 vs 54 μ m; choroid: 124 vs 29 μ m, p<0.05). These findings provide evidence for the involvement of abnormal ocular rhythms in the growth stimulation underlying myopia development.

Keywords

ciliary ganglion; pterygopalatine; superior cervical ganglion; ultradian; myopia

Introduction

The eye uses visual input to modulate its growth to achieve emmetropia, but the molecular cascade mediating the signal from the retina through the retinal pigment epithelium (RPE) and choroid to the scleral effector tissue underlying the changes in eye growth (and size) is as yet unknown (review: (Wallman and Winawer 2004). There is evidence that the choroid plays an important role in influencing sclera growth. For instance, co-culturing pieces of

sclera with thicker choroids from slow-growing (recovering) eyes results in a decrease in scleral proteoglycan synthesis compared to co-culture with choroids from “normal” eyes. Conversely, co-culture with thin choroids from fast-growing eyes results in increased proteoglycan synthesis (Marzani and Wallman 1997). Also, thicker choroids of experimentally-induced slower growing eyes synthesize more retinoic acid, which decreases scleral proteoglycan synthesis. There is also increasing evidence that ocular circadian rhythms are important in the regulation of ocular growth. First, ocular growth itself exhibits a diurnal rhythm in normal eyes, being faster during the day than at night (Weiss and Schaeffel 1993; Nickla et al. 1998; Papastergiou et al. 1998). Both choroidal thickness (Nickla et al. 1998; Papastergiou et al. 1998) and anterior chamber depth (Nickla et al. 1998) also show diurnal oscillations. The choroidal rhythm is in approximate anti-phase to the rhythm in axial length, becoming thicker at night and thinner during the day. In eyes that have slowed their growth rate in response to myopic defocus imposed by positive spectacle lenses, the rhythm in axial length phase-delays, and that of choroidal thickness phase-advances, suggesting that the phase relationships between the two might influence ocular growth rate (Nickla 2005). Alternatively, the phase shifts might be an effect, as opposed to a cause, of changes in ocular growth rate.

In birds, the choroid is innervated by all three branches of the autonomic nervous system: the adrenergic sympathetic inputs come from the superior cervical ganglion, and the cholinergic (muscarinic) and nitrergic (nitric oxide) parasympathetic inputs from the ciliary ganglion (CG) and pterygopalatine microganglia (PPG). In humans, the pterygopalatine ganglion is a discrete ganglion located within the pterygopalatine fossa, and comprises the main parasympathetic input to the choroid. In birds, however, the PPG is a series of microganglia that lie within the orbit adjacent to the Harderian gland. In both species, the postganglionic fibers target a number of ocular structures, including the choroidal vasculature (mediates vasodilation), non-vascular smooth muscle, and intrinsic choroidal neurons (review: (Nickla and Wallman 2010). Because the sclera lacks innervation by the autonomic system, it is likely that these central inputs to the choroid, which may play a role in the diurnal oscillations in choroidal thickness, may also influence the diurnal rhythm in scleral proteoglycan synthesis that underlies ocular growth rate changes.

It is generally accepted that ocular growth is largely locally-regulated: disconnecting the retina from the brain by optic nerve section has little effect on the eye's response to form deprivation (growth stimulation and myopia) or the compensation to either positive or negative spectacle lenses (Wildsoet and Wallman 1995). However, more selective lesions have yielded evidence for the involvement of autonomic influences; for instance, sectioning the ciliary nerve, the major parasympathetic input to the avian choroid, reduced, but did not eliminate the eye's growth stimulation in response to form deprivation, but had little effect on the compensatory stimulatory response to negative lenses (Schmid and Wildsoet 1996). Ciliary ganglionectomy reversed the phase of the rhythm in choroidal thickness, causing it to thin at night and thicken during the day (based on 12-hr interval measures), and increased ocular growth rates (Schmid et al. 1999). Perhaps unsurprisingly, complete parasympathectomy (lesioning both the ciliary ganglion and the pterygopalatine ganglion) had a greater effect on the emmetropization responses: it prevented the growth stimulatory

response to form deprivation and inhibited the choroidal thickening response to myopic defocus (Nickla and Schroedl 2012).

