ARTICLES

Diurnal Rhythms in Intraocular Pressure, Axial Length, and Choroidal Thickness in a Primate Model of Eye Growth, the Common Marmoset

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PURPOSE. In chickens, there are circadian rhythms in axial length, choroidal thickness, and intraocular pressure (IOP), the phases of which differ, depending on visual manipulations that alter ocular growth rate. In this study, these same rhythms were sought in the common marmoset, a primate model of eye growth, to establish whether these may play a role in ocular growth regulation across species.

METHODS. IOP was measured by applanation tonometry in 14 untreated marmosets ranging in age from 24 to 259 days. High-frequency A-scan ultrasonography was used to measure ocular dimensions (axial length and choroidal thickness) in 12 marmosets ranging in age from 24 to 572 days. Four monkeys were measured when they were juveniles (<110 days of age) and again later, when they were adolescents. Measurements were typically made at 12-hour intervals, although three animals were measured at 6-hour intervals. Nine monkeys had both IOP and axial dimensions measured in the same experiment.

RESULTS. There was a diurnal rhythm in IOP in the marmosets: IOP was higher during the dark period and lower during the light period (mean change, 3.6 mm Hg; P < 0.005). There were also rhythms in axial length and choroidal thickness. The rhythm in axial length was dependent on age, with faster-growing eyes of juveniles increasing in length during the day and decreasing at night (+25 μm vs. -22 μm; P < 0.001) and slower-growing eyes of adolescents showing the opposite pattern (-27 μm vs. +46 μm; P < 0.0001). The choroid thickened during the night and thinned during the day, at all ages measured (+19 μm vs. -16 μm; P < 0.0001).

CONCLUSIONS. Diurnal rhythms in IOP, axial length, and choroidal thickness exist in primates. Age-related differences in the phase relationships of these rhythms may be associated with differences in the rates of ocular growth. (Invest Ophthalmol Vis Sci. 2002;43:2519–2528)

Many physiological processes oscillate with a 24-hour periodicity; these diurnal rhythms may be endogenous (driven by an internal clock) and entrained by the 24-hour cycle of light and dark, or they may require the cycle of light and dark for their expression. The eye, the main source of photic information to the central pacemaker, expresses circadian rhythms in various processes at all levels of organization from the molecular (e.g., melatonin synthesis1–3) through the cellular (retinomotor movements, rod outer segment phagocytosis4), whole organ (intraocular pressure, [IOP])5), and visual system (visual sensitivity6–8) levels.

Recent evidence in chickens7,8 suggests that some ocular diurnal rhythms may play a role in emmetropization (the visual regulation of ocular growth rate to attain emmetropia). It has been shown, for instance, that axial length fluctuates in a diurnal rhythm, increasing during the day and decreasing during the night.7–9 This rhythm has also been found in rabbits; however, the phase of the rhythm is opposite that of chicks, so that rabbit eyes increase in length during the night and decrease during the day.10 In both species, the phase of the rhythm in axial length generally coincides with that of the rhythm in IOP,10,11 which in chicks is high during the day11 and in rabbits is high during the night.12,13–15 This coincidence in phase is consistent with IOP playing a role in the diurnal fluctuations in axial length. However, various lines of evidence indicate that, although IOP may be one factor involved, it is not the sole one.10,11 Other possible underlying factors are the diurnal rhythm in scleral extracellular matrix synthesis16,17 or the rhythm in choroidal thickness.7

In chicks, the thickness of the choroid, the vascular tissue behind the retina, undergoes diurnal oscillations—normally, thickening during the night when the eye is shortest and thinning during the day when the eye is longest. These rhythms in axial length and choroidal thickness are thus in antiphase, thereby increasing the amplitude of the fluctuations in vitreous chamber depth and, consequently perhaps, refractive error. Although the functional significance of these two rhythms is as yet unknown, it has been speculated that they form part of a negative-feedback loop through which the associated fluctuations in refractive error are sensed and translated into changes in ocular growth.16 Further evidence that they may be involved somehow in emmetropization is that their phase relationships appear to vary in a consistent manner, depending on experimentally induced changes in ocular growth rate. Specifically, in eyes that are growing slower than normal, the rhythms shift into phase with one another,18 whereas in faster-growing eyes, they shift completely out of phase.19 Whether these phase shifts are a cause or a result of the changes in ocular growth rate is unknown. However, the rhythm in choroidal thickness could be a component of a rhythm in choroidal “stiffness” that may modulate the mechanical influence of IOP on the sclera (see, for instance, Ref. 19). This is supported by the finding that thicker choroids synthesize more glycosaminoglycans (GAGs, presumably proteoglycans) than thinner ones,20 and that increased proteoglycan synthesis is associated with increased stiffness in some connective tissues.21

The purpose of the present study was to determine whether rhythms in axial length, choroidal thickness, and IOP are...
The study. The horizontal line separates the animals into two age groups referred to in the text as juvenile (ages 24 and left eyes, respectively) is the change in axial length divided by the number of days between two successive US measurements made before than 110 days).

