Published in final edited form as:

Exp Eye Res. 2016 May ; 146: 189–195. doi:10.1016/j.exer.2016.03.003.

Brief light exposure at night disrupts the circadian rhythms in eye growth and choroidal thickness in chicks

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Abstract

Changes in ocular growth that lead to myopia or hyperopia are associated with alterations in the circadian rhythms in eye growth, choroidal thickness and intraocular pressure in animal models of emmetropization. Recent studies have shown that light at night has deleterious effects on human health, acting via “circadian disruptions” of various diurnal rhythms, including changes in phase or amplitude. The purpose of this study was to determine the effects of brief, 2-hour episodes of light in the middle of the night on the rhythms in axial length and choroidal thickness, and whether these alter eye growth and refractive error in the chick model of myopia.

Starting at 2 weeks of age, birds received 2 hours of light between 12:00 am and 2:00 am for 7 days (n=12; total hours of light: 14 hrs). Age-matched controls had a continuous dark night (n=14; 14L/10D). Ocular dimensions were measured using high-frequency A-scan ultrasonography on the first day of the experiment, and again on day 7, at 6-hour intervals, starting at noon (12pm, 6pm, 12am, 6am, 12pm). Measurements during the night were done under a photographic safe-light. These data were used to determine rhythm parameters of phase and amplitude. 2 groups of birds, both experimental (light at night) and control, were measured with ultrasound at various intervals over the course of 4 weeks to determine growth rates. Refractive errors were measured in 6 experimental and 6 control birds at the end of 2 weeks.

Eyes of birds in a normal L/D cycle showed sinusoidal 24-hour period diurnal rhythms in axial length and choroid thickness. Light in the middle of the night caused changes in both the rhythms in axial length and choroidal thickness, such that neither could be fit to a sine function having a period of 24 hours. Light caused an acute, transient stimulation in ocular growth rate in the subsequent 6-hour period (12 am to 6 am), that may be responsible for the increased growth rate seen 4 weeks later, and the more myopic refractive error. It also abolished the increase in choroidal thickness that normally occurs between 6 pm and 12 am.

We conclude that light at night alters the rhythms in axial length and choroidal thickness in an animal model of eye growth, and that these circadian disruptions might lead to the development of ametropias. These results have implications for the use of light during the night in children.

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Keywords

circadian; chicks; refractive error; choroid; axial length

Introduction

Until the advent of artificial lighting over the past 2–3 centuries, life was adapted to daily 24-hour cycles of darkness and light, and animals (including man) have developed endogenous circadian rhythms synchronized to this cycle, to optimize survival strategies. The modern use of artificial light indoors during both day and night has permanently altered the natural lighting cycle. The negative impact of the increasing prevalence of nighttime illumination resulting from city lights and modern conveniences such as luminous signs on the night environment (Cinzano et al. 2001) and nocturnal species may extend to the realm of human health concerns (Chepesiuk 2009; Stevens et al. 2013). For instance, bedroom night-lights were associated with the development of myopia in children (Quinn et al. 1999) (however this remains controversial as two subsequent studies found no such association (Gwiazda et al. 2000; Zadnik et al. 2000)). Scientific studies have recently linked light at night to increased risk for cancers (review: Feillet et al. 2015), metabolic changes leading to obesity, mood disorders and cardiovascular disease (Chepesiuk 2009; Anisimov et al. 2012; Stevens et al. 2013). Even dim light at night, for instance, from computer screens or televisions, can adversely affect the rhythm in melatonin, a hormone that influences sleep and daily rhythms of physiology, which then leads to alternations in the rhythms in locomotor activity and core body temperature (Fonken et al. 2013; Borniger et al. 2014). The physiological basis of these adverse effects is the impingement of light on the clock driving endogenous rhythms (Wyse et al. 2011; Anisimov et al. 2012). It is well established that brief periods of light at certain times of night (dark part of the L/D cycle) have effects on the phases of circadian systems; these effects are generally most robust when close to dawn (lights on) or dusk (lights off), causing phase advances and delays, respectively. However, light in the middle of the night can also affect the clock, and cause a dampening of the rhythm amplitude, and/or acute affects.

