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

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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 ($P = 0.0003$, $R^2 = 0.62$). 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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Presented in part at the Optical Society of America Vision Meeting in Rochester, New York, October 2008, and in part at annual meetings of the Association for Research in Vision and Ophthalmology, Fort Lauderdale, Florida, May 2009, May 2010, and May 2011.

Supported by the Natural Sciences and Engineering Research Council (NSERC), Ontario Photonics Consortium (OPC), Canada Research Chair (CRC), Canadian Foundation for Innovation (CFI), and Ontario Innovation Trust (OIT).

Submitted for publication October 21, 2011; revised February 15, June 29, and August 3, 2012; accepted August 6, 2012.

Disclosure: **M.C.W. Campbell**, None; **K. Bunghardt**, None; **M.L. Kisilak**, None; **E.L. Irving**, None

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Investigative Ophthalmology & Visual Science, September 2012, Vol. 53, No. 10

In the chicken, day-to-day refractive changes have been measured during normal development, and in response to defocus and to visual deprivation.[1–4] In response to imposed defocus, chick eyes change refractive error within a short time (minutes or hours).[5] Thus, it is important to know if these rapid changes overlay normal circadian rhythms or represent an increased amplitude in a rhythm of refractive error. Circadian rhythms in mean ocular refraction (MOR)[6] would be expected from the rhythms seen in optical aberrations, anterior biometrics, axial length, and/or choroidal thickness. Any rhythmic fluctuations in the curvature or position of the refracting surfaces, refractive indices, or pupil diameter could also contribute to a rhythm in MOR.

It is known that, in a variety of species, many ocular parameters, which could potentially influence MOR, show circadian variations, with close to a 24-hour cycle. In humans, circadian fluctuations have been reported in intraocular pressure (IOP, see Sit[7] for review), axial length,[8–10] choroidal thickness,[8,11] anterior chamber depth,[8,10,12] corneal topography,[13–15] optical aberrations,[15–18] pupil diameter,[19,20] and corneal thickness.[8,10,13,21] In marmoset, a primate myopia model, circadian rhythms of IOP, axial length (cornea to sclera), and choroidal thickness have been observed.[22] Circadian variations of various ocular parameters have also been shown in nonprimate mammals such as rabbit[23–27] and rat.[28]

In chick, a nonmammalian myopia model, normal ocular growth requires a diurnal light cycle.[4,29,30] This, as well as evidence of growth rate changes at night in experimentally induced myopia,[4,31] suggests that knowledge of normal (and abnormal) circadian rhythms may be important to understanding normal and abnormal growth in the chick eye. Circadian rhythms in IOP,[32–34] axial length,[4,6,34–36] choroidal thickness,[6,32,35,36] anterior chamber depth,[6,35] and pupil size[6] have been demonstrated in this species. Johnson and colleagues[37] have reported that 2-week-old chickens were more myopic at 9 AM than 6 PM. To date, measured diurnal variations in chick in MOR are inconsistent with measured variations in axial length.[6,37] However, these measurements were performed only during the day.[6,37] Since retinal blur due to MOR is believed to be important in the control of eye growth, in this study we quantified diurnal rhythms in MOR throughout the day and night in normal chicks. We also examined the relation of diurnal rhythms in MOR to diurnal changes in pupil size and optical axial length (OAL; anterior cornea to anterior retina).

Methods

Nine Ross-Ross (*Gallus gallus domesticus*) chicks of mixed sex were obtained on the day of hatching and developed naturally under a fluorescent light cycle of 14-hour light/10-hour dark until day 7. Beginning on day seven, 4 hours after the lights were turned on (time 0), measurements were taken every 4 hours for 24 hours with an additional measurement at 36 hours, for a total of eight measurement times. Retinoscopy, A-scan ultrasound, and Hartmann-Shack wavefront