Present in the marmoset model of eye growth, and if so, to determine whether the phase relationships of these rhythms showed similar growth-rate–dependent differences as in the chick. We measured juvenile animals with fast-growing eyes and adolescent animals with slow-growing eyes, and we found diurnal fluctuations in axial length, choroidal thickness, and IOP. Furthermore, the phase at which axial elongation is at its maximum differs with age (and ocular growth rate). Parts of these results have been reported in abstract form.22–24

Methods

Animals

Fifteen normal (untreated) marmosets ranging in age from 24 to 572 days were used in the study (Table 1); both males (n = 9) and females (n = 6) were studied. Marmosets were housed in family groups in our breeding facility. Artificial lighting (Vita-Light, Fairfield, NJ) was on a 12:12 light–dark cycle, with lights on at 7:30 AM and off at 7:30 PM. Temperature was maintained at 24 ± 2°C; humidity was maintained at 45% ± 10%. Food and water were provided ad libitum, with the food consisting of dry pellets (Marmoset Lite; Animal Spectrum, North Platt, NE) with fresh fruit and protein supplements. Before the study, high-frequency A-scan ultrasonography was performed at various intervals (2–4 weeks) to determine ocular dimensions (and growth rates, Table 1). Care and use of animals conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Intraocular Pressure

Measurements. IOP was measured in 14 monkeys ranging in age from 24 to 259 days (mean, 121) by applanation tonometry (Tono-pen; Mentor O&O, Norwell, MA). Measurements were performed in animals under halothane or isoflurane inhalation anesthesia, and a local anesthetic was topically applied to the cornea (AK-Taine, 0.5% proparacaine hydrochloride; Bausch & Lomb, Tampa, FL). Generally, IOP was measured at 6 AM and 6 PM (n = 10) during a period of 48 hours. Sampling at 12-hour intervals with diurnal rhythms can be difficult in that measurements made near the mesor of the rhythm show little variation. However, we also made measurements at other times and intervals: IOP in three monkeys (M1, J1, and K2) was measured at 12 PM, 12 AM, and 12 PM (one full 24-hour cycle), and in one monkey (R1) at 6-hour intervals starting at 2 PM over 24 hours (one full cycle). The measurement schedule for all animals is shown in Table 1. In all except 4 of the 14 monkeys (asterisk and dagger, Table 1), both eyes were measured. All monkeys were measured at only one age.

To minimize the possible effect of brief exposures to light on circadian rhythms, all nighttime measurements were made under a dark yellow photographic safe light that provided approximately 0.5 lux at the monkey’s eye. The animals were immediately returned to the dark after measurement. The same protocol was used for the ultrasound measurements, described later.

We did not calibrate the tonometer for the marmoset eye; therefore, the data may not be an accurate representation of the actual IOP for marmosets. Because the purpose of the study was to ascertain the existence (or absence) of a diurnal rhythm in IOP, relative measures were sufficient, and we therefore considered the killing of a monkey for calibration purposes unwarranted.

Data Analysis. The tonometer (Tono-pen; O&O Mentor) provides confidence interval information based on successive readings. The data shown for each eye per time point represent the averages of at least two readings, with confidence intervals of less than 0.05%. Most of the data were obtained at 12-hour intervals over 48 hours (i.e.,
two cycles and four measurements per eye). The data for corresponding time points over two cycles (for example, 6 AM) were averaged for presentation and analyses. The paired data from right and left eyes (regardless of time of day, n = 10 animals) correlated significantly (r = 0.83; P < 0.01). Thus, in statistical analyses, the averages for the two eyes of individual animals were pooled with the individual eye data for the four cases in which only one eye was measured. A paired t-test was used to compare changes during the day versus those during the night.