Circadian disturbances, including phase shifts and effects on circadian clock outputs (e.g., melatonin or dopamine) (Stevens et al. 2013) may have profound effects on refractive development. This was first shown in chickens that were reared under constant light conditions: eyes grew excessively long and the corneas flattened (Laubert et al. 1961; Laubert and McGinnis 1966; Li et al. 1995). Constant darkness too, caused excessive eye growth (Gottlieb et al., 1987). The resulting ametropias (refractive errors) depended on the length of time in the abnormal lighting condition, but it was undisputable that eye growth was altered. In chickens (Weiss and Schaeffel 1993; Nickla et al. 1998; Papastergiou et al. 1998) as well as in primates (Nickla et al. 2002) including humans (Stone et al. 2004; Wilson et al. 2006; Chakraborty et al. 2011), the length of the eye oscillates in a diurnal manner, increasing in length during the day and stopping growth, or even decreasing in length, at night. The thickness of the choroid also shows a diurnal rhythm, the phase of which is opposite that of eye length: choroids thicken at night and thin during the day (Nickla et al. 1998; Brown et al. 2009; Chakraborty et al. 2011). It has been found that circadian disruptions in these rhythms

are associated with alterations in ocular growth patterns and refractive development: their phases and/or amplitudes are altered in eyes growing too fast, in response to form deprivation (no image) or negative lens-induced hyperopic defocus (image focused behind the retina), or too slowly, in response to positive lens-induced myopic defocus (image focused in front of the retina) (Nickla et al. 1998; Papastergiou et al. 1998; Nickla et al. 2001; Nickla 2006). In all of these previous experiments documenting the effects of abnormal visual conditions on eye growth, eyes were continuously exposed to the visual condition, which could be argued does not approximate the common visual experience of children, whose nighttime exposure to light would likely not be continuous, but rather for relatively brief periods. With this in mind, we examined the effects of 2 hours of light exposure, in the middle of the night, on the circadian rhythms in axial length and choroid thickness, and on eye growth rate, in young chicks. We report that light at night disrupts the sinusoidal diurnal rhythms in axial length and choroidal thickness. Parts of this manuscript have been presented in abstract form (Nickla and Totonelly 2013).

Methods

Subjects

White Leghorn chicks (*Gallus gallus domesticus*) were hatched in an incubator and raised from day one in temperature – controlled brooders. The light cycle was 14L/10D; the light level in the brooders was about 500 lux. Food and water were supplied *ad libitum*. Care and use of the animals conformed to the ARVO Resolution for the Care and Use of Animals in Research.

Paradigm

Diurnal rhythms in axial length and choroidal thickness—Starting at 12-days of age, birds received 2 hours of light (700 lux) between 12:00 am and 2:00 am for 7 days (n=12; only one eye was used in data analyses). The “daytime” (period of light) was changed from 14 hours to 12 hours, from 7:30 am to 7:30 pm, which together with the 2 hours of light at midnight totaled 14 hours of light and 10 hours of dark, as in the previous 2 weeks of life. Chicks were put into temperature controlled light-tight chambers at 7:30 pm, immediately before the time of “lights off”; the timer was set to turn the light on for the 2-hour interval, then off. Two groups of birds comprise these data. The first (n=6) were otherwise untreated except for the light regimen; only the right eyes were used in the data analysis. The second (n=6) wore monocular –10 D lenses for 10 out of the 12 hours of light during the day (i.e. lenses were NOT worn during the 2 hours of light at night); the fellow untreated left eyes (*not* wearing lenses) comprise the second group of experimental eyes, for a total of 12 eyes: 6 right and 6 left. 2-way ANOVAs comparing the two data sets as a function of time showed no significant differences ($p>0.1$) so these data were combined.¹ However, in the interest of diligence and accuracy, data and the statistical analyses are

¹We justify the inclusion of these fellow eyes by the following: First, 10 out of 12 daily hours of minus lens-wear has a negligible effect on emmetropization (Schmid & Wildsoet, 1996) hence the likelihood of the lenses affecting the *fellow* eyes is negligible, especially as they were not worn during the night exposure. Second, there is no substantive effect on the sinusoidal nature of either rhythm by the wearing of negative lenses (Nickla, 2005), and finally, both rhythms in fellow eyes of monocular minus lens-wearing birds are normal, i.e. there is no evidence of “yoking” (unpublished data).

reported for both the combined data (“Full data set”) as well as for only the 6 eyes of the six otherwise untreated birds (“Subset”). Controls were age-matched birds that received no light at night (“dark night”) on a 14L/10D cycle, with lights turned on at 7:30 am and off at 9:30 pm (n=14); only the right eye for each bird was used in the analyses. Two experiments make up these control data.

Ocular dimensions were measured using high-frequency A-scan ultrasonography at noon at the start of the experiment, under inhalation anesthesia (for details see: (Nickla et al. 1998)). On day 7 of the experiment, eyes were re-measured with ultrasound at 12 pm, 6 pm, 12 am, 6 am and 12 pm to obtain the parameters of the diurnal rhythms in axial length and choroidal thickness (see below). Measurements at night were done under a photographic safe light; measurements typically took about 5 minutes per eye, after which the birds were returned to the dark cage. Refractive errors were measured at the end of the experiment for a cohort of 6 experimental and 6 control birds (the same cohort for which “short term” growth rates were measured; below) using a Hartinger’s refractometer.

