Endogenous Rhythms in Axial Length and Choroidal Thickness in Chicks: Implications for Ocular Growth Regulation

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PURPOSE. To determine whether the diurnal rhythms in axial length and choroidal thickness in the chick eye are endogenous circadian rhythms.

METHODS. Six chickens, 14 days of age, were put into darkness for 4 days. Beginning on the 3rd day, ocular dimensions were measured using high-frequency A-scan ultrasonography, in darkness, at 6-hour intervals over 48 hours. Five age-matched chickens reared in a normal light/dark (L/D) cycle and measured at 6-hour intervals for 5 days were controls.

RESULTS. The rhythms in axial length and choroidal thickness persist in constant darkness. The phases of these rhythms are approximately in antiphase to one another, similar to those of eyes in a L/D cycle; however, the peak of the rhythm in axial length occurs slightly earlier relative to that of eyes in L/D (12 PM versus 3 PM; P < 0.05, one-tailed t-test). By the 3rd day in darkness, the rate of growth is significantly higher than that in L/D (117 versus 72 μm/24 hours; P < 0.01), and the choroid becomes significantly thinner (159 versus 210 μm; P < 0.0001).

CONCLUSIONS. The rhythms in axial length and choroidal thickness are circadian rhythms, driven by an endogenous oscillator. The phase of the rhythm in axial length in constant darkness is slightly phase-advanced relative to eyes in L/D and thus is similar to eyes that are deprived of form vision. These findings suggest that in the absence of visual input, the eyes revert to a “default” growth state and that the similarities between the effects of constant darkness and of form deprivation suggest that deprivation may represent a type of “constant” condition.


The eyes of young chickens show a diurnal rhythm in axial elongation, growing more during the day than during the night.1–3 The thickness of the choroid, the tissue behind the retina, also shows a diurnal rhythm, thickening during the day and thinning during the day, in approximate antiphase to the rhythm in axial length.2–3 It has been shown that several visual manipulations that alter the rate of ocular growth are associated with consistent effects on the phase relationships between these rhythms: specifically, myopic defocus, induced either by prior form deprivation or positive spectacle lenses, both of which cause a compensatory decrease in growth rate, is associated with the two rhythms shifting into phase with one another; this is effected by a phase delay in the rhythm in axial length and a phase advance in the rhythm in choroid thickness.2–4 Conversely, form deprivation, which causes an increase in growth rate, results in a phase advance in the rhythm in elongation that shifts the two rhythms into “exact” antiphase with one another.2

To more fully understand the significance of the above observations in the context of ocular growth regulation, it is important to know whether these rhythms in dimension are circadian (i.e., driven by an endogenous clock) or whether they are strictly diurnal (driven by the cycle of light and dark [L/D]). To answer this question, we measured eyes at 6-hour intervals for 48 hours, after 2 days in constant darkness and compared these to age-matched animals in a diurnal L/D cycle from a previous study.2 If the phase relationships between the rhythms in axial length and choroidal thickness are related to altered growth, as our previous studies suggest, it is possible that the phases of the endogenous rhythms may differ from those seen in L/D, because it has long been known that rearing chickens in constant darkness results in abnormally large eyes.5–7 Such results would provide further evidence linking shifts in phase with altered ocular growth rates.

We here show that both the rhythms in axial elongation and in choroidal thickness persist in constant darkness. The peak in the rhythm in axial length occurs slightly earlier than that for normal eyes in L/D, similar to form-deprived eyes in L/D.2 Furthermore, constant darkness results in a rapid decrease in choroidal thickness and an increase in ocular growth rate, also similar to form-deprived eyes in L/D. We conclude that the absence of form vision and/or temporal cues in constant darkness causes the eyes to revert to a “default” growth state, in which growth rate is faster than would be required for emmetropization. We speculate that the similarities to eyes deprived of form vision suggest that form deprivation causes eyes to revert to the default state. It follows that form vision, not light per se, exerts a restraining influence on growth rate, possibly via subtle influences on the phases of these two rhythms. Parts of this work have been presented in abstract form.8,9

METHODS

Six untreated birds were raised in a normal L/D cycle (12 hours L/12 hours D) until 2 weeks of age, after which they were put into constant darkness for the duration of the study. Food and water were supplied ad libitum. Large amounts of food were scattered on the floor to provide more ready access in darkness. Birds were monitored daily for feeding by checking their crops for the presence of food. After 48 hours in darkness, ocular dimensions were measured at 6-hour intervals for 48 hours (6 AM, 12 PM, 6 PM, and 12 AM) using high-frequency A-scan ultrasonography (for details, see Ref. 2). This protocol for assessing the circadian nature of a rhythm follows standard procedure whereby the organism (or tissue) is “released” into constant darkness and measured after 1 to several days. For example, most of the studies on the circadian nature of the rhythm in intraocular pressure measure within 3 days in darkness.10–12

