Diurnal Rhythms of Spherical Refractive Error, Optical Axial Length, and Power in the Chick

Melanie C. W. Campbell,1,3 Kaitlin Bunghardt,1 Marsba L. Kisilak,1,2 and Elizabeth L. Irving2

PURPOSE. To measure the diurnal variation of spherical equivalent refractive error (mean ocular refraction or MOR) and to investigate factors contributing to it in chick, an important animal myopia model.

METHODS. Nine chicks developed naturally on a 14-hour light/10-hour dark cycle. Optical axial length (OAL) and Hartmann-Shack wavefront error (HSWE) measurements, including pupil size, were taken starting on day 7, at eight times during the following 32 hours. MOR was calculated for a constant pupil size from HSWE measurements.

RESULTS. MOR, OAL, and pupil size showed significant diurnal variation (P < 0.0001). Most eyes showed significant sinusoidal variations in MOR and in pupil size with periods close to 24 hours. On average, MOR oscillated ±0.84 diopters. OAL varied with a period not different from 12 hours. Diurnally varying MOR and OAL were correlated (P = 0.0003, R2 = 0.62). However, as previously reported, the variation in OAL did not account for the variation in MOR. From these results, we derived the diurnal variation in ocular power necessary to give the measured MOR variation.

CONCLUSIONS. We confirmed a diurnal variation in OAL and found diurnal variations in pupil size and MOR. Although changes in OAL explain the MOR previously observed in response to lenses and diffusers, they do not completely account for the observed diurnal variation of MOR nor for the reduction in hyperopia during normal development. We infer that the diurnal variation in MOR and normal emmetropization both result from small differences in the relative changes of OAL and ocular power. (Invest Ophthalmol Vis Sci. 2012;53:6245–6253) DOI:10.1167/iovs.11-8844

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After subtraction of the linear fits, residual pupil sizes plotted versus time for left (solid) and right (open circles, covered by the solid symbols) eyes, with nonsignificant linear fits that were subtracted from the data points (left solid, and right dashed, overlapping lines). Note that the averages of the linear fits across all left and right eyes were significant. After subtraction of the linear fits, residual pupil sizes plotted versus time for left (solid) and right (overlapping open circles) eyes. These were significantly fitted by sine curves for each of the left (solid) and the right eyes (dashed, overlapping) \( (P < 0.02, R^2 = 0.8) \). When averaged across individual fits (Table), periods were not significantly different from 24 hours, and amplitudes were not significantly different from the average amplitude here or from the fits of the residual average pupil size.

FIGURE 1. Shaded regions highlight periods of darkness with error bars giving standard errors. (a) The average minimum pupil size across all eyes at each time, for left (solid) and right (open circles, covered by the solid symbols) eyes, with nonsignificant linear fits that were subtracted from the data points (left solid, and right dashed, overlapping lines). Note that the averages of the linear fits across all left and right eyes were significant. (b) After subtraction of the linear fits, residual pupil sizes plotted versus time for left (solid) and right (overlapping open circles) eyes. These were significantly fitted by sine curves for each of the left (solid) and the right eyes (dashed, overlapping) \( (P < 0.02, R^2 = 0.8) \). When averaged across individual fits (Table), periods were not significantly different from 24 hours, and amplitudes were not significantly different from the average amplitude here or from the fits of the residual average pupil size.

