Published in final edited form as:

Optom Vis Sci. 2018 April ; 95(4): 323–331. doi:10.1097/OPX.0000000000001198.

The ipRGC-Driven Pupil Response with Light Exposure, Refractive Error and Sleep

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Abstract

Significance—We investigated links between the intrinsically photosensitive retinal ganglion cells, light exposure, refractive error, and sleep. Results showed that morning melatonin was associated with light exposure, with modest differences in sleep quality between myopes and emmetropes. Findings suggest a complex relationship between light exposure and these physiological processes.

Purpose—Intrinsically photosensitive retinal ganglion cells (ipRGCs) signal environmental light, with pathways to the midbrain to control pupil size and circadian rhythm. Evidence suggests that light exposure plays a role in refractive error development. Our goal was to investigate links between light exposure, ipRGCs, refractive error, and sleep.

Methods—Fifty subjects, aged 17–40, participated (19 emmetropes and 31 myopes). A subset of subjects ($n = 24$) wore an Actiwatch Spectrum for one week. The Pittsburgh Sleep Quality Index (PSQI) was administered, and saliva samples were collected for melatonin analysis. The post illumination pupil response (PIPR) to 1 second (s) and 5s long and short wavelength stimuli was measured. Pupil metrics included the 6s and 30s PIPR and early and late area under the curve.

Results—Subjects spent 104.8 ± 46.6 minutes outdoors per day over the previous week. Morning melatonin concentration (6.9 ± 3.5 pg/mL) was significantly associated with time outdoors and objectively measured light exposure ($P = 0.0099$ and 0.0016 , respectively). Pupil metrics were not significantly associated with light exposure or refractive error. PSQI scores indicated good sleep quality for emmetropes (score 4.2 ± 2.3) and poor sleep quality for myopes (5.6 ± 2.2 , $P = 0.036$).

Conclusions—We found that light exposure and time outdoors influenced morning melatonin concentration. No differences in melatonin or the ipRGC-driven pupil response were observed between refractive error groups, although myopes exhibited poor sleep quality compared to emmetropes. Findings suggests that a complex relationship between light exposure, ipRGCs, refractive error and sleep exists.

Keywords

intrinsically photosensitive retinal ganglion cells; pupil; melanopsin; light exposure; melatonin; sleep; refractive error

Myopia is an epidemic, reaching a prevalence of over 90% in some urbanized countries.¹ Myopia represents a large socioeconomic burden and poses a risk for associated ocular diseases including retinal detachment, neovascularization, and glaucoma.² Evidence suggests that refractive development and eye growth are regulated by a complex interaction between genetic and environmental factors.^{3,4} Time outdoors and light exposure have been shown to be protective of myopia in children.^{5,6}

Light exposure is associated with dopamine and melanopsin signaling. The intrinsically photosensitive retinal ganglion cells (ipRGCs), located in the inner retina, are directly stimulated by light through activation of the photopigment melanopsin, with a peak sensitivity around 482 nm.⁷ IpRGCs account for less than 1% of the ganglion cell population, with widespread dendritic coverage encompassing the entire retina (except at the fovea),^{8,9} and are responsible for non-image forming functions including circadian rhythm entrainment and pupil size control.¹⁰ Axons project to the olivary pretectal nucleus for control of pupil size,¹¹ and to the suprachiasmatic nucleus in a pathway ultimately leading to the pineal gland to control the release of the sleep hormone melatonin. The ipRGCs are considered environmental irradiance detectors, and have been implicated in a myriad of physiological processes, including metabolism, mood, and sleep.¹²⁻¹⁴

In addition to direct stimulation, the ipRGCs receive extrinsic synaptic input through the rod and cone pathway, with connections to bipolar and amacrine cells.¹⁵ Studies utilizing animal models have demonstrated that increased light exposure leads to an increase in retinal dopamine,¹⁶ which may, in part, be mediated by synaptic connections between ipRGCs and dopaminergic amacrine cells.¹⁷ Single cell recordings show that extrinsically driven ipRGC responses are rapid and transient, while intrinsic ipRGC responses show a longer latency and sustained firing during stimulation, and continue to fire following stimulus offset.¹⁸ In vivo ipRGC activity can be measured indirectly through the pupil response to short wavelength light.¹⁹ Following short wavelength stimulation offset, the post illumination pupil response (PIPR) redilation dynamics have been shown correlate with the sustained firing pattern seen in single cell recordings,¹⁹ and are an indicator of ipRGC activity.