To summarize, our knowledge of the roles of the various branches of the autonomic nervous system in emmetropization, and specifically in the ocular diurnal rhythms that are related to emmetropization, is incomplete, due to a dearth of studies using combined lesions of autonomic inputs, or because data sampling was insufficient to determine precise phase or frequency for other than diurnal (24-hour period) rhythms (Schmid et al. 1999). If the ciliary ganglion influences the phase of the diurnal rhythm in choroidal thickness, as reported by Schmid et al., then the pterygopalatine ganglia may also play a role in one or the other rhythms (although the superior cervical ganglion apparently does not) (Schmid et al. 1999). The present study sought to answer some of these questions by expanding on the aforementioned seminal study, via lesions of both parasympathetic inputs (double lesions) and lesions of all three inputs (parasympathectomy plus lesions of the superior cervical ganglion: triple lesions). We used high frequency ultrasound measurements of eyes at 4-hour intervals over 24 hours to allow the determination of potential higher-frequency (ultradian) rhythms, and asked whether these were associated with altered growth rates. We looked at the parameters of frequency and amplitude for the rhythms in axial length, choroidal thickness, and anterior chamber depth. In brief, we found that double and triple lesions resulted in increased ocular growth rates and shorter periods in both the rhythms in axial length and choroidal thickness. In double-lesions only (parasympathectomy), there was an increase in amplitude for both rhythms. Parts of this manuscript have been published in abstract form (Nickla and Schroedl 2012).

Methods

Subjects

White Leghorn chicks (*Gallus gallus domesticus*; Cornell-K strain) were hatched in an incubator and raised from day one in temperature – controlled brooders. The light cycle was 12L/12D (8:30 am to 8:30 pm). Surgeries were done under general anesthesia, using an intramuscular injection of a mixture of ketamine and xylazine (100 mg/kg, 5 mg/kg respectively). Food and water were supplied *ad libitum*. Care and use of the animals conformed to the ARVO (Association for Research in Vision and Ophthalmology) Resolution for the Care and Use of Animals in Research.

Surgeries:

Double lesions: In birds, the pterygopalatine ganglion consists of a chain of interconnected microganglia, which cannot be excised without major damage to surrounding structures. Therefore, we sectioned the autonomic facial nerve (N VII) pre-synaptic to the pterygopalatine ganglia¹. The ciliary ganglion was accessed from the inferior temporal aspect of the orbit, and the post-synaptic fibers were cut rostral to the ganglion. Details for this surgery followed those in Lin et al. (Lin et al. 1996). Surgery was done on the right eyes

¹We do not know how long the cell bodies in the pterygopalatine ganglia remain intact following the pre-synaptic lesion, but it is generally accepted that a pre-synaptic lesion will substantially reduce transmission via the post-synaptic pathway.

of eight 12d-14d old chicks as previously described (Nickla and Schroedl 2012). The pterygopalatine ganglion (N VII) was always lesioned first, followed by ciliary ganglionectomy. 7–9 days later (age 19d-23d), high frequency ultrasonography was done on both eyes at 4-hour intervals starting at 10:00 (10:00, 14:00, 18:00, 22:00, 2:00, 6:00, 10:00). For all experiments, measurements at night were done under a photographic safe light, and generally took less than 5 minutes per eye.

Triple lesions: Surgery was done on the right eyes of eight 19d-old chicks.

Parasympathectomy (N VII lesion and ciliary ganglionectomy; described above) was done first, followed by lesions of the superior cervical ganglion. The superior cervical ganglion was accessed via an incision into the neck musculature that exposed the ganglion between cranial nerves IX and X, according to Lauber et al. (Lauber et al. 1972). The ganglion was completely excised, to ensure elimination of the post-ganglionics. Two weeks later (age 33 days), ultrasonography was done on both eyes at 4-hour intervals, as noted above. In addition, sham surgeries were done for triple lesions (n=5), in which the target ganglia were accessed as described, but the pathways remained intact. This group served as “sham operated” controls for both double and triple lesion groups for all analyses of rhythm parameters.