FIGURE 2. Shaded regions highlight periods of darkness with error bars giving standard errors. (a) OAL as a function of time for left (solid) and right (open circles) eyes of a sample bird. The linear fit to this left eye data (solid) increases significantly over time \( (P = 0.03) \) while that to this right eye (dashed) does not. Both lines were subtracted from the raw data. (b) The sinusoidal fits (left solid and right dashed) to the residual data for this bird were both significant \( (P = 0.005, R^2 = 0.88; P = 0.01, R^2 = 0.84, \text{ respectively}) \) with periods not significantly different from 24 hours, as in a total of three eyes. (c) Eyes of another bird showed a sinusoidal variation in residual OAL data with periods not significantly different from 12 hours \( (P = 0.01, R^2 = 0.84, P < 0.04, R^2 = 0.75) \), as in 15 eyes, giving an average period across all left and right eyes not different from 12 hours (Table).
Table. Linear and Sinusoidal Fits to Ocular Parameters

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<thead>
<tr>
<th>Linear Fits</th>
<th>Sinusoidal Fits</th>
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<td><strong>No. of Eyes for which ( P \leq 0.05 )</strong></td>
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<td><strong>Slope</strong></td>
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The number of eyes with significant linear fits and the number with subsequent significant sinusoidal fits are shown. Average and standard errors across all left and right eyes of the slopes of the linear fits and of parameters of the sinusoidal fits to residual data are given. Phase is \( c \) in equation (1) in radians, where 6.28 radians correspond to one period. Phase in hours was estimated by using the average period.

* Significant slope.
† Not significantly different from 24 hours.
§ Rayleigh test indicates a significant clustering.
‡ Not significantly different from 12 hours.

\[
f = a \sin \left( \frac{2\pi t}{b} + c \right), \tag{1}\]

where \( f \) is the property considered; \( t \) is time; and \( a, b, \) and \( c \) are constants, representing the amplitude, period, and phase of the oscillation, respectively. Fits were considered to be significant for \( P \leq 0.05 \).

Linear and sinusoidal variations fitted to data from each eye were averaged separately across right and left eyes. Averaging sinusoidal parameters across eyes is preferable to fitting averaged data when performing circadian analyses.39 The acrophase (first peak position in time) was calculated for each eye from the sinusoidal fits.

Results

A univariate ANOVA (SPSS; IBM, Armonk, NY) showed a significant difference in pupil size, OAL, and MOR with time (\( P < 0.0001 \)) but no significant difference with eye. Therefore, the statistical analyses were performed for left and right eyes both separately and combined.

A summary of the number of eyes with significant linear and sinusoidal fits, the average linear slopes, and the amplitudes, periods, phases, and acrophases of the sinusoidal fits (separately across all right and left eyes) are given in the Table for OAL, pupil size, and MOR. We used one sample \( t \) tests to compare periods to 12 and 24 hours where data were normally distributed (SPSS; IBM). OAL was not normally distributed and a Wilcoxon test was performed to compare its period to 12 and 24 hours (Table). Slopes for OAL, pupil size, and MOR versus times were tested against zero by using a Wilcoxon test. For pupil size and OAL, linear changes were similar in magnitude to the sinusoidal changes; for MOR, sinusoidal changes were larger.

Pupil size data averaged across right and left eyes (Fig. 1) are shown because they have periods and amplitudes not significantly different from the averages across individual fits. All other sinusoidal plots are representative individual eyes (Fig. 2; see also Figs. 4, 5, 6).

Pupil Size

Pupil size was not correlated with OAL or MOR. None of the eyes showed a significant linear variation of minimum pupil size over time, but the average of slopes across eyes was
significantly larger than zero for the right ($P = 0.007$) and left ($P = 0.0001$) eyes (Table). Plots of minimum pupil size, averaged separately across right and left eyes, are shown before the subtraction of the linear fits (Fig. 1a). For minimum pupil size against time, sinusoids fitted to the residual data were significant for most eyes, with an average period for left and right eyes not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b). Sinusoidal fits to the averaged residual data (Fig. 1b) for minimum pupil size also had periods not significantly different from 24 hours and significant for most eyes, with an average period for left and right eyes not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b). Sinusoidal fits to the averaged residual data (Fig. 1b) for minimum pupil size also had periods not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b). Sinusoidal fits to the averaged residual data (Fig. 1b) for minimum pupil size also had periods not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b). Sinusoidal fits to the averaged residual data (Fig. 1b) for minimum pupil size also had periods not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b). Sinusoidal fits to the averaged residual data (Fig. 1b) for minimum pupil size also had periods not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b). Sinusoidal fits to the averaged residual data (Fig. 1b) for minimum pupil size also had periods not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b). Sinusoidal fits to the averaged residual data (Fig. 1b) for minimum pupil size also had periods not significantly different from 24 hours and average amplitudes of 0.11 mm (Table, Fig. 1b).
amplitudes not significantly different from the average across individual fits, consistent with clustering in time of the first peaks (Table and see section on “Timing of the Peaks”). Fits to the average pupil size across left and right eyes were not significantly different from those to the minimum pupil size.