**Axial Dimensions**

**Measurements.** Axial dimensions were measured in 12 untreated marmosets at ages ranging from 24 to 572 days (mean, 177) using high-frequency A-scan ultrasonography in animals under halothane anesthesia (for details, see Ref. 7). Four of these 12 monkeys were treated monkeys at ages ranging from 24 to 52 days (mean, 17) using high-frequency A-scan ultrasonography in animals under halothane–indirect measure reflation as the distance from the cornea to the front of the sclera was used instead. Because the identification of the retina–choroidal boundary is sometimes difficult, choroidal thickness was defined as the sum of the retinal and choroidal thicknesses. This indirect measure reflects true choroidal thickness, because retinal thickness is fairly constant over the ages used in this study (Troilo D, Nickla DL, unpublished data, 2000). Data for choroidal thickness in two animals (M1 and J1) was excluded, based on the poor quality of the ultrasound traces.

Because a wide range of ages, and hence eye sizes, were used in this study, to best illustrate the fluctuations in length as a function of time, the data for each eye per time point were normalized to the mean of all measurements for that eye. The data shown in the line graphs (Figures 3A, 4A, 4B, and 7) represent the mean of normalized data for all eyes. In this way, the large age-related variability in eye size was eliminated. The bars represent the standard errors for the normalized data.

Another way to represent the mean diurnal fluctuations in eye size and choroidal thickness is to subtract the ‘length’ at each time point from the length at the next time point for each eye. This procedure was used with 12-hour-interval data. Individual eye data for both cycles were combined (for instance, from 6 AM to 6 PM for both cycles), and data for individual eyes were then pooled to derive the mean ‘raw’ changes in length (and standard errors) across the ‘day’ and ‘night’ (for example, Fig. 3A, inset). Two-sample t-tests were used to compare the data for day and night.

Data collected more frequently than the Nyquist limit (12-hour intervals for a 24-hour period) can be subjected to an analysis that yields phase and amplitude (for example, Fig. 2B). To assess these parameters, a sine wave having a period of 24 hours (the diurnal period) can be fit to the data. Because most of our data were collected at 12-hour intervals, this analysis was possible on only a small subset of data, and no statistics were possible. Despite the absence of accurate ‘phase’ determinations for our 12-hour interval data, we found differences in the temporal pattern of these various rhythms that were related to age. We take the liberty of referring to these pattern differences as ‘phase’ differences.

**RESULTS**

**Rhythm in IOP**

Marmoset eyes exhibit a diurnal rhythm in IOP: IOP was higher during the dark phase, regardless of whether the measurement was obtained midway through (midnight) or late into (6 AM) the dark phase of the diurnal cycle (Fig. 2). The mean IOP during the dark-versus-light phases was 18.0 mm Hg versus 14.4 mm Hg, respectively (paired t-test, P < 0.005; Fig. 2A). A similar trend was evident in the data from the monkey measured at 6-hour intervals (R1, Fig. 2B): the peak occurred at approximately 11 PM, and the amplitude was 6 mm Hg (data for two eyes were averaged and a sine wave fit to these data). Neither the amplitude nor the phase of the diurnal rhythm in IOP differed as a function of age; the mean light–dark differences for juvenile and adolescent animals were 3.6 and 3.5 mm Hg, respectively (n = 7 each). Finally, IOP per se did not differ as a function of age, although it tended to be somewhat higher in older monkeys (means for adolescents versus juveniles, +17.5 mm Hg vs. +14.9 mm Hg, P = 0.06).
Rhythms in Axial Length and Choroidal Thickness

Marmoset eyes exhibited diurnal rhythms in axial length and choroidal thickness, extending earlier findings in chicks to a primate model of eye growth. We found that the diurnal pattern for the rhythm in axial length differed between juvenile and adolescent monkeys \((n = 16\) eyes for both groups, Figures 3, 4, and 5), whereas that for the rhythm in choroidal thickness did not (Fig. 6).

**Axial Length**

**Juvenile Monkeys.** The eyes of juvenile monkeys increased in length during the day (6 AM–6 PM) and decreased during the night (6 PM–6 AM; Fig. 3). The (normalized) means and standard errors for axial length as a function of time for juvenile monkeys are shown in Figure 3A \((n = 16\) eyes). The raw mean diurnal changes in axial length are also shown (Fig. 3A, inset). There was a mean increase of 25 \(\mu m\) from 6 AM to 6 PM and a mean decrease of 22 \(\mu m\) from 6 PM to 6 AM \((t\text{-test}, P < 0.001)\). As an indication of interindividual variability, the data from both eyes of four representative monkeys are shown in Figure 3B. The overall trend of elongation during the day and shrinkage at night prevailed, although both interocular and interanimal differences in the magnitude of the changes were apparent.