Single lesions: These surgeries were initially done for a different purpose, and so the diurnal measurement times are not at 4 hour intervals. Single lesions of the ciliary ganglion (CGX) or pterygopalatine ganglia (N VII) (PPGX) were done on the right eyes of 4 birds for each type of lesion, at 12 days of age. 19 days later (age 31 days), measurements were done at 10:00, 18:00, 22:00 and 6:00. We analyzed the day (10:00–18:00) versus night (22:00–6:00) 8-hour intervals for comparison to those of the double and triple lesion groups (Fig. 4), as an estimation of the day versus night (i.e. diurnal) differences. Note that our measurement protocol did not include the light/dark transition points (i.e. 8:30 and 20:30), precluding 12-hour interval data for day versus night.

Data analysis:

The parameters measured were axial length (front of the cornea to the front of the sclera), vitreous chamber depth (back of the lens to the front of the retina), choroidal thickness and anterior chamber depth (front of the cornea to the anterior surface of the lens) over a 24-hour measurement interval, from 10:00 to 10:00. Growth rates were defined as the changes in dimension over the 24-hour period for axial length, vitreous chamber depth and anterior chamber depth. These data are presented as interocular differences (experimental – fellow eyes; subsequently denoted as X-C) in rate. Paired t-tests were used for analysis of interocular differences. For choroids, thicknesses of experimental and fellow eyes at the end of the experiment are shown (i.e. these are not rates).

Diurnal rhythms were assessed in several ways to determine periodicity. We calculated the changes over each of the six successive 4-hr intervals as well as the changes over the two 8-hour intervals comprising the day (light part of the cycle; 10:00 to 18:00), versus the night (dark part of the cycle; 22:00 to 6:00). (12-hour interval data was precluded due to choice of

measurement times). In order to analyze period, we subtracted the “steady state” growth rate (slope) from the raw data for individual eyes, to yield the “cyclic component” (Nickla et al. 1998). These data were normalized to their means, and the means of these are plotted as line graphs in Figs. 2, 5 and 7. A “best fit” sine function was fit to these data using an automated program (Igor Pro; Wavemetrics Inc.). To determine frequency (period) and amplitude for statistical analysis of axial length and choroidal thickness, “best fit” sine functions were used on the data for all individual eyes. Frequency was taken to the nearest 0.5 cycles (e.g. an automated fit of 1.3 cycles/day was noted as 1.5 cycles). Only those eyes whose data could be fit to a sine function were used in the statistical determination of frequency. “Goodness of fit” required that the mean standard deviation of the residuals of the sine wave fit was less than 80% of the standard deviation of the raw data.

We assessed statistical differences in amplitude in three ways: First, we took the minimum versus maximum differences from the 7 diurnal measurements regardless of time of day; this is referred to as “Max-Min” in figures. Second, we restricted the maximum to either the day, for axial length, or night, for choroid thickness, and the minimum to either night, for axial length, or day, for the choroid thickness; this is referred to as “Day-Night”. This is the more accurate means of assessing if there is a diurnal (day versus night) excursion. Third, we used the amplitudes of the automatic sine wave fits to the individual data.

Results

Lesion effects on ocular dimensions:

The surgical procedures caused mydriasis and a slight ptosis on the ipsilateral side; no other effects were observed. Corneal transparency remained unaltered, and function of the nictitating membrane was conserved. Recovery from anesthesia occurred within several hours, and birds began food intake and showed normal behavior.

Both double lesions (parasympathectomy) and triple lesions resulted in a significant increase in growth rate of the vitreous chamber relative to that of fellow eyes (Fig. 1a, black bars: X-C: 69 μm and 104 μm , respectively; $p=0.037$; $p=0.007$). For the triple lesions, axial length also showed a significant increase relative to fellow controls (Fig. 1a, white bars: 80 μm ; $p=0.02$), but this was not true for double lesions due to a significant lesion-induced *decrease* in anterior chamber growth (Fig. 1b: X-C: -111 μm ; $p=0.01$) resulting in shallower anterior chambers, as previously reported (Nickla and Schroedl 2012). Finally, double lesions, but not triple lesions, resulted in significantly thicker choroids compared to fellow controls (Fig. 1c: 349 vs 259 μm ; $p=0.016$), replicating previous results (Nickla and Schroedl 2012).