**Optical Axial Length**

Five of 18 eyes showed a significant increase in OAL over the course of the experiment. The (predominantly positive) slopes of OAL versus time averaged across all left and right eyes separately were significantly different from zero (Table). Residual plots of OAL versus time were made for each individual eye. Most of the eyes (Table) showed significant sinusoidal fits with average periods of 14 and 15 hours, significantly shorter than 24 hours ($P = 0.9991$ for left and 0.002 for right eyes) but not different from 12 hours (Table). Three eyes had periods greater than 22 hours (Fig. 2b), while 0.002 for right eyes) but not different from 12 hours (Table).

**Timing of the Peaks of Sinusoidal Variations of Optical Properties of the Eyes**

The amplitudes versus time of the first peaks (acrophases) for the sinusoidal variation of selected properties for each individual eye are shown in the clock plots in Figure 5. For pupil size (Fig. 5a), all of the first peaks were in daylight with an average position of 5.7 hours. A Rayleigh test confirmed significant clustering ($P < 0.001$) as well as significant clustering of the phases ($P < 0.001$) around 10 hours (Table).

For OAL, the phases were significantly clustered ($P < 0.05$) at around 5 hours for right and left eyes but the acrophases were not. However, the acrophases happened in the light for 16 of 18 eyes (Fig. 5b), on average at 7 hours. Neither phases nor acrophases of MOR were significantly clustered. Acrophases of MOR (Fig. 5d) were spread more evenly between daylight and darkness with most MOR peaks in daylight, on average at 11 hours, while phases averaged around 9 hours.

For some eyes, OAL and MOR had second peaks within the 24 hours but pupil size did not. When second peaks were combined with acrophases, for OAL (Fig. 5c) there appeared to be two clusters near 0 (or 24) hours and near 12 hours.

**DISCUSSION**

**Circadian Measurements and Calculations**

Our data were acquired every 4 hours over day and night unlike previous circadian rhythm studies, where measurements were performed at longer intervals (minimum 6 hours during day and night or just during the day). Our data were initially fitted with straight lines because we expected a linear change with growth and emmetropization. The lack of significance in linear fits to most of the individual eyes likely arises from the short time over which measurements were made and the overlaid sinusoidal variation. Averages across individual linear fits for pupil size and OAL were significant as expected. If MOR measurements were made over a longer period, a significant negative slope would be expected.1–4 After subtraction of the linear fits, all individual data sets were analyzed, sine curves fitted to most of the eyes were significant (Fig. 4b, Table). The average period of the fits across all eyes was 20 hours, different from 24 hours at $P = 0.05$ for all eyes ($P = 0.45$ for right eyes and $P = 0.05$ for left eyes). Right eyes had periods significantly different from 12 hours ($P = 0.014$), while left eyes did not ($P = 0.055$). For all eyes, periods were significantly different from 12 hours ($P = 0.001$). Average amplitudes of right and left eyes were 0.8 and 0.9 D (Fig. 4a, Table).
For pupil size variation, the parameters are not significantly different for a sinusoidal fit to averaged residual data than for the average of individual fits. However, for MOR and OAL, more accurate estimates of sinusoidal parameters (including larger amplitudes) were obtained by fitting data from individual eyes and then averaging the sinusoidal parameters. The surprising finding of similar, significantly clustered acrophases for pupil size but not for MOR or OAL.