Growth rate—Two separate sets of 12-day old birds were subjected to the same lighting protocols (2 hours of light at night vs no light at night starting at age 12 days) and measured with ultrasound at various intervals to obtain detailed growth rate data. The “short term” set were measured using ultrasonography at noon on the first day (12 days of age), and then again at 24, 96, 120, 144 and 168 hours later (n=6 birds in each condition; only right eyes used)(Figure 3A). The “long term” set were exposed to the lighting protocols (2 hours of light at night vs no light at night starting at age 12 days) for 28 days, and were measured on day 1, day 7, day 21 and day 28 (control, n=12 birds; experimental, n=22 birds; only left eyes used).

Data Analysis

Ocular growth rate was defined as the change in axial length (from the front of the cornea to the front of the sclera) over the different intervals, from noon to noon. The diurnal rhythm in eye length, as determined by the 6-hour interval measurements on day 7 of the experiment, was assessed in 2 ways: first, we calculated the change over each of the four 6-hour intervals (figures 1A and 2A: “Interval” data), which includes the “steady state” eye growth occurring over the 24 hour period (i.e the slope of the longitudinal data). Second, the “steady state” growth rate (regression) was subtracted from the data for each individual eye, as described in Nickla et al. (Nickla et al. 1998). To control for the large between-animal variability, these data were then normalized to their mean, and the means of these are plotted as line graphs in figures 1B and 2B (“regression data”), with the standard errors. For control eyes, because of the assumption that these data would be similar to published results (Nickla et al. 1998), we fit a sine wave with a fixed 24-hour period to the means. This analysis was used to determine phase and amplitude. Two-way ANOVAs were used to analyze the effect of time of day.

For the choroids, the longitudinal data did not require the subtraction of the regression, because unlike eye length which reflects normal growth over 24 hours, choroid thickness does not change in any discernable way (i.e. the slope would be about zero). For consistency, the data were normalized to the mean, similar to that for axial length. One- and two-way

ANOVAs were used to analyze the effect of time of day, as indicated. Post-hoc tests were done between groups when ANOVAs showed between-group significant differences.

Results

Axial length

Time-of-day accounts for the differences in “interval” changes in axial length between chicks given light at night versus those in a dark night (Figure 1A, 2-way ANOVA: Full data set (white bars): $F_{3,72}=3.93$; $p=0.012$; Subset (circles): $F_{3,54}=6.5$; $p=0.0008$). Light at night caused an “acute” growth stimulation over the 6-hour interval starting with the period of lights-on (between mid-night and 6 am) for both data sets (48 and 91 μm vs $-28 \mu\text{m}$; $p=0.012$; $p=0.0019$ respectively). There was also a significant difference in the interval immediately after this, between 6 am and 12 pm for the data subset (circles: $-78 \mu\text{m}$ vs 21 μm ; $p=0.019$) which did not reach significance for the full data set. There were no significant between-group differences at the other two 6-hour intervals.

The acute growth stimulation between 12 am and 6 am is also illustrated in the “regression” data (normalized to the mean) in figure 1B: light at 12 am caused an increase in length in experimental eyes in both data sets (solid lines), at the time when the length of control eyes was decreasing (dashed line). Note that neither experimental data set could be fit to a 24-hour period sine wave, unlike the good sine-wave fit for control eyes (dashed line and curve). A two-way ANOVA (groups $\times$ time-of-day) shows a statistically significant interaction using both data sets (full data set (bold line): $F_{4,96}=3.18$; $p=0.016$; subset (without symbols): $F_{4,72}=3.72$; $p=0.0083$). In control eyes, the mean peak (obtained from the sine wave) occurs at 7:45 pm, slightly later than previous published results (3:30 pm; (Nickla et al. 1998), and the mean amplitude is 26 μm .

We also analyzed these data by taking the difference between the maximum and minimum axial length, regardless of time (Figure 1C; left), and found that experimental eyes show a mean “excursion” of 94 μm , which does not differ from the value of 65 μm for controls ($p=0.361$). There is similarly no significant between-group difference if we restrict the two axial length values to the maximum for the day (12 pm or 6 pm) and the minimum for the night (12 am or 6 am): 66 μm vs 38 μm ; $p=0.146$ (figure 1C, right). All “amplitudes” are significant (i.e. differ from 0: Exp: $p<0.00001$ for both; Con: day-night: $p=0.036$; max-min: $p=0.005$).