Effects on rhythms:

In general, both double and triple lesions altered the rhythms in axial length and choroidal thickness, changing them from one cycle per day (diurnal) (Weiss and Schaeffel 1993; Nickla et al. 1998; Papastergiou et al. 1998) to higher frequency (ultradian) oscillations in both parameters. For double lesions, the axial length rhythm in 7/8 experimental eyes had higher than diurnal frequencies: 1.5 cycles/day, $n=3$; 2 cycles/day, $n=3$; 3 cycles/day, $n=1$ (one eye had a diurnal frequency). For triple lesions as well, 7/8 experimental eyes showed

higher frequencies: 1.5 cycles/day, n=4; 3 cycles/day, n=3 (one eye was not adequately fit to a sine function). The mean longitudinal data for axial length for the double lesions showed 1.7 cycles/day, with a peak at 22:15 (Fig. 2a, red lines), while the triple lesions showed 2.8 cycles/day, with peaks at 13:35, 22:25 and 6:45 (Fig. 2b, red lines).

For choroidal thickness too, both lesions resulted in ultradian frequencies that were variable within lesion groups (blue lines in Fig. 2a and 2b). For double lesions, 4/8 choroids showed higher frequencies: 1.5 cycles/day, n=4; 1.0 cycles/day, n=4. For triple lesions, 7/8 choroids showed higher frequencies: 1.5 cycles/day, n=4; 2 cycles/day, n=2; 2.5 cycles/day, n=1 (one eye was not adequately fit to a sine function). The mean longitudinal data for choroidal thickness for both lesion groups showed 1.5 cycles/day. For double lesions, the peak was at 23:00 (Fig. 2a, blue lines), while for triple lesions it was at 21:45 (Fig. 2b, blue lines). It is notable that the peaks for both groups occurred in the middle of the night, similar to normal (intact) eyes (Nickla et al. 1998; Papastergiou et al. 1998). Data for both rhythms from the sham surgery group is shown for comparison in Fig. 2c. Both can be fit to a diurnal sine function, with the peaks for axial length occurring in late morning, and for choroidal thickness, in the middle of the night.

The higher frequency oscillations in both experimental groups is also demonstrated by the 4-hour interval data (Fig. 3a and b, top). For axial length, the double lesion eyes (Fig. 3a, black bars) show 2 cycles per 24-hours, while the triple lesion eyes (Fig. 3a, white bars) show 3 cycles per 24-hours. For choroid thickness, the double lesions (Fig. 3b, black bars) show about 2 cycles, and the triple lesions (Fig. 3b, white bars) show about 3 cycles. The sine wave fits for both rhythms and the sham surgery controls are shown in the bottom panels, for comparison.

Figure 4 shows 8-hour interval data for day (white bars) versus night (black bars) for single lesions (PPGX and CGX), double lesions and triple lesions. A significant result of the higher frequency oscillations is the elimination of the daytime versus nighttime (diurnal) differences, for both axial length (Fig. 4a) and choroidal thickness (Fig. 4b) in all groups (i.e. no significant day versus night differences). This could also result from the measurement times (10:00, 18:00, 22:00 and 6:00) being close to the mesor (rhythm adjusted mean). For comparison, fellow eyes (combined) exhibited the normal diurnal differences: for axial length, greater daytime growth, for choroids, greater nighttime thickening.

The rhythms in anterior chamber depth are shown in Fig. 5. The data for the double-lesion group (blue line), while not well-fit to a sine function, had a peak at 22:00. The triple lesion data (red line) was sinusoidal, with a mean frequency of 1.5 cycles/day, and a peak at 23:10. For comparison, the rhythm for sham controls (green lines) was sinusoidal with a 24-hour period and a peak in late afternoon.