**Pupil Size**

The observed linear increase in pupil size over time is consistent with previous reports. Both the average and minimum pupil sizes (measured during H-S illumination) show a circadian variation with their peak significantly clustered around 6 hours after lights on, consistent with reports of increased size in daylight hours and suggesting a light-entrained rhythm (Fig. 5a). This diurnal variation agrees with that found in humans but not with randomly occurring pupil diameter acrophases or maximum pupil size in the dark in other species. There does not appear to be a contribution from differential accommodation as pupil size is not correlated with MOR.

**Optical Axial Length**

Axial length and choroidal thickness, which fluctuate approximately in antiphase in normal chicks, both influence the OAL to the retinal surface. OAL increased linearly at a faster rate than, but within one standard deviation of, previously reported growth rates. The residual sinusoidal variation in OAL, whose amplitude is a large fraction of its linear variation, is consistent with earlier results for OAL and axial length. Not surprisingly, our average amplitude is significantly larger than the average variation reported by Tian and Wildsoet, measured only through daylight hours. However, our average amplitude of OAL oscillation is also larger than that predicted by Nickla and colleagues (0.1-mm amplitude peak to trough of averaged data, based on an assumption of perfectly out-of-phase oscillations of axial length and choroidal thickness) and Papastergiou and colleagues (0.08 mm). A larger amplitude, closer to the actual value, is expected from our more frequent measurements and averages across individual fits. Our larger amplitude may also be due to our use of a faster-growing strain of bird, which might also explain the larger linear increase in OAL. Differences in ocular parameters in growing chicks between measurements with repeated anesthesia (as used in previous diurnal measurements) and without (ours) have also been reported.

Most eyes had OAL periods close to 12 hours with a minority close to 24 hours. It is difficult to compare with previous chick studies, as they have constrained the period of fits to 24 hours. Our averaged data show the previously reported trend of increasing daytime length between 4 and 12 hours and little increase in darkness. This is predicted by the combination of average period and phase of oscillation and the linear changes measured. For the average 14-hour period, the phases significantly clustered around 5 hours and produced maximum amplitudes close to both lights on and off (Figs. 5b, 5c), and thus acrophases were not significantly clustered. As expected, larger standard deviations were found in the acrophases of OAL (Table) than in those of axial length or choroidal thickness, which contribute to OAL. Variation in our (unconstrained) periods also increased the variability of acrophases above that of phases. However, the average position of the acrophase occurred 7 hours after lights on, consistent with Nickla and colleagues’ inference for the maximum optical length to the retina at 6 hours after lights on and axial length peaks 7 hours after lights on, without assessing clustering. In humans, significant clustering of OAL acrophases has been found in the morning, 3.5 hours after lights on by some, but others show variable acrophases mostly in daylight, including early evening.

**Diurnal Variation of MOR**

We mapped, for the first time, a full diurnal cycle of the variation of MOR, whose average period of 20 hours suggests a circadian rhythm. Others measuring only during the day, have suggested that chick eyes become more hyperopic in the evening, but we found that acrophases (most hyperopic MOR) spread throughout the day and night with insignificant clustering, although most peaks were in daylight hours (Fig. 5d).

**Dependence of MOR on OAL and Power**

For 36 hours, our results showed no significant linear change in MOR, while there was a significant linear increase in OAL (Table). However, residual circadian changes in MOR and OAL were significantly correlated in each eye, as was the pooled raw data. However, the slopes of raw and residual plots of MOR versus OAL (Figs. 3b, 3c) are opposite in sign to that given by a schematic eye model, confirming their previously suggested “paradoxical” relationship. That is, when the eye lengthens, it surprisingly becomes more hyperopic.