**Adolescent Monkeys.** In marked contrast to eyes of juvenile monkeys, eyes of adolescent monkeys decreased in length during the day (6 AM–6 PM) and increased during the night (6 PM–6 AM; compare Figs. 4 and 3; see Fig. 5). Figure 4 shows separately the axial length data from eyes measured at 12-hour intervals—at 6 AM and 6 PM \((n = 9\) eyes, Fig. 4A)—and more frequently—at 8 AM, 2 PM, 8 PM, and 2 AM \((n = 6\) eyes, Fig. 4B). The mean raw diurnal changes in axial length, shown in the insets of the figures, exhibited similar diurnal patterns of changes in length \((-20 \mu m\) vs. \(+38 \mu m; Fig. 4A, inset; \(-49 \mu m\) vs. \(+68 \mu m, Fig. 4B, inset; both differences are significant, \(t\text{-test}; P < 0.0001\) and \(P < 0.01,\) respectively). For an estimation of phase, sine waves were fit to the mean data of the eyes that were sampled more frequently than at 12-hour intervals (R1, solid lines, J1 and M1, dashed lines; Fig. 4B). The period of the best-fit sine wave was approximately 24 hours for both sets of data, and the mean phases were similar (peaks at 5:30 AM, 6:00 AM).

As an illustration of the consistency of the age-related change in the phase of the rhythm in axial length, Figure 5 shows the data for two of the four monkeys measured as juveniles and again as adolescents. The data for the two eyes of each animal are averaged in this representation. The juvenile and adolescent patterns were approximately antiphase in both cases.
Choroidal Thickness

The choroid showed a diurnal rhythm, thickening during the night (6 PM–6 AM) and thinning during the day (6 AM–6 PM; Fig. 6). This pattern was the same in juvenile and adolescent monkeys (Fig. 6A). The averaged data from the two eyes of one monkey (R1, an adolescent) measured on a different schedule is shown by the solid symbols and dashed lines. The phase and amplitude were similar to that of both other groups. In juvenile monkeys (n = 8), the mean day-versus-night change in choroidal thickness was -12 µm versus +18 µm (P < 0.0001), whereas in adolescent monkeys (n = 6), the mean change was -22 µm versus +21 µm (P < 0.0001; Fig. 6B). The mean diurnal change in choroi-
The net result of the age-related differences in the rhythms in axial length was that in juvenile monkeys, the rhythms in axial length and choroidal thickness were (approximately) out of phase (Fig. 7A, top), whereas in adolescent monkeys they were (approximately) in phase (Fig. 7A, bottom). Similarly, the phase relationship of the rhythms in axial length and IOP...
showed age dependence (Fig. 7B). In adolescents, the two rhythms were approximately in phase, whereas in juveniles they were approximately out of phase.

**DISCUSSION**

Marmoset eyes in our study showed diurnal fluctuations in IOP, axial length, and choroidal thickness. IOP was higher during the dark period and lower during the light period at all ages measured. Also in both age groups, choroids thinned during the day and thickened during the night. In juvenile monkeys, axial length increased during the day and decreased at night, whereas in adolescent monkeys the opposite occurred. The net result is that the phase relationships between these parameters differed with age.

**Diurnal Rhythm in IOP**

Diurnal rhythms in IOP have been documented in many species, including rats, rabbits, chicks, and humans. The phase and amplitude of the rhythm in IOP differ between species.

We found that in our marmosets, IOP was higher during the dark period and lower during the light period at all ages measured. Also in both age groups, choroids thinned during the day and thickened during the night. In juvenile monkeys, axial length increased during the day and decreased at night, whereas in adolescent monkeys the opposite occurred. The net result is that the phase relationships between these parameters differed with age.