We assessed amplitude for the axial length and choroidal rhythms in three ways (Methods). For axial length (Fig. 6a), the amplitude for the double lesion group was significantly larger than that of sham surgery controls in the “sine fit” analysis (black bars vs grey bars: 107 vs 54 μm ; ANOVA $p=0.025$; post-hoc Bonferroni $p=0.038$). The double lesion amplitude

tended to be larger than that of the triple lesion group as well (compare black to white bars; sine fit analysis: 107 vs 62 μm ; $p=0.059$; Day-Night analysis: 84 vs 41 μm ; $p=0.060$). Triple-lesion amplitudes did not differ from sham controls in any analysis. For choroidal thickness too (Fig. 6b), amplitudes of the double lesion group were larger than those of both sham controls and triple lesions (black bars vs white and grey bars: ANOVA $p=0.00001$; post hoc Bonferroni $p<0.00005$ for both comparisons).

Effects on fellow eyes:

The rhythms in axial length and choroidal thickness were altered in fellow eyes in a similar manner to that of experimental eyes (Fig. 7). The mean longitudinal data were not as well-fit to a sine function as that for experimental eyes, reflecting greater variability in phase. For the double lesions (Fig. 7a), both the rhythms in axial length (red line) and choroidal thickness (blue line) had a best-fit sine function of 1.5 cycles/day, however, the axial rhythm was similar to that of normal eyes, with a peak at 16:25. The choroidal rhythm too, was similar to normal in showing a peak at 22:45, but the rhythm wasn't sinusoidal. For triple lesions (Fig. 7b), fellow eye rhythms were even more abnormal: axial length showed 3 cycles/day, and choroidal thickness 2 cycles/day. These results evince a "yoking" influence of experimental eyes on contralateral fellow eyes, a phenomenon that has been described for various visual manipulations in chicks (Ohngemach et al. 2001; Rucker et al. 2009).

Discussion

The purpose of these studies was to examine in more detail than previously tested, the parameters of the rhythms in ocular dimensions that are associated with altered ocular growth rates. To study periodicity for potential ultradian rhythms, it is necessary to use measurement intervals of less than the Nyquist frequency for circadian rhythms, which is 12 hours, or 2 sampling times per day. Previous work used 6-hour intervals (Nickla et al. 1998; Nickla 2005), which may or may not be sufficient to reveal 12-hour period rhythms, depending on the measurement times (i.e. if measurement times fall near the mesor, rhythms would not be detected). Sampling at 4-hour intervals is sufficient for detecting periods of 12–16 hours (1.5 or 2 cycles per day), but not necessarily for 3 cycles per day. Our main findings are that lesions of both parasympathetic inputs, and lesions combining parasympathetic and sympathetic inputs, result in ultradian rhythms in choroidal thickness and axial length that are associated with an increased rate of ocular growth. For the double (parasympathetic) lesion, the mean frequency was 1.5 cycles per day for both rhythms (16 hour periods); for the triple lesions, the axial length rhythm was approximately 3 cycles per day (8 hour periods). Because our sampling at 4-hour intervals cannot reveal higher-frequency components than 3 cycles per day, we cannot eliminate the possibility that these exist. Finally, double lesions caused an increased amplitude for both the rhythms in axial length and choroidal thickness. These effects on both rhythm parameters strengthen the hypothesis associating altered ocular rhythms with the development of ametropias (Chakraborty et al. 2018), and implicate the involvement of the autonomic nervous system in ocular rhythm control and eye growth regulation.