Choroid thickness

Time-of-day accounts for the differences in “interval” changes in choroidal thickness between chicks given light at night versus those in a dark night (Figure 2A, 2-way ANOVA: Full data set (white bars): $F_{3,72}=16.9$; $p<0.00001$; Subset (circles): $F_{3,54}=6.3$; $p=0.0009$). Light at mid-night abolished the 24-hour sinusoidal rhythm in choroidal thickness: the mean 6-hour interval data (white bars) shows that choroidal thickness in experimental eyes oscillated by a mean of 9 μm (s.d.= 33 μm) at each successive 6-hour interval. While control eyes in a dark night increase in thickness from 6 pm to midnight, eyes given light at night do not, in either data set (57 μm vs -21 and $-7 \mu\text{m}$ respectively; $p<0.0001$ for full and subset

comparisons respectively). There is also a significant difference between groups over the subsequent interval from 12 am to 6 am, when control choroids are thinning ($-37\text{ }\mu\text{m}$ vs $18\text{ }\mu\text{m}$; $p=0.0004$ and $p=0.04$ respectively).

In the regression data for control eyes (figure 2B, dashed lines), choroidal thickness shows the typical sinusoidal pattern (Nickla et al. 1998) of increasing between noon and midnight, and decreasing between midnight and noon. The mean peak (from the sine wave fit) is at 1:45 am, and the mean amplitude is $46\text{ }\mu\text{m}$, both parameters of which are similar to previously published data (Nickla et al. 1998). ANOVA shows that “time of day” accounts for the variance in control eyes ($F_{4,60}=8.73$; $p=0.00001$), but not for that in experimental eyes (Full set: $F=2.54$; $p=0.3$; Subset: $F=0.057$; $p=0.99$). A two-way ANOVA shows a statistically significant difference between experimental and control eyes with regard to “time of day” in both sets of data (Full data set (bold line): $F_{4,96}=6.18$; $p=0.0002$; Subset (without symbols): $F_{4,72}=4.91$; $p=0.0015$).

We also analyzed the data by taking the difference between the maximum and minimum thicknesses, regardless of time, and found that experimental eyes show a mean “excursion” of $58\text{ }\mu\text{m}$, which is similar to the $68\text{ }\mu\text{m}$ excursion in controls ($p>0.1$; figure 2C, left). However, if we do the same but restrict the two values to the minimum value for the day (12 pm and 6 pm) and the maximum value for night (12 am and 6 am), we find that the “amplitude” for the experimental eyes is significantly smaller than that of the controls ($31\text{ }\mu\text{m}$ vs $62\text{ }\mu\text{m}$; $p=0.015$; figure 2C, right). Both amplitudes are significant (i.e. differ from 0: $p=0.049$; $p<0.00001$, respectively). This implies that despite the loss of the sinusoidal rhythm in thickness, experimental choroids thickened at one of the two nighttime measures, albeit to a smaller degree than that of controls.

Growth rate

The growth rates in experimental eyes showed a complex pattern that varied over the 4-week time period measured in the two groups of birds (figure 3): In the “short term” set (Figure 3A), the only interval in which there was a significant between-group difference was over the first 24 hours, in which experimental eyes grew faster than controls ($124\text{ }\mu\text{m}$ vs $79\text{ }\mu\text{m}$; $p=0.007$). In the “long term” set (Figure 3B), the rate of growth in experimental eyes was initially inhibited compared to controls (day 1–8: $698\text{ }\mu\text{m}$ vs $758\text{ }\mu\text{m}/7\text{d}$; $p=0.020$), but by the 4th week (day 21–28), growth rate was stimulated ($827\text{ }\mu\text{m}$ vs $703\text{ }\mu\text{m}/7\text{d}$; $p<0.0001$). For the 6 animals per group (experimental and controls) in which refractive error was measured at 2 weeks (figure 3C), eyes receiving light at night were slightly but significantly more myopic than controls (0.8 vs 1.8 D ; $p=0.03$). Whether this was a result of the increased growth over the last week is uncertain, as we did not measure corneal curvature, which could be a contributor to the refractions.

Discussion

We report that daily 2-hour exposures to light in the middle of the night cause “acute” episodes of eye growth stimulation immediately upon exposure to the stimulus, that lasts for at least 6 hours. This acute stimulation occurs within one week of the start of the night light exposure, and may be responsible for the increased growth rate seen four weeks later. The

acute stimulation results in the loss of the diurnal sinusoidal rhythm in axial length found in control eyes in an uninterrupted night. Nightly light exposure also abolishes the 24-hour sinusoidal rhythm in choroidal thickness.