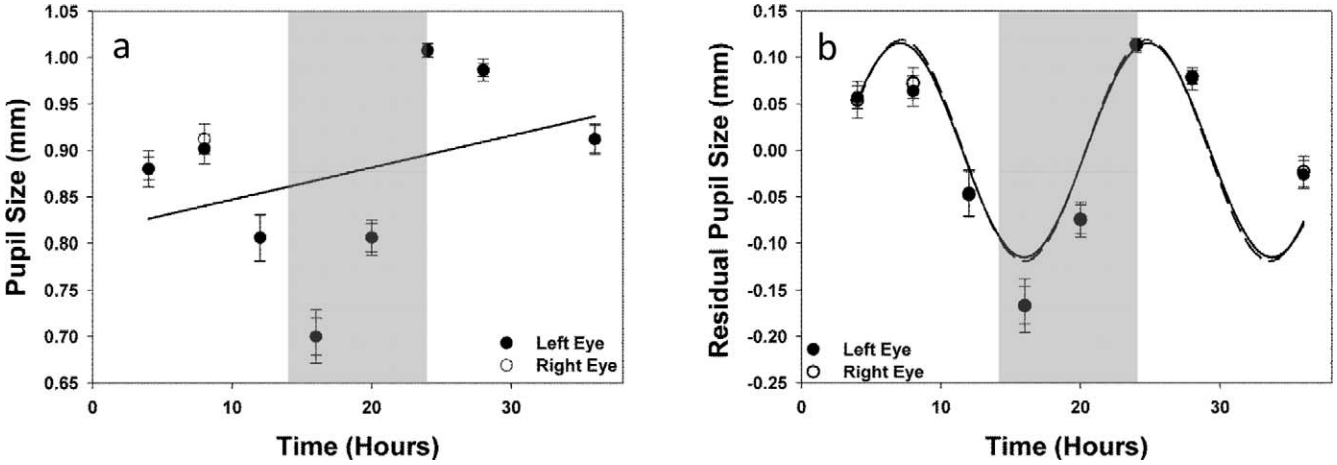

Figure 1. *Shaded regions* highlight periods of darkness with error bars giving standard errors. (**a**) The average minimum pupil size across all eyes at each time, for left (*solid*) and right (*open circles, covered by the solid symbols*) eyes, with nonsignificant linear fits that were subtracted 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.

error (HSWE) measurements[3] were performed at each measurement time. Retinoscopy (precision of 0.5 diopters [D]) was used to specify a spherical lens correction during HSWE measurements. OAL was measured from the anterior cornea to the retinal surface with A-scan ultrasound to a precision of 0.05 mm. The sensitivity of the HSWE measurement of spherical refractive error was 0.25 D. During the day, chicks were kept in the light and at night, in the dark, with the exception of the short time needed for measurement. Ambient illumination at the eye was between 0.5 and 2.5 lux during all measurements. Red light (633 nm) was used for HSWE measurements, which took less than 5 minutes. Birds were lightly restrained during measurements without the use of anesthesia or lid retractors. Owing to a lack of bird cooperation, at two measurement times, data are missing from two different eyes, and at one measurement time, data are missing from one eye. All experiments received ethics clearance from the University of Waterloo Animal Care Committee and adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

To minimize the influence of accommodation and off-axis measurements, only Hartmann-Shack (H-S) images with the largest round pupils within a measurement session were analyzed. Entrance pupil diameter was measured from the H-S images, after thresholding, to make the edges of the pupil visible. Values of MOR, determined from HSWE measurements[38] (see Appendix) were determined from an average of 23 images (minimum 5) for each eye at each measurement time. Both minimum and average pupil sizes across frames for each eye were analyzed as a function of time. To avoid the influence of any sinusoidal diurnal rhythm of pupil size, MOR was calculated for the fixed largest common pupil size across all eyes and all measurement times.

Data for pupil size and MOR were averaged across frames at each measurement time for individual eyes. OAL, MOR, and pupil size were then plotted as a function of time. Most plots displayed sinusoidal variations superimposed on linear variations (e.g., see Fig. 2a). Because the normal eye is growing and undergoing emmetropization, any circadian rhythm in OAL, pupil size, or MOR is expected to overlay a linear change. The linear slopes averaged across eyes were significant for OAL and pupil size (Table). Although the MOR shows an insignificant linear change, for consistency, we subtracted the individual fitted linear variations before fitting the sinusoidal variation.