**Diurnal Rhythm in Axial Length and IOP**

We found that in our marmosets, the eyes showed diurnal fluctuations in axial length, extending this phenomenon to a primate model of eye growth. In chicks, eyes elongate more during the day than during the night, whereas in rabbits, the reverse is true. In normal (untreated) eyes of both these species, the time of maximal elongation approximately coincides with the time of highest IOP, implicating a possible influence of the rhythm in IOP on the rhythm in axial length. Indeed, IOP has long been postulated to play a role in ocular development and ocular growth as a source of inflationary pressure (see, for example, Refs. 36–38). In the marmosets, however, this phase correspondence held true only in the adolescent animals, with their slower-growing eyes increasing in length during the night, when IOP was highest. The faster-growing eyes of juveniles, on the other hand, increased in length during the day, when IOP was lower. This age-related difference in the phases of the rhythm in axial length is not associated with a concurrent age-related phase difference in the rhythm in IOP that might account for this (Fig. 7B). These findings support the notion that IOP is not the only (or the main) influence on axial length, in agreement with several other studies showing a noncorrespondence in the phases of these two rhythms. In rabbits, for instance, the rhythms in IOP and axial length are only approximately in phase: IOP peaks early in the dark phase, whereas axial length peaks late in the dark phase. Furthermore, in form-deprived myopic chick eyes, the rhythm in IOP is no
longer synchronized to the light–dark cycle, yet the phase of the rhythm in axial elongation does not change accordingly. Evidence for the probable involvement of various other ocular growth-related rhythms in emmetropization make the findings of phase differences in IOP and axial length less paradoxical than they at first might appear. Specifically, the synthesis of extracellular matrix glycosaminoglycans in chick sclera exhibits a circadian rhythm that presumably influences the rhythm in axial elongation. It is plausible that the rate of biosynthesis in the sclera is dependent on the phase of the rhythm in IOP, because cyclic forces have been shown to effect changes in growth activities in several connective tissues (for a review, see Ref. 39). Furthermore, it is known that the proteoglycan content of connective tissue influences its mechanical stiffness (e.g., cartilage). Thus, it is reasonable to assume that there is also a rhythm in scleral compliance associated with the rhythm in GAG synthesis. The net effect of IOP on eye size is thus determined, at least in part, by the phase relationships between the rhythms in IOP and in scleral compliance. Finally, if choroidal thickness changes are associated with changes in stiffness (in chick, thicker choroids are associated with increases in proteoglycan synthesis), then the rhythm in choroidal thickness could also play a significant role in modulating the influence of IOP on the sclera (and hence eye size). In summary, in any of these scenarios, the net effect of IOP on eye size is dependent on the phase relationships of these various other rhythms. Some of these rhythms—for example, the rhythm in GAG synthesis—are presumably

![Figure 6. Diurnal rhythm in choroidal thickness. (A) Mean choroidal (choroid+retina) thickness ± SE, as a function of time of day. Left y-axis, data from juvenile and adolescent monkeys; right y-axis: average of both eyes of R1 (an adolescent). The much larger error bars for the adolescent group reflect a much greater variability in choroidal thickness in the older animals. Marmoset choroids thickened during the night (6 PM–6 AM or 8 PM–8 AM) and thinned during the day (6 AM–6 PM or 8 AM–8 PM). (B) Mean raw changes ± SE in choroidal thickness for the 12-hour intervals of day versus night in all juveniles and all adolescents.](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932916/)
more active in young, fast-growing eyes. We speculate that in older animals in which eye growth has almost ceased, the coincidence between the peaks in IOP and axial length may reflect an increasing contribution of IOP to the changes in axial length.

Diurnal Rhythms in Choroidal Thickness

We find that the thickness of the choroid in the marmosets also showed a diurnal rhythm, increasing during the night and decreasing during the day, similar to the rhythm in normal chicken eyes. 

Diurnal Rhythms in Choroidal Thickness

We find that the thickness of the choroid in the marmosets also showed a diurnal rhythm, increasing during the night and decreasing during the day, similar to the rhythm in normal chicken eyes. Unlike the rhythm in axial length, the choroidal thickness rhythm did not differ as a function of age. The net consequence of the age-dependent phase shift in the rhythm in axial length and the age independence of the phase of the rhythm in choroidal thickness is that the phase relationship between these two rhythms changed with age. Specifically, in younger, faster-growing eyes the two rhythms were approximately out of phase, whereas in older, slower-growing eyes they were approximately in phase. This finding has an interesting parallel in chickens: In chicks as well, in rapidly growing eyes, the two rhythms are typically out of phase, whereas in slower-growing eyes they are in phase. 

A true test of the relevance of these different phase relationships to growth rate changes in marmosets would be to study these rhythms in eyes in which the growth rates had been experimentally altered by spectacle lenses or diffusers.

We conclude that diurnal rhythms in IOP, axial length, and choroidal thickness exist in primates, supporting the hypoth-

![Figure 7](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932916/)
esis that these rhythms may subserve similar functions in the control of eye growth across species. The similarities between the different phase relationships for axial length and choroidal thickness as a function of ocular growth rate in both chicks and marmosets further support this idea.

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