Light influences physiological processes in two distinct ways: it can cause acute changes in the level of the parameter, or it can induce phase shifts in the underlying circadian oscillator. The phase response curve for most rhythms indicate that light in the beginning of the dark phase causes a phase delay while light at the end causes phase advances. In humans, there are circadian rhythms in many physiological processes, including intraocular pressure (IOP) (Liu 1998), core body temperature, levels of circulating hormones (Arendt 2006), and aqueous humor production (Anjou 1961; Anjou 1961), to name a few. In chicks, we had previously demonstrated circadian rhythms in axial length, choroidal thickness, anterior chamber depth (Nickla et al. 1998), IOP (Nickla et al. 1998) and scleral proteoglycan synthesis (Nickla et al. 1999). This study addressed the question of the influence of light at night on two of these rhythms. Our results for axial length are consistent with light having an acute effect on ocular elongation, stimulating growth over the 6 hours following the 2-hour light pulse. Experimental eyes also decrease their growth between 6 am and noon compared to controls, but only in one data set. The end effect is that axial length appears to oscillate, but certainly not in a normal 24-hour rhythm. Light at night also alters the diurnal rhythm in choroidal thickness compared to controls, but does not seem to cause an acute effect. While the longitudinal data for choroids of control eyes can be nicely fit to a 24-hour sine wave identical to that of published results (Nickla et al. 1998), data for experimental eyes cannot. For both rhythms, 2-way ANOVAs indicate that time of day accounts for the variance between the two groups.

What mechanisms might underlie the effects on the rhythm in axial length?

Melatonin is an output of the clock, and is synthesized by pinealocytes and photoreceptors. Its release is circadian in all species studied, being high at night and low during day, and has a reciprocal relationship to dopamine, the release of which is high during the day and low at night (reviews: (Wiechmann and Summers Rada 2008; Toscini et al. 2012). In most species, they are mutually inhibitory (Dubocovich 1983). Light at night has acute suppressive effects on melatonin levels, and causes phase delays when given at the beginning of the dark cycle, and phase-advances when given at the end. Light also causes acute transient increases in dopamine (Iuvone et al. 1978; Witkovsky and Deary 1992). Light in the middle of the night has lesser effects, both acutely and on phase shifting. Our paradigm exposed eyes to light near the middle of the dark phase. If light at night resulted in acute releases of dopamine, it would be most parsimonious that this would result in an overall ocular growth inhibition, as daily injections of dopamine agonists inhibit eye growth. While it is possible that this accounts for the growth inhibition seen between 24–96 hours, it is highly unlikely to account for the rapid, transient (6 hour) elongation. It is possible that the acute suppression of melatonin by light causes the acute growth response, as melatonin injections into form-deprived eyes tend to reduce the amount of myopia (Hoffmann and Schaeffel 1996), and blockade of melatonin receptors with the antagonist luzindole results in increased eye growth (Wiechmann and Rada 2003; Wiechmann and Summers Rada 2008).

If the mechanism underlying the acute increase in eye size is indeed via ocular (scleral) growth, then the response to the stimulus would by necessity be very rapid. Evidence in support of this possibility is that eyes increase their growth within hours after the wearing of a negative lens: by 3 hours post-lens wear, eyes are longer than fellow control eyes (Kee et al. 2001). Indeed, the effects of even much briefer stimuli (minutes) were found to cause “saturating” effects on growth (i.e. as great as that of much longer stimulation) within minutes of the eye growth-enhancing stimulus (Zhu and Wallman 2009). Hence it is possible that what we are observing within the 6-hour interval from midnight to 6 am is in fact, ocular (scleral) growth.

Alternatively, the ocular elongation we see upon light stimulation at midnight could be a result of an increase in IOP. In most species IOP is highest at night, and aqueous production is low. In chickens however, IOP is low at night, coincident with the shortest eye length, and high during the day, coincident with the longest eyes (Nickla et al. 1998). Evidence suggests that the rhythm in IOP at least partially influences the rhythm in axial length in normal eyes. In constant darkness, the rhythm continues to oscillate, however, IOP is significantly reduced during subjective day compared to the daytime IOP in eyes in a normal L/D cycle. Hence, light *per se* increases IOP, which supports the hypothesis that IOP may be involved, although we know of no evidence for acute effects of light on IOP. Furthermore, we have shown that the mean compliance in axial length in chicks is 8 $\mu\text{m}/\text{mm Hg}$, therefore, the increase in length of 40 μm that we find over this time would require a 5 mm Hg increase in IOP. This value is well within the range of the 8 mm Hg amplitude for the diurnal rhythm in IOP in chickens (Nickla et al. 1998), making it plausible that an increase in IOP at midnight, when IOP is normally lowest, could cause the acute elongation.

What mechanisms might underlie the effects on the rhythm in choroidal thickness?