After both significant and nonsignificant linear fits (Table) were subtracted from the time-dependent data,[36] the resulting residual data were then plotted (e.g., see Fig. 2) and fitted with a three-parameter sine curve:

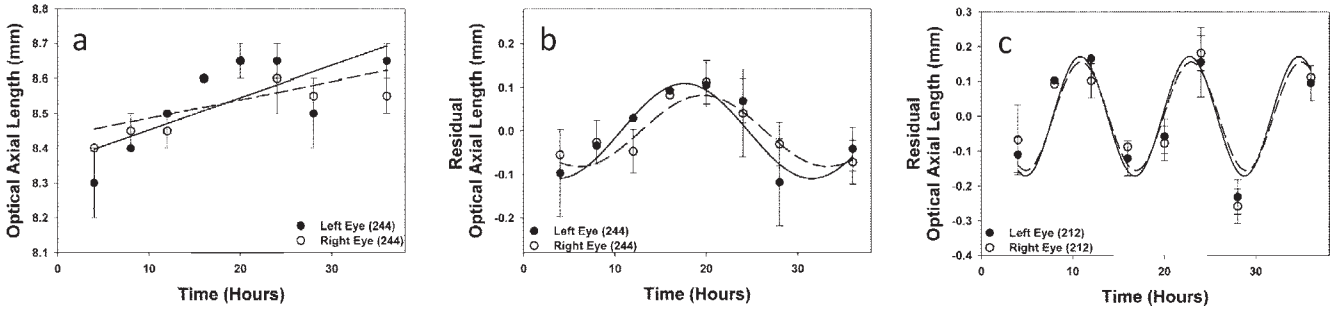

Figure 2. *Shaded regions* highlight periods of darkness with error bars giving standard errors. (**a**) OAL as a function of time for left (*solid*) and right (*open circles*) eyes of a sample bird. The linear fit to this left eye data (*solid*) increases significantly over time ($P = 0.03$) while that 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 ($P = 0.005$, $R^2 = 0.88$; $P = 0.01$, $R^2 = 0.84$, 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 ($P = 0.01$, $R^2 = 0.84$; $P < 0.04$, $R^2 = 0.73$), 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

	Linear Fits		Sinusoidal Fits				
	No. of Eyes for which $P \leq 0.05$	Slope	No. of Eyes for which $P \leq 0.05$	Amplitude	Period, h	Phase, rad	Acrophase, h
Pupil size							
OD	0/9	0.08 ± 0.01 mm/d*	6/9	0.107 ± 0.006 mm	22 ± 1†	3.3 ± 0.9§	5.7 ± 0.7§
OS	0/9	0.05 ± 0.01 mm/d*	7/9	0.110 ± 0.008 mm	22 ± 1†	2.6 ± 0.8§	5.7 ± 0.7§
OAL							
OD	2/9	0.16 ± 0.04 mm/d*	6/9	0.16 ± 0.02 mm	14 ± 2‡	2.5 ± 0.6§	8 ± 2
OS	3/9	0.17 ± 0.04 mm/d*	8/9	0.16 ± 0.02 mm	15 ± 2‡	2.1 ± 0.3§	7 ± 2
MOR							
OD	0/9	0.1 ± 0.2 D/d	3/9	0.8 ± 0.1 D	22 ± 3†	3.2 ± 0.6	12 ± 2
OS	0/9	0.1 ± 0.1 D/d	8/9	0.9 ± 0.1 D	18 ± 3‡	2.4 ± 0.5	10 ± 2

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.