Effects on ocular dimensions

We have previously reported that double parasympathectomy resulted in an increase in choroidal thickness 5 days after the surgery (Nickla and Schroedl 2012); this finding was replicated in the current study. Because ciliary ganglionectomy alone resulted in choroidal thickening (Lin et al. 1996; Schmid et al. 1999), and lesions of N VII (PPGX) did not (Nickla and Schroedl 2012), the ciliary ganglion is presumably the major influence opposing choroidal expansion, possibly via maintaining the tonus of the non-vascular smooth muscle cells spanning the stroma, or modulating capillary permeability to inhibit movement of water into the stromal matrix (Wallman et al. 1995; Nickla and Wallman 2010). It is notable that in parasympathectomized eyes, choroidal thickening was associated with an *increase* in ocular growth rate, and not a decrease. We reported the same in our previous study: Double lesions prevented the development of form-deprivation-myopia, but choroidal thinning was retained, however, when the diffuser was removed, the choroid *thickened* back to normal, and ocular growth rate *increased* (Nickla and Schroedl 2012). This may imply that choroidal thickening is not mechanistically linked to ocular growth inhibition. However, it is equally possible that the denervations caused the dissociation, and the two processes are, in fact, linked in an intact eye.

The parasympathetic system appears to be required for normal growth of the anterior chamber, because parasympathectomy (double lesion) resulted in a shallower than normal anterior chamber, replicating our previous study (Nickla and Schroedl 2012). Again, because reduced anterior chamber growth was not found with single lesions of N VII (Nickla and Schroedl 2012), the ciliary ganglion is probably the major effector. Possible underlying mechanisms include a parasympathectomy-induced decrease in intraocular pressure (Schmid et al. 1999; Nickla and Schroedl 2012), or altered tonus of the ciliary or corneal accommodative muscles.

We found that the double- and triple lesions caused a significant increase in ocular growth rate by 7 days post-surgery, which was not apparent 5 days post-surgery in our prior study using double lesions, or in single lesions of N VII (PPGX) (Nickla and Schroedl 2012). However, at 5 weeks post-surgery, both ciliary ganglionectomy and superior cervical ganglionectomy resulted in an increase in eye size (Schmid et al. 1999), implying that our prior negative results were a result of insufficient time for the effect. Together the findings suggest a redundancy within the three autonomic inputs in restraining ocular growth, and that the sympathetic and parasympathetics do not have opposing influences on ocular growth control systems.

Effects on rhythm amplitude

Parasympathectomy caused an increase in amplitude in both the axial and choroidal rhythms, and removal of all three inputs (triple lesion) returned both to normal. This implies that the parasympathetic branches restrain the rhythm amplitudes. Because the mechanisms underlying changes in choroidal thickness (either diurnal or compensatory) are unknown, we can only speculate on the influences of these inputs. The loss of an excitatory cholinergic input (Meriney and Pilar 1987) onto choroidal non-vascular smooth muscle (De Stefano and

Mugnaini 1997) effected by parasympathectomy is a plausible underlying cause of the increase in the choroidal thickness amplitude. The superior cervical ganglion also innervates the non-vascular smooth muscle, but the effects of noradrenaline on these are unknown (Schroedl et al. 2001; Stubinger et al. 2010). If the two branches have opposing roles, and if the non-vascular smooth muscle mediates the changes in thickness, this would imply that the sympathetics mediate muscle relaxation.

The finding that parasympathectomy increased the amplitudes of both the choroidal and axial rhythms, and that triple lesions re-normalized both, suggests that the choroidal amplitude is “driving” the increase in the axial rhythm amplitude, because the sclera itself is not innervated by the autonomics. Perhaps the large oscillations in choroidal thickness directly influence the oscillations in scleral proteoglycan synthesis, which exhibits diurnal oscillations (Nickla et al. 1999) and underlies the diurnal rhythms in axial length. The fact that both lesion conditions result in higher growth rates, but only one alters amplitude, indicates that amplitude is not mechanistically-related to altered growth rates.

Previous work reported that ciliary ganglionectomy “reversed” the diurnal rhythm in choroidal thickness, so that choroids thickened during the day and vice versa (Schmid et al. 1999). In our hands, the 8-hour interval data showed no diurnal rhythm for either of the single lesion groups. This discrepancy between studies is likely a result both of our small sample size and of the different sampling times. Specifically, the older study measured at the times of lights on and lights off (12 hour intervals), whereas our measurements straddled the light/dark transitions, making the light and dark intervals only 8 hours. If, however, the ciliary ganglion influences the rhythm amplitude and frequency, as suggested above, it is possible that the apparent reversal of phase in the 12-hour interval study is a reflection of an increase in frequency, in which “day” and “night” measurements do not align with the shorter periods.