The ipRGCs provide environmental light information to the suprachiasmatic nucleus to synchronize sleep/wake patterns.²⁰ Recent studies have found that myopia may contribute to decreased sleep quality.^{21,22} Jee, et al, found an inverse relationship between sleep duration and myopia in Korean adolescents.²² Similarly, another study reported that children with high myopia exhibited the poorest scores on the Pittsburgh Sleep Quality Index (PSQI), a sleep quality questionnaire.²¹ The authors attributed the observed decreased sleep quality to high demands in school, distress over poor vision, or decreased ipRGC function in myopic eyes. Sleep, circadian rhythm, and ipRGC activity have also been shown to be affected in diseases which affect the inner retina, such as glaucoma,²³ and in outer retinal diseases, including age related macular degeneration.²⁴ Another study reported a positive association between morning melatonin concentration and the magnitude of myopia, with myopes demonstrating up to three times greater melatonin concentration than non-myopes.²⁵ Therefore, it is possible that ipRGCs could also be affected with axial myopic eye growth, in which the retina is subject to structural and functional changes.²⁶ A recent report found no effect of refractive error on ipRGC inputs to the pupil control pathway.²⁷ However, another

investigation found a significant positive correlation between a more hyperopic refractive error and a slower rate of pupil redilation to 0.1 Hz flashing stimuli, which varied as a function of light exposure over the previous five days.²⁸ Differing results and current interest in light exposure and myopia warrant further investigation into potential relationships between light exposure, refractive error and pupil responses.

The goal of this study was to investigate relationship between the ipRGC-driven pupil response and light exposure to begin to understand the interaction between environmental factors and potential mechanisms influencing eye growth and circadian rhythm. We also examined downstream relationships between light exposure, sleep quality and melatonin in myopic and emmetropic individuals.

METHODS

Subjects, ages 17–40, were recruited for this study. All subjects provided informed consent after the purpose of the study and the risks were explained. The study was approved by the Committee for Protection of Human Subjects at the University of Houston and followed the tenets of the Declaration of Helsinki. Exclusion criteria included ocular disease and the use of melatonin or other pharmacological sleep aids.

Lab visits occurred between 9:00 am to 11:00 am to avoid potential effects of circadian variation.^{29, 30} Visual acuity was measured with habitual correction, and an anterior eye exam using slit lamp biomicroscopy was performed to confirm angles were open and suitable for dilation. Acuity for all subjects was 20/25 or better and no subject had ocular pathology, including cataracts or glaucoma. No subjects were taking prescription medications known to affect pupil size.

The experimental protocol is shown in Figure 1A. The Pittsburgh Sleep Quality Index questionnaire (PSQI) was administered to measure sleep quality subjectively, and saliva samples were collected to measure melatonin concentration. Prior to collection, subjects were instructed not to eat, drink coffee, or brush their teeth within an hour of the lab visit. In addition, subjects were asked to abstain from alcohol and nicotine 12 hours prior to the lab visit. Approximately 1 ml of saliva was collected and immediately placed in a –20° C freezer for later analysis using a melatonin ELISA kit (Salimetrics, CA, USA). All samples were run in duplicate.

Objective Light Exposure Measurement

A subset of subjects (n = 24) wore an Actiwatch Spectrum (Philips Resipronics, OR) for one week prior to the lab visit. The Actiwatch Spectrum is a non-invasive wrist-worn activity monitoring method that measures ambient light exposure and activity continuously at 32 Hz. This device has been actively used in both children and adults in various applications, including light exposure, activity, and sleep related studies.^{31, 32} The light sensor in the Actiwatch Spectrum consists of color sensitive photodiodes to measure illuminance of white light in units of lux (range 0.1 to 200,000 lux), and the irradiance of the red (400–500 nm), green (500–600 nm), and blue (600–700 nm) components in $\mu\text{W}/\text{cm}^2$. Each subject was asked to wear the device for 7 days, which was set to average over one minute epochs.

Subjects were instructed not to remove the device for the entire measurement period, including during sleep. Days were excluded from the analysis if the subject removed the device for more than 30 minutes, or if the light exposure dropped to zero for 30 minutes or more during daylight hours, indicating obstruction of the light sensor by clothing. An “off-wrist” sensor in the device and absence of missing data showed that all subjects were compliant with the requested wear regimen.

Actiwatch data were analyzed with the device software, Philips Actiware 6.0.8, as described previously.³² Time outdoors was defined as mean minutes per day exposed to > 1000 lux. For subjects who did not wear the Actiwatch, a brief survey was administered to determine time outdoors over the previous week. Light exposure was analyzed in terms of natural log cumulative white (broad band) light (lux), as well as for the blue and red spectral output ($\mu\text{W}/\text{cm}^2$). To understand the integration time for potential relationships between melatonin and pupil metrics with light exposure, we calculated the cumulative light exposure binned into four intervals, 1 day, 3 days, 5 days and 7 days. Sleep duration and efficiency were calculated for the week.

Pupillometry

Following saliva collection, the left eye was dilated with 2.5% phenylephrine and 1% tropicamide. Dilation of the left eye was employed in an effort to maintain consistent retinal illumination within and between subjects during stimulation. After a 20 minute dilation period, the ipRGC driven pupil response was measured via the post-illumination pupil response (PIPR) in the right eye, as described previously.³³ Briefly, a frame mounted binocular eye tracker with infrared illumination (ViewPoint EyeTracker, Arrington, AZ) was used to record the pupil size of the right eye at 60 Hz. The IR LED light source has a λ_{max} of 943 nm with a half-max width of 46 nm (Ocean Optics Spectrometer, Largo, FL). Pupil size was calibrated for each subject by first capturing an image of a 5mm artificial pupil. Following calibration, subjects dark adapted for 5 minutes (< 1 lux), then placed their head in a chinrest with an LED-driven Ganzfeld system (Color Burst, Espion, Diagnosys LLC, MA) centered in front of the left eye at 10 mm providing full field stimulation. Subjects viewed a red fixation point directed at the wall at 10 feet with the right eye during measurements.