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

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

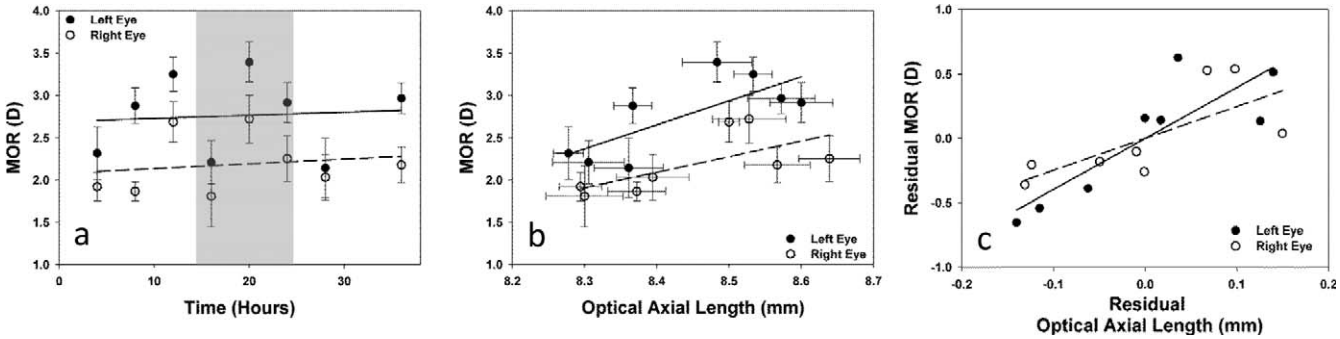

Figure 3. (**a**) Plots of the initial MOR data averaged across eyes versus time, with error bars giving standard errors and the *shaded region* showing times of darkness. Neither the fit for the left ($P = 0.8$, *solid symbols* and *line*) nor the right ($P = 0.7$, *open symbols*, *dashed line*) eye was significant. (**b**) Plots of MOR averaged across eyes at each measurement time versus OAL averaged across eyes at the same time for left and right eyes with the same *symbols* as in (**a**) with error bars giving standard errors. The fit for the left eye was significant ($P = 0.04$, $R^2 = 0.55$), it was not for the right eye ($P = 0.07$, $R^2 = 0.44$) but was for right and left eyes combined ($P < 0.03$, $R^2 = 0.29$). (**c**) Average residual values of MOR were plotted versus average residual values of OAL for each measurement time for left and right eyes with the same *symbols* as in (**a**). Error bars would be the same as in (**b**) and are left off for clarity. The fits were significant for the left (*solid*) ($P < 0.007$, $R^2 = 0.73$) and right (*dashed*) ($P < 0.05$, $R^2 = 0.51$) eyes and overall ($P = 0.0003$, $R^2 = 0.62$), and as in (**b**), are of opposite sign to that expected.

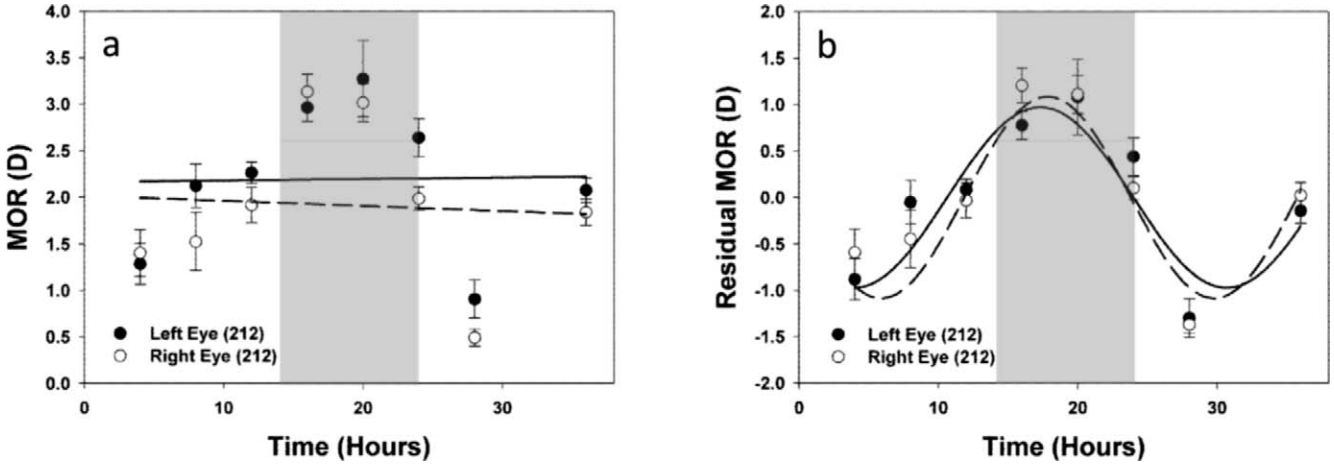

Figure 4. *Shaded regions* highlight periods of darkness with error bars giving standard errors. (**a**) MOR versus time for left (*solid*) and right (*open circles*) eyes of a sample bird. The linear fit for neither the left (*solid*) nor the right (*dashed*) eye data was significant, as was the case for all eyes. Both *lines* were then subtracted from the raw data. (**b**) Residual MOR versus time for the left (*solid*) and right (*open circles*) eyes of the bird in (**a**). The sinusoidal fits (*left solid*, and *right dashed*) were significant ($P < 0.02$, $R^2 = 0.8$; $P < 0.006$, $R^2 = 0.87$, respectively), as they were for most eyes.