Effects on rhythm frequency

Ultradian rhythms are defined as rhythms with periods shorter than 24 hours; many of these fall between 2 and 6 hours. They are not as well-documented as circadian rhythms, but they exist in all species, from fruit flies to man. The most commonly studied are rhythms in locomotor activity, sleep, feeding, body temperature and hormone levels. In general, their periods are less stable than circadian ones, and can be altered by variations in external cues that are unrelated to the light/dark cycle (reviews: (Blessing and Ootsuka 2016; Bourguignon and Storch 2017). Of relevance to our current findings, there is an ultradian component (1.875 cycles/day) in the endogenous circadian rhythm in scleral proteoglycan synthesis in isolated chick scleras from fast-growing myopic eyes that is not as prevalent in normally-growing eyes (Nickla et al. 1999). Because scleral proteoglycan synthesis in chicks mediates the changes in eye growth rates (Rada et al. 1990; Rada et al. 1991; Rada et al. 1992), it is plausible that the ultradian rhythms in scleral matrix molecules in the faster-growing eyes underlie the ultradian rhythms in axial length in our lesion-induced faster-growing eyes, evincing a mechanistic link between ultradian growth rhythms and eye growth stimulation. Because the sclera is not innervated by the autonomics, it is likely that the ultradian rhythm

in the choroid plays a role in driving the ultradian rhythm in axial length, similar to what we proposed for the choroid driving the increased amplitudes of both rhythms.

It is also possible that the increased frequency in these rhythms is ultimately driven by the loss of input from the master clock in the hypothalamus, the suprachiasmatic nucleus. In birds, the parasympathetic circuit has been documented: the suprachiasmatic nucleus projects to the (medial) Edinger Westphal nucleus, which projects to the ciliary ganglion that innervates the uvea (Reiner et al. 1983). This circuit is involved in modulating choroidal blood flow. The sympathetic circuit is less definitive, but may involve the hypothalamic pre-sympathetic paraventricular nucleus input to the intermediolateral nucleus of the spinal cord, which projects to the choroid via the superior cervical ganglion (Buijs et al. 2003; McDougal and Gamlin 2015). The sympathetic system plays a major role in driving/ maintaining the rhythms of various peripheral tissue functions (Vujovic et al. 2008); perhaps this includes the choroidal thickness rhythms. Of note, the choroid contains endogenous clocks (Stone et al. 2018); the potential influence/role of these on the choroidal thickness oscillations is an interesting area for future study.

Finally, we found that the rhythms in fellow eyes were yoked in some way to their contralateral experimental eyes. This effect was larger in the triple lesion condition, in which both rhythms were ultradian in fellow eyes. This yoking phenomenon has been previously reported, primarily in the growth rates of fellow eyes of lens-wearing or differ-wearing eyes in chicks and marmosets (Rucker et al. 2009). Of greater relevance to the present study, the eye growth rhythms in fellow eyes of form-deprived eyes were reported to be reversed in phase compared to normal, evincing what the authors termed an interocular “interaction” (Ohngemach et al. 2001). While the mechanism whereby this yoking occurs remains mysterious, the scleras of the two eyes in chicks are closely apposed, with only a thin septum between, conceivably allowing diffusion of a humoral signal from one to the other (Wallman et al. 1995; Smith et al. 2002). Alternatively, perhaps the lesions produced a systemic serum-borne signal affecting both eyes.

In summary, both double- and triple lesions resulted in alterations in ocular rhythm parameters: both caused ultradian oscillations and an increase in growth rate. For parasympathectomy only, the amplitudes of both rhythms were larger than normal. These results provide further evidence for the involvement of abnormal ocular rhythms in the growth stimulation resulting in the development of myopia. We conclude that the autonomic nervous system plays a role in driving ocular rhythms, and thereby in regulating ocular growth.