There are three possible explanations for the pattern of thickness changes shown by the choroids of the experimental eyes over 24 hours. The first, and most plausible, is that individual choroids no longer oscillate in a sinusoidal manner, that is, they are arrhythmic. In support of this, only 4/18 choroids show clear “normal” sinusoidal oscillations in thickness. Second, individual choroids may be rhythmic, but have different phases (i.e. become desynchronized from one another), thus “dampening” the mean amplitude of the rhythm (a decrease in the peak, and increase in the trough). This may be supported by the fact that choroids in experimental eyes are generally thicker at one of the nighttime points (either 12 am or 6am), than during one of the daytime points when computing the maximum day (12 pm or 6 pm) vs night (12 am or 6 am) differences, and that this day-night difference is significantly smaller than that for controls. However, the mean choroidal thickness does not differ for day (both times) versus night (both times) in experimental eyes (392 μm vs 400 μm ; $p=0.41$), while for control eyes there is a significant difference (397 μm vs 421 μm ; $p=0.0036$). Third, choroids may oscillate with a different (12 hour?) period. Observation of the alternating oscillations in thickness around 6 hour intervals in figure 2A may support this hypothesis, however, the fact that a one-way ANOVA is *not* significant, weakens this hypothesis. As the mechanism(s) underlying neither the diurnal nor the vision-evoked changes in choroid thickness are known, we cannot speculate upon the underlying molecular or cellular mechanism.

In conclusion, evidence indicates that the increasing and widespread existence of light at night has deleterious effects on human health. Our findings add to this body of knowledge by showing that light at night results in circadian disruptions in the rhythms in axial length and choroidal thickness in chicks, which might be associated with the development of ametropias. Further studies are required in order to identify the longer-term influences of these circadian disruptions on ocular growth, the emmetropization system, and ocular health in general.

Acknowledgments

This work was funded by NIH-NEI-013636. The authors thank Dr. Li Deng (NECO) for help with the statistical analyses, and Pearl Thai and Rinita Zanzierka for some data collection.

References

- Anisimov V, Vinogradova I, Panchenko A, Popovich I, Zabezhinski M. Light-at-night-induced circadian disruption, cancer and aging. *Curr Aging Sci.* 2012; 5:170–177. [PubMed: 23237593]
- Anjou C. Influence of light on the 24-hour variation in aqueous flare density and intraocular pressure in the normal rabbits' eyes. *Acta Ophthalmol.* 1961; 39:852–873. [PubMed: 13861660]
- Anjou C. Physiological variations of the aqueous flare density in normal human eyes. *Acta Ophthalmol.* 1961; 39:525–539. [PubMed: 13683639]
- Arendt J. Melatonin and human rhythms. *Chronobiol Int.* 2006; 23:21–37. [PubMed: 16687277]
- Borniger JC, Maurya SK, Periasamy M, Nelson RJ. Acute dim light at night increases body mass, alters metabolism, and shifts core body temperature circadian rhythms. *Chronobiol Int.* 2014; 31:917–925. [PubMed: 24933325]
- Brown JS, Flitcroft DI, Ying G, Francis EL, Schmid GF, Quinn GE, Stone RD. In vivo human choroidal thickness measurements: Evidence for diurnal fluctuations. *Invest Ophthalmol Vis Sci.* 2009; 50:5–12. [PubMed: 18719079]
- Chakraborty R, Read SA, Collins MJ. Diurnal variations in axial length, choroidal thickness, intraocular pressure, and ocular biometrics. *Invest Ophthalmol Vis Sci.* 2011; 52:5121–5129. [PubMed: 21571673]
- Chepesiuk R. Missing the dark: Health effects of light pollution. *Environ Health Perspect.* 2009; 117:20–27.
- Cinzano P, Falchi F, Elvidge C. The first world atlas of the artificial night sky brightness. *Mon Not R Astron Soc.* 2001; 328:689–707.
- Dubocovich ML. Melatonin is a potent modulator of dopamine release in the retina. *Nature.* 1983; 306:782–784. [PubMed: 6656879]
- Feillet C, van der Horst G, Levi F, Rand D, Delaunay F. Coupling between the circadian clock and cell cycle oscillators: implication for healthy cells and malignant growth. *Frontiers in Neurology.* 2015; 6:1–7. [PubMed: 25699006]
- Fonken L, Aubrecht T, Melendez-Fernandez O, Weil Z, Nelson R. Dim light at night disrupts molecular circadian rhythms and affects metabolism. *J Biol Rhythms.* 2013; 28:262–271. [PubMed: 23929553]
- Gwiazda J, Ong E, Held R, Thorn F. Myopia and ambient night-time lighting. *Nature.* 2000; 404:144. [PubMed: 10724158]
- Hoffmann M, Schaeffel F. Melatonin and deprivation myopia in chickens. *Neurochem Int.* 1996; 28:95–107. [PubMed: 8746769]
- Iuvone PM, Galli CL, Garrison-Gund CK, Neff NH. Light stimulates tyrosine hydroxylase activity and dopamine synthesis in retinal amacrine neurons. *Science.* 1978; 202:901–902. [PubMed: 30997]
- Kee, C-s; Marzani, D.; Wallman, J. Differences in time course and visual requirements of ocular responses to lenses and diffusers. *Invest Ophthalmol Vis Sci.* 2001; 42(3):575–583. [PubMed: 11222513]