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

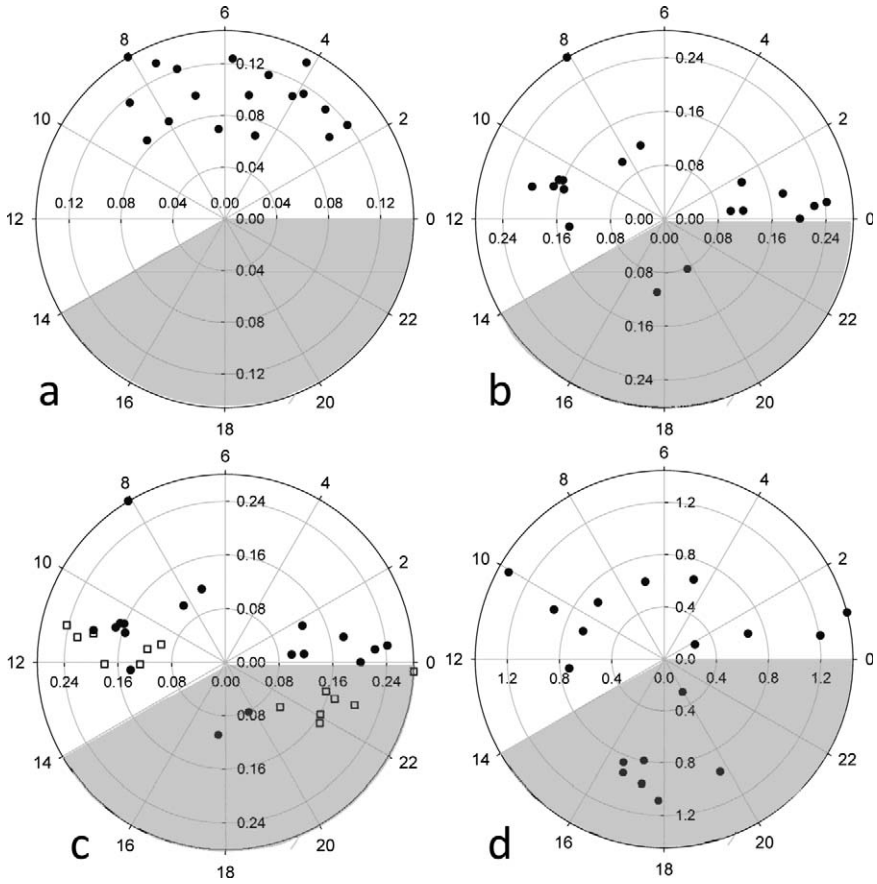

Figure 5. *Shaded regions* highlight periods of darkness. Clock plots of the amplitude (labeled on the rings) versus time (hours labeled on the outer ring) of the first peak (acrophase) of (**a**) pupil size variation (mm) for which the acrophase was significantly clustered in daylight around 6 hours, (**b**) OAL (mm) for which the acrophases were mainly in daylight, (**c**) peaks for OAL including acrophases (*filled circles*) and any second peaks (*open squares*) within 24 hours, and (**d**) MOR (D) for which acrophases were spread throughout daylight and darkness up to 20 hours, with most in daylight.