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For ultrasonography, birds were anesthetized with halothane (1% in oxygen). Measurements were done in darkness, with the aid of a dark yellow photographic safe light (the level of illumination was 0.5 lux at the bird’s eye); measurements typically took less than 5 minutes, after which birds were returned to darkness. Controls were 5 normal age-matched birds from a previous study that were reared in L/D and measured at 6-hour intervals over 5 days. All animal use was in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Data Analysis**

Because these measurements are done on growing eyes, we devised a method to separate the ‘cyclic component’ from the ‘steady state’ growth component to assess the parameters of the rhythms: phase, amplitude, and period. In brief, we assume that the growth curve (as in Fig. 1A) is composed of a diurnal rhythm superimposed on a steady rate of growth. To separate these, a regression line is fit to the data for each eye; the slope of this line yields the steady state rate of growth (these data are used to compare rates of growth between groups). The regression is then subtracted from the raw data; these residuals represent the pure cyclic component for each eye. From these, one can obtain phase, amplitude, and period by fitting a sine wave to these data. For statistical comparisons of phase, a sine wave with a period of 24 hours is fit to the data for each eye for the 4th day in darkness. This cycle was chosen because the phase of the rhythm is less variable over this last 24-hour period and because it could possibly be a more accurate reflection of the true circadian (free-running) rhythm. For statistical tests of phase, only those data meeting our inclusion criterion, that the mean SD of the residuals of the sine wave fit to the data were less than the SD of the raw data, and less than 60% of the amplitude of the sine wave fit to the data (a similar method was used in Ref. 2). These data and the number of eyes used are shown in Table 1 (‘From individual eyes’). All descriptions of phase in Results are obtained from these data. For graphical depictions, we show the sine waves fit to the mean of the residuals for all eyes; the period used for these fits was either 24 hours or was left unconstrained, as indicated.
TABLE 1. Phase for Axial Length and Choroidal Thickness

<table>
<thead>
<tr>
<th>Condition</th>
<th>Component</th>
<th>From Averaged Data*</th>
<th>From Individual Eyes†</th>
</tr>
</thead>
<tbody>
<tr>
<td>L/D</td>
<td>Axial length</td>
<td>3:30 PM</td>
<td>3:00 PM ± 3.5 (8)</td>
</tr>
<tr>
<td></td>
<td>Choroid</td>
<td>12:00 AM</td>
<td>10:30 PM ± 3.7 (6)</td>
</tr>
<tr>
<td>Dark</td>
<td>Axial length</td>
<td>11:00 AM</td>
<td>12:00 PM ± 2.8 (10)</td>
</tr>
<tr>
<td></td>
<td>Choroid</td>
<td>1:00 AM</td>
<td>12:00 AM ± 3.4 (8)</td>
</tr>
</tbody>
</table>

* Values are the phases derived from the sine waves fit to the mean of the residuals for all the data (both cycles) and are the sine waves shown in all of the figures.
† Values are the mean phases derived from the sine waves fit to the residuals for each individual eye, with ±SD: number of eyes in parentheses. These are the data used in statistical comparisons of phase and include only those eyes that meet our criterion for “goodness of fit” (see Methods).

The phases obtained from these “mean” sine fits are shown in Table 1 for comparison (“From averaged data”). Amplitude is derived from these fits to the mean of the data. t-Tests were used for all statistical comparisons.

RESULTS

Axial Length

The rhythm in axial length persists in constant darkness (Fig. 1). Figure 1A shows the mean axial length and SEs for all eyes as a function of time over the measurement period: at both midnight points there is a mean reduction in length compared with the previous time point (arrows), similar to eyes in L/D. However, after 48 hours in constant darkness the growth rate is significantly higher than for eyes in L/D (117 versus 72 μm/d, t = 5.1, P < 0.01). The cyclic component is shown in the inset on the bottom of the graph (with symbols) with two sine waves fit to the data: the solid wave is the diurnal (i.e., 24 hour) fit wave, and the dashed wave is the best fit to the data, which yields a shorter period (20 hours). This shorter period is the result of four eyes showing anomalous 12-hour oscillations over the first cycle, which skews the period. The mean peak occurs at about noon (11 AM from averaged data, 12 PM from individual eyes; Table 1).