Acknowledgements:

This work was funded by NIH-NEI-013636 and NIH-NEI-025307. The authors thank Kristen Totonelly and Jonathan Elin-Calcedor for collecting some of the data. Care and use of animals conformed to the ARVO Resolution for the Care and Use of Animals in Research.

List of Abbreviations

ARVO	Association for Research in Vision and Ophthalmology
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CG	Ciliary ganglion
CGX	Ciliary ganglion lesion
N VII	Cranial nerve VII
PPG	Pterygopalatine ganglia
PPGX	Pterygopalatine ganglion pre-synaptic (NVII) lesion
RPE	Retinal pigment epithelium

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Ocular dimensions

Figure 1.

Changes in ocular dimensions. a. Interocular (experimental minus control; X-C) differences in growth rates (change per 24 hours) for vitreous chamber depth (black bars) and axial length (white bars) for double and triple lesions. b. Interocular differences (X-C) in growth rates of the anterior chamber for double and triple lesions. Double lesions caused a decrease in anterior chamber growth relative to that of fellow eyes. c. Choroidal thickness for experimental (black bars) and fellow (white bars) eyes at the end of the experiment. Error bars are standard errors of the mean. Asterisks denote statistical significance ($p < 0.05$; paired t-tests) in the interocular differences.

Rhythms in lesioned eyes and sham surgery controls

Figure 2.

Mean rhythms in axial length (red) and choroidal thickness (blue) for double lesions (parasympathectomy) (a) and triple lesions (b) measured at 4-hr intervals. (c) Sham surgery controls measured at 4-hr intervals. The dashed lines show the automated best fit by the Igor program. Time is on the x axis. For double lesions, both rhythms have a mean period of 16 hours (1.5 cycles/day). For triple lesions, the choroidal rhythm period is 16 hours (1.5 cycles/day), and the axial rhythm is 8 hours (3 cycles/day). Error bars are standard errors of the mean.

4-hour interval data

Figure 3.

Changes over the 4-hour intervals for axial length (top, a) and choroid thickness (top, b) in the double (black bars) and triple (white bars) lesions. For axial length, note the difference in oscillations between the two lesion conditions: the triple lesions show 3 cycles, and the double lesions show 2 cycles. Bottom panels for both parameters show the sine wave fits from figure 2 for comparison. Error bars are standard errors of the mean.

Day versus night 8-hour interval data

Figure 4.

Changes over the 8-hour intervals of day (white bars) and night (black bars) for axial length (a) and choroid thickness (b) for single lesions (PPGX and CGX), double and triple lesions. The fellow eyes (all experimental groups combined) are also included at far right. For all lesion conditions, note the lack of diurnal (day vs night) differences for both rhythms, reflecting the increase in cycle frequency. Error bars are standard errors of the mean.

Anterior chamber depth rhythms

Figure 5.

Mean rhythms in anterior chamber depth for double lesions (blue line), triple lesions (red line) and sham surgery controls (green line). The dotted lines are the “automated best fit” by the Igor program. Time is on the x axis. Note that the double lesion data (blue) cannot be fit to a sine function. Error bars are standard errors of the mean.

Amplitude

Figure 6.

Amplitude derived from 3 analyses (Methods) for double lesions (black bars), triple lesions (white bars) and sham surgery controls (grey bars), for axial length (a) and choroid thickness (b). Note that double lesions result in an increase in amplitude for both axial length (a) and choroid thickness (b) using the sine wave fit analysis. Error bars are standard errors of the mean. * $p < 0.05$; ** $p < 0.00005$

Fellow eye rhythms

a. Double lesion

b. Triple lesion

Figure 7.

Rhythms in axial length (red) and choroid thickness (blue) in fellow eyes for double lesions (a) and triple lesions (b). The dotted lines are the “automated best fit” by the Igor program. Time is on the x axis. Note that rhythms are abnormal, indicating a yoking by the contralateral experimental eyes for both lesions. Error bars are standard errors of the mean.