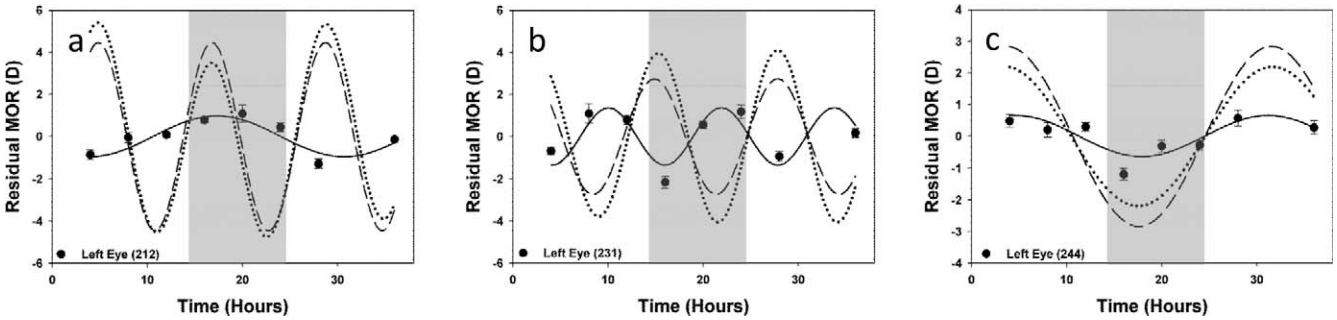

Figure 6. *Shaded regions* highlight periods of darkness. (**a**), (**b**), and (**c**) show plots where the temporal variation of ocular power can be inferred for different sample eyes. The *dashed line* is the predicted variation of residual MOR, calculated from the variation of residual OAL for that eye. The data points are the experimental measurements of residual MOR with a *solid line* sinusoidal fit. Note that in each case, the measured variation in residual MOR is not equal to the variation predicted from the measured variation in residual OAL, implying that the residual ocular power also varies with time. The *dotted line* is the residual ocular power variation, predicted from the other two curves. In other words, the *dashed line* (MOR variation from OAL variation) minus the *dotted line* (predicted power variation) gives the observed MOR variation (*solid line*). In each case, the predicted variation in ocular power is approximately sinusoidal with a similar phase to the variation in MOR from OAL, such that their difference gives the measured, smaller variation in MOR, on average out of phase with the prediction from OAL as in (**b**).

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 ($P = 0.0001$ 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 15 of the eyes had periods less than 16 hours (Fig. 2c). The average amplitudes for left and right eyes were 0.16 mm.

Mean Ocular Refraction

Linear fits of MOR versus time were not significant for any individual eye (Table, Fig. 3) nor were the averages of the slopes across left or right eyes. When averaged across eyes, MOR correlated with OAL over time only for left eyes (Fig. 3b) but was significant when data points from both eyes were combined ($P < 0.03$, $R^2 = 0.29$). After subtraction of linear fits, residual MOR was significantly correlated with residual OAL for the left and right eyes (Fig. 3c) and when data points from both eyes were combined ($P < 0.0003$, $R^2 = 0.62$). The slope of average MOR versus average OAL (Fig. 3b) for both eyes combined was 2.2 D/mm and for the residual data, the slope of 3.2 D/mm (Fig. 3c) was not significantly different.

When residual MOR data from each individual bird 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).

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 fitted with sinusoidal curves (R^2 between 0.4 and 0.96), with none showing the high-frequency components observed previously by others in abnormal rearing conditions.[31] We averaged across all fits but sinusoidal parameters were similar to averages across the subset of significant fits; periods differed by less than 2% and amplitudes by less than 7%.

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.[39] This is not surprising given our 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.[3,6] 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[6,19] and suggesting a light-entrained rhythm (Fig. 5a). This diurnal variation agrees with that found in humans[20] but not with randomly occurring pupil diameter acrophases[40] or maximum pupil size in the dark in other species.[26,41] 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,[31–33,36] 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.[34,36] The residual sinusoidal variation in OAL, whose amplitude is a large fraction of its linear variation, is consistent with earlier results for OAL[4,6,32] and axial length.[31,33,36] Not surprisingly, our average amplitude is significantly larger than the average variation reported by Tian and Wildsoet,[6] measured only through daylight hours. However, our average amplitude of OAL oscillation is also larger than that predicted by Nickla[31] 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[32] (0.08 mm). A larger amplitude, closer to the actual value,[39] 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.[42]

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.[31,35,36] Our averaged data show the previously reported trend of increasing daytime length between 4 and 12 hours and little increase in darkness.[4,6,32] 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,[31] 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[35] and axial length peaks 7 hours after lights on,[36] without assessing clustering. In humans, significant clustering of OAL acrophases has been found in the morning, 3.5 hours after lights on by some,[10] but others show variable acrophases mostly in daylight, including early evening.[9,43]

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,[6,37] 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,[44] confirming their previously suggested "paradoxical" relationship. That is, when the eye lengthens, it surprisingly becomes more hyperopic.[6,37]

A change in MOR can be expressed in terms of changes in optical length and in ocular power[45] (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[44] (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 (Fig. 6a) or a similar period (Fig. 6b) in MOR.