To assess the diurnal changes in growth rate as a function of time of day, we subtracted the axial length at each time point from the length at the next time point for each eye (data for both cycles combined); this gives the mean rate per interval (Fig. 1B). Similar to eyes in L/D, the rate of growth is lowest (negligible) in the interval from 6 PM to midnight (Fig. 1B, midpoint 9 PM: gray bars and white square). These differences between groups are significant (dark versus L/D, midpoint 9 PM: gray bars and white square). However, after 48 hours in constant darkness the growth rate is significantly higher than for eyes in L/D (117 versus 72 μm/d, t = 5.1, P < 0.01).

Although the means of the data for the diurnal changes in axial length for eyes in L/D appear sinusoidal, the means for eyes in constant darkness do not (Fig. 1B, cf. bars and white squares, and see Ref. 2). This is the result of several eyes over the first 24 hours (3rd day in dark) showing anomalous 12-hour oscillations, which is apparent in the mean residuals in the inset of Figure 1A (first 5 data points). However, if we use the data from the 4th day in darkness, the residuals can be well fit to a sine wave with a 24-hour period (solid symbols and solid wave, Fig. 1C). A comparison of these eyes with eyes in L/D (solid symbols and waves versus open symbols and dashed waves) indicates that the mean peak occurs slightly earlier than in L/D (Table 1; 12 PM versus 3 PM; t = 1.76, df = 16, P < 0.05, one-tailed t-test). The mean amplitude in darkness is 30 μm (peak to peak, Fig. 1A, right axis), similar to that for eyes in L/D (37 μm; Ref. 2).

Choroid Thickness

The rhythm in choroidal thickness also persists in constant darkness: choroids become thicker during subjective night and thinner during subjective day (Figs. 2 and 3), with the mean peak occurring at 12 AM (Table 1; data from individual eyes). This is similar to the phase for eyes in L/D, which also show a peak in the middle of the night (Table 1). The rhythm is approximately sinusoidal, having a mean amplitude of 13 μm (Fig. 2, right axis), also similar to that of eyes in L/D (16 μm, Ref. 2), and a diurnal period (best fit sine wave period = 24.5 hours). Another effect of constant darkness is that the thickness of the choroid decreases over this period by approximately 7 μm/d (159 μm at start versus 140 μm at end; paired t-test, t = 2.1, P = 0.06). This thinning must have begun shortly after the chicks were put into darkness: by the first measurement in darkness (after 48 hours), choroids were already significantly thinner than those in eyes in L/D (159 versus 210 μm, measurement at 6 AM in both conditions; Ref. 2; t = 5.2, P < 0.0001).

We conclude that the endogenous rhythms in axial length and choroidal thickness are approximately in antiphase to one another (12 hours apart; Fig. 3), similar to eyes in L/D. This phase difference is significant (t = 7.7; df = 16, P < 0.001).

DISCUSSION

Rearing chickens in constant darkness produces excessive ocular elongation and thinning. The default ocular growth rate is approximately sinusoidal, having a mean amplitude of 13 μm, with a mean amplitude of 30 μm (peak to peak, Fig. 1A, right axis), similar to that for eyes in L/D (37 μm; Ref. 2). This thinning must have begun shortly after the chicks were put into darkness: by the first measurement in darkness (after 48 hours), choroids were already significantly thinner than those in eyes in L/D (159 versus 210 μm, measurement at 6 AM in both conditions; Ref. 2; t = 5.2, P < 0.0001).
An understanding of the role of visual input on these rhythms requires understanding their expression in the absence of visual or temporal cues. We report that the rhythms in axial length and choroid thickness persist in constant darkness, which defines them as circadian, endogenous rhythms.

We show that eyes “released” into constant darkness show several differences from eyes in L/D: (1) there is an increase in the rate of growth, (2) there is a decrease in choroidal thickness, and (3) the peak in the rhythm in axial length occurs slightly earlier. These results imply that in the absence of visual input eyes return to a “default” condition of excessive growth. Similar effects are also found in eyes that are deprived of form vision by translucent occluders, implying that deprivation may resemble a “constant condition” under which eyes also revert to the “default” state. Because diffusers do not appreciably reduce the amount of light reaching the retina (0.6 log units, Ref. 13) but do reduce spatial contrast, it follows that visual transients, in addition to light per se, may function as a Zeitgeber for these rhythms and may influence ocular growth. We hypothesize that the effects of visual transients on ocular growth are effected (at least in part) by shifts in phase in one or more ocular rhythms. We speculate that the small phase advance in the rhythm in axial length in constant darkness and in form deprivation may be causally related to the faster rate of growth in both conditions. This would imply that form vision phase-delays a rhythm underlying the rhythm in elongation (e.g., the rhythm in scleral proteoglycan synthesis14), perhaps influencing the growth rate by virtue of altering the phase relationships between this rhythm and others (such as IOP15).

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### References


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