A change in MOR can be expressed in terms of changes in optical length and in ocular power (see Appendix equation A1). If the power does not change, the constant of proportionality, based upon a schematic eye model of a normally growing 7-day-old chick eye, can be calculated as –26 D/mm optical length change (see Appendix equation A2). However, our slopes are positive, indicating a large concurrent contribution of the second term in equation A1, a change in power. We predicted the variation of power by rearranging equation A1 (see Appendix equation A3) and substituting the measured variations of MOR and OAL.

In Figure 6, we predict the variation of MOR due to the variation in OAL. The variation in power is the difference between this prediction and the actual variation of MOR. The curves for power for each of the three sample eyes shown (Fig. 6) are approximately sinusoidal. In these examples, the variation of MOR predicted from OAL variation and the variation of power are in phase, producing a reduction in the amplitude (Fig. 6c) or even a phase reversal of MOR variation (Fig. 6b) from that expected from OAL variation alone (Appendix, equation A1). On average, the change in MOR is opposite in direction to that predicted by OAL changes (Figs. 4, 6b). However, the variation of MOR is sometimes dominated by the length contribution (Fig. 6c) and sometimes shows a more complex relationship over time (Fig. 6a). An approximately 12-hour period in OAL can produce a longer (~24 hour) period in MOR (Fig. 6a) or a similar period in MOR (Fig. 6b).

Over 24 hours, MOR has an amplitude of variation of >30% and OAL of only 2%. However, a 2% decrease in eye power with a concurrent 2% increase in OAL gives a 25% increase in MOR. Adjusting the change in power to 2.5% predicts the observed amplitude change in MOR. This emphasizes the relative amplitudes of length and power oscillation, which produce the observed MOR oscillation, and the fact that any direct measurement of power oscillation used to predict MOR will need to be quite precise.

Since shorter time-course diurnal changes do not follow the expected relationship between MOR and OAL (Fig. 4), we considered published longer-term changes during normal emmetropization of the chick eye. During normal growth,
the power decreases and OAL increases, with active tuning of the retinal position leading to a less hyperopic MOR. However, MOR and OAL change on different time courses. During the first 16 days post hatching, the average rate of change in MOR is $-2.55$ D/mm change in OAL, much less than the value predicted (Appendix, equation A2) but with the expected sign, indicating that OAL contributes more than power. The small rates of change of MOR for both short-term diurnal changes and longer-term emmetropization indicate that in normal growth, changes in the power of the eye (the second term in Appendix equation A1) are as important to MOR changes as are OAL changes.

In form-deprivation myopia, on average, 13.47 D of myopia is induced for a 0.47-mm change in vitreous chamber depth, giving a proportionality constant of $-28.6$ D/mm, not significantly different from that calculated from OAL (Appendix, equation A2). For induced differences between treated and untreated eyes for lenses between $-10$ D and $+18$ D, MOR and axial length have a linear dependence with a slope equal to the $-26$ D/mm, as predicted (Appendix, equation A2). Thus, length changes almost completely account for long-term experimental induction of MOR, without considering changes in power.

There are several possible explanations for the observed circadian dependence of MOR on OAL and power, where power decreases as length increases. This could correspond to a flattening of the cornea, a decrease in crystalline lens power, and/or an increase in the anterior chamber depth (ACD), consistent with MOR, lens, ACD, and length changes found by Tian and Wildsoet and their postulated flattening of the cornea. However, others find out-of-phase ACD and length changes for both short-term and untreated eyes for lenses between $-10$ D and $+18$ D. For our experimental induction of MOR, without considering changes in power, there are other possible explanations for the observed dependence of MOR on OAL and power. For example, melatonin has been shown to regulate corneal thickness and to affect corneal hydration and to affect corneal thickness and diurnal fluctuations in normal emmetropization, and long-term response to either form deprivation or lens-induced refractive error. Thus, the influences of lighting conditions and the induction of refractive error on diurnal variations in MOR, OAL, and power should be explored.

**References**