- Lauber JK, McGinnis J. Eye lesions in domestic fowl reared under continuous light. *Vision Res.* 1966; 6:619–626. [PubMed: 6003385]
- Lauber JK, Shutze JV, McGinnis J. Effects of exposure to continuous light on the eye of the growing chick. *Proc Soc Exp Biol Med.* 1961; 106(4):871–872. [PubMed: 13759412]
- Li T, Troilo D, Glasser A, Howland HC. Constant Light Produces Severe Corneal Flattening and Hyperopia in Chickens. *Vision Res.* 1995; 35(9):1203–1209. [PubMed: 7610581]
- Liu J. Circadian rhythm of intraocular pressure. *J Glaucoma.* 1998; 7:141–147. [PubMed: 9559503]
- Nickla D. The phase relationships between the diurnal rhythm in axial length and choroidal thickness and the association with ocular growth rate in chicks. *J Comp Physiol A.* 2006; 192:399–407.
- Nickla D, Wildsoet C, Troilo D. Endogenous rhythms in axial length and choroidal thickness in chicks: Implications for ocular growth regulation. *Invest Ophthalmol Vis Sci.* 2001; 42:584–588. [PubMed: 11222514]
- Nickla D, Wildsoet C, Troilo D. Diurnal rhythms in intraocular pressure, axial length, and choroidal thickness in a primate model of eye growth, the common marmoset. *Invest Ophthalmol Vis Sci.* 2002; 43:2519–2528. [PubMed: 12147579]
- Nickla DL, Rada JA, Wallman J. Isolated chick sclera shows a circadian rhythm in proteoglycan synthesis perhaps associated with the rhythm in ocular elongation. *J Comp Physiol [A].* 1999; 185(1):81–90.
- Nickla DL, Totonelly K. The efficacy of brief periods of “vision” in chick eyes wearing negative lenses is dependent on time of day. *ARVO.* 2013 E-Abstract #5175.
- Nickla DL, Wildsoet C, Wallman J. The circadian rhythm in intraocular pressure and its relation to diurnal ocular growth changes in chicks. *Exp Eye Res.* 1998; 66:183–193. [PubMed: 9533844]
- Nickla DL, Wildsoet C, Wallman J. Visual influences on diurnal rhythms in ocular length and choroidal thickness in chick eyes. *Exp Eye Res.* 1998; 66:163–181. [PubMed: 9533843]
- Papastergiou GI, Schmid GF, Riva CE, Mendel MJ, Stone RA, Laties AM. Ocular axial length and choroidal thickness in newly hatched chicks and one-year-old chickens fluctuate in a diurnal pattern that is influenced by visual experience and intraocular pressure changes. *Exp Eye Res.* 1998; 66:195–205. [PubMed: 9533845]
- Quinn GE, Shin CH, Maguire MG, Stone RA. Myopia and ambient lighting at night. *Nature.* 1999; 399:113–114. [PubMed: 10335839]
- Schmid K, Wildsoet CF. Effects of the compensatory responses to positive and negative lenses of intermittent lens wear and ciliary nerve section in chicks. *Vision Res.* 1996; 36:1023–1036. [PubMed: 8736261]
- Stevens R, Brainard G, Blask D, Lockley S, Motta M. Adverse health effects of nighttime lighting: Comments on AMA Policy Statement. *Am J Prev Med.* 2013; 45:343–346. [PubMed: 23953362]
- Stone RD, Quinn GE, Francis EL, Ying G, Flitcroft DI, Parekh P, Brown J, Orlov J, Schmid G. Diurnal axial length fluctuations in human eyes. *Invest Ophthalmol Vis Sci.* 2004; 45:63–70. [PubMed: 14691155]
- Toscini G, Kenkichi B, Hwang C, Iuvone P. Melatonin: An underappreciated player in retinal physiology and pathophysiology. *Exp Eye Res.* 2012; 103:82–89. [PubMed: 22960156]
- Weiss S, Schaeffel F. Diurnal growth rhythms in the chicken eye: relation to myopia development and retinal dopamine levels. *J Comp Physiol A.* 1993; 172(3):263–270. [PubMed: 8510054]
- Wiechmann A, Rada J. Melatonin receptor expression in the cornea and sclera. *Exp Eye Res.* 2003; 77:219–225. [PubMed: 12873453]
- Wiechmann A, Summers Rada J. Circadian rhythms in the eye: The physiological significance of melatonin receptors in ocular tissues. *Prog Retin Eye Res.* 2008; 27:137–160. [PubMed: 18316227]
- Wilson LB, Quinn GE, Ying G, Francis EL, Schmid G, Lam A, Orlov J, Stone RA. The relation of axial length and intraocular pressure fluctuations in human eyes. *Invest Ophthalmol Vis Sci.* 2006; 47:1778–1784. [PubMed: 16638981]
- Witkovsky, P.; Deary, A. Functional roles of dopamine in the vertebrate retina. In: Osborne, NN.; Chader, GJ., editors. *Progress in Retinal Research.* Vol. 11. Oxford: Pergamon Press; 1992. p. 247–292.