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.[44,46] During the first 16 days post hatching, the average rate of change in MOR is −2.55 D/mm change in OAL,[44] 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,[47] 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,[48,49] 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,[48,49] 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[6] and their postulated flattening of the cornea. However, others find out-of-phase ACD and length changes in chick[32,35] and humans.[8,10]

Approximately out-of-phase changes in power and OAL could be due to a change in IOP, which could simultaneously lengthen the chick eye[4,32–34] and flatten the cornea[6,50] owing to passive expansion.[10,46] IOP changes could also change lens power.[51] In chick, corneal flattening (giving a decrease in corneal power of 1.2 D) corresponds to a 9-mm Hg increase in IOP[50] and for the same IOP increase, OAL increases by 0.11 mm.[32] For our observed diurnal MOR variation, there needs to be a larger relative change 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 hydration[52] and to affect corneal thickness[21] and diurnal fluctuations in ACD,[53] which in turn, would affect eye power. Melatonin, considered the most reliable marker of circadian clock timing, peaks at night in all species studied.[54,55] Dopamine, which has a reciprocal relationship with melatonin (retinal dopamine levels are lower at night than during the day in chicks[56]), has been implicated in the control of eye growth[4,57,58] and is thought to influence diurnal variation of axial length.[4]

Blur on the Retina

Diurnal fluctuations of MOR produce changes in the blur on the retina. It is important to know whether these changes are large enough to be detected by retinal photoreceptors and neurons. From MOR and pupil size variations, the average amplitude of equivalent blur, which has been shown to be a good approximation to the exact angular point spread function on the chick retina,[46] can be calculated as 1.6 ± 0.2 arcmin in both the left and right eyes. The average amplitude of linear retinal blur can also be estimated[46] from equivalent blur combined with OAL as 2.7 ± 0.4 μm in both left and right eyes. Calculated sinusoidal variations of both equivalent blur and linear retinal blur are dominated by the sinusoidal variation of MOR. Amplitudes of oscillation of linear retinal blur are similar to the spacing of cone photoreceptors in chick.[59]

Conclusions

New circadian rhythms were shown in chick for pupil size and MOR. We concluded that MOR shows a diurnal fluctuation due to fluctuations in OAL and power. We showed that relationships between MOR, power, and OAL differ between diurnal fluctuations, 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.

Acknowledgments

Our appreciation is extended to Jeanette O'Hara Hines and Jock Mackay for helpful discussions, Linda Lillakas for proofreading, and Ian Andrews for his assistance with data analysis.

Appendix 1. Calculation of MOR and Its Relationship to OAL and Power

Wavefront errors were fitted to Zernike polynomials up to fourth order.[38] MOR was calculated for each eye of each bird from the corresponding Zernike coefficient and adjusted for any correcting lens at each measurement time for the largest common pupil size for images across eyes and across time. For images with pupils larger than this, the Zernike polynomials were rescaled for this smaller pupil size.[60]

MOR varies with the OAL of the eye and with ocular power[45]

$$\Delta MOR = \frac{-K'^2}{n'}\Delta k' - \Delta F_e, \qquad (A1)$$

where $\Delta k'$ is the change in OAL, ΔF_e is the change in the power of the eye, K' is the dioptric length, and n' is the vitreous refractive index.

Assuming that the variation in residual MOR was due solely to the variation in eye length,

$$\Delta MOR(k') = \frac{-K'^2}{n'}\Delta k'. \qquad (A2)$$

However, the measured relationship between diurnally varying OAL and MOR in chick is not as predicted in equation A2, given the value of K' in a schematic eye model on day 7,[44] indicating a simultaneous variation in power. We calculated this variation by rearranging equation A1 to

$$\Delta F_e = \frac{-K'^2}{n'}\Delta k' - \Delta MOR \qquad (A3)$$

and calculating the first term from literature values of K' for day 7.[44]

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