- Wyse C, Selman C, Page M. Circadian desynchrony and metabolic dysfunction; did light pollution make us fat? *Med Hyp*. 2011; 77:1139–1144.
- Zadnik K, Jones LA, Irvin BC, Kleinstei RN, Manny RE, Shin JA, Mutti DO. Myopia and ambient night-time lighting. *Nature*. 2000; 404:143–144. [PubMed: 10724157]
- Zhu X, Wallman J. Temporal properties of compensation for positive and negative spectacle lenses in chicks. *Invest Ophthalmol Vis Sci*. 2009; 50:37–46. [PubMed: 18791175]

Highlights

Brief light at night alters the rhythms in axial length and choroid thickness.

Brief light at night causes “acute” growth stimulations.

Brief light at night abolishes the 24-hour rhythm in choroidal thickness.

Alterations in the rhythms in axial length and choroid thickness may cause increased eye growth.

Figure 1.

Effects of light at night on axial length. A. Change in axial length over 6-hour intervals on the last 24 hours of the 7-day experiment. For experimental groups, lights went on from 12 am to 2 am (full data set=white bars; subset=circles); controls were in a dark night (black bars). Light at night caused significant differences between both experimental vs control groups as a function of time of day (ANOVA $p=0.012$, 0.0008 ; respectively). Experimental eyes grew significantly faster than controls from 12 am to 6 am (full data set; $p=0.012$). B. Longitudinal "regression" data normalized to the mean, for both experimental groups (full data set=bold line; subset=non-bold line) and control eyes (dashed line and sine curve) on day 7. Note that neither experimental data can be fit to a 24-hour sine wave function. Time of day accounts for the differences between experimental and control groups (ANOVA

p=0.016, 0.0083). C. “Amplitude” derived in two ways from the raw (not regressed) data. Left: the difference between the maximum and minimum values regardless of time of day. Right: the difference between the maximum value at either 12 pm or 6 pm (day) and the minimum at either 12 am or 6 am (night). Error bars are standard errors of the mean in all graphs.

Figure 2.

Effects of light at night on choroidal thickness. A. Change in choroidal thickness over 6-hour intervals on the last 24 hours of the 7-day experiment. For experimental groups, lights went on from 12 am to 2 am (full data set white bars; subset=circles); controls were in a dark night (black bars). Light at night caused significant differences between both experimental vs control groups as a function of time of day (ANOVA $p < 0.00001$; $p = 0.0009$ respectively). Experimental choroids thinned between 6 pm and 12 am while controls were thickening (full data set: $p < 0.0001$), and thickened between 12 am and 6 am when controls

were thinning (full data set: $p=0.0004$). B. Longitudinal data normalized to the mean, for both experimental groups (full data set=bold line; subset=non-bold line) and control eyes (dashed line and sine curve) on day 7. Note that neither experimental data set can be fit to a 24-hour sine wave function. Time of day accounts for the differences between both experimental vs control groups (ANOVA $p=0.0002$, 0.0015). C. “Amplitude” derived in two ways from the raw data. Left: the difference between the maximum and minimum values regardless of time of day. Right: the difference between the maximum value at either 12 pm or 6 pm (day) and the minimum at either 12 am or 6 am (night). Error bars are standard errors of the mean in all graphs. $P<0.05$

Figure 3.

Growth rates (changes in axial length) and refractive errors. A. Growth rates (elongation over time) in the “short term” set. $n=6$ in each group; only right eyes used. B. Growth rates in the “long term” set. Experimental: $n=22$; Controls: $n=12$. Only one eye per bird was used. C. Refractive errors at the end of the experiment in the cohort of 6 birds in each condition shown in figure 3A. * $p<0.05$.