Stimuli presented to the left eye consisted of long wavelength (red) and short wavelength (blue) narrowband 1 second (s) and 5 s pulses of light (Figure 1B). Previous studies have utilized stimulus durations of 4 ms to 30 s; here, two durations that were within the range of previously published studies were utilized.³⁴ While previous studies have concluded that a 1 s stimulus is sufficient to stimulate the melanopsin pathway, we also included the 5 s stimulation to understand if PIPR metrics would be different for longer stimulations. Long wavelength light was 651 nm with a half-max width of 25 nm (Spectroradiometer CS1W, Minolta, Tokyo, Japan), and corneal irradiance was 5.58×10^{13} photons/ cm^2/s (Power Meter, Newport Corporation, Andover, MA). Short wavelength light was 456 nm (half-max width of 20 nm), with a corneal irradiance of 5.85×10^{13} photons/ cm^2/s . After dark adaptation, the baseline pupil diameter was measured in the dark for 10 s. A long wavelength 1 s pulse was presented to the left eye, and the consensual pupil diameter was

measured for 60 s. Then a long wavelength 5 s pulse was presented and the consensual pupil diameter was again measured for 60 s. The subject dark adapted for 5 minutes, and the protocol was repeated with short wavelength light. Previous evidence suggests that prior long-wavelength light exposure enhances short-wavelength induced pupil constriction.³⁵ Therefore, both long wavelength stimuli were presented, followed by a dark adaptation period, and then blue stimuli were presented, similar to previously published experiments.²⁹

Following ipRGC measurements, the right eye was dilated. After 20 minutes, axial length (LenStar, Haag-Streit) and refractive error (WAM-5500, Grand-Seiko, Tokyo, Japan) were measured in both eyes. For axial length, three measurements were recorded and averaged for each eye, then both eyes were averaged together. For refraction, the spherical equivalent refractive error (SER) was calculated for the average of 5 measurements for each eye, and both eyes were averaged together. Subjects were grouped based on refractive error; the emmetropic group included SER from +1.25 to -0.5 D, and the myopic group included SER < -0.50. Emmetropic and myopic groups were age and sex matched.

Data Analysis

Results are presented as mean $\pm$ standard deviation. Primary outcomes were pupil metrics, sleep quality and melatonin. Statistical analysis was performed in MedCalc (MedCalc Software, Ostend, Belgium). Normality was confirmed with the Kolmogorov-Smirnov test, and outliers were detected with a Tukey outlier filter. The Kolmogorov-Smirnov test showed that light exposure, pupil metrics and melatonin were normally distributed, so statistical analyses of these variables were performed with simple linear regression models and significance was determined based on the p value for slope less than 0.05. Refractive groups were compared with unpaired two tailed t-tests and Mann-Whitney tests for independent samples.

Pupil data were analyzed off line using a custom MATLAB program (MathWorks, Natick, MA). Blinks were identified as intervals of pupil-aspect ratio outside 6 SD of the mean pupil aspect ratio during stable fixation, or samples deemed poor quality by the instrument, and removed from the trace. Pupil metrics are defined in Table 1. The baseline pupil diameter was calculated by averaging pupil diameter during the 10 s pre-stimulus period. Normalized responses were determined by dividing pupil diameter by the baseline pupil diameter (expressed in percent). Peak constriction was calculated as the percentage of the maximally constricted pupil diameter to baseline pupil diameter. The 6 s and 30 s PIPR were calculated as the normalized pupil size averaged over 6–7 s and 30–31 s, respectively, after each stimulus offset. Early and late area under the curve (AUC) were computed for the recovery intervals 0 to 10 s and 10 to 30 s following stimulus offset.³⁶ The areas were computed as the trapezoidal approximation of the integral of 100% minus the interpolated percent pupil diameter (i.e. the difference between the pupil and baseline) for the respective intervals (unitless). The pupil metrics were analyzed in terms of SER, morning melatonin concentration, time outdoors, and natural log cumulative light exposure.

RESULTS

Fifty subjects participated (24 female, 26 male), with a mean age of 26.9 ± 6.2 years (mean $\pm$ SD; range 17–40, Table 2). Mean cycloplegic SER was -2.5 ± 2.7 D (range -7.8 to $+1.2$ D) and mean axial length was 24.6 ± 1.2 mm (range 22.3 to 26.9 mm). Mean SER of the emmetropic group was $+0.39 \pm 0.48$ D ($+1.2$ to -0.50 D, $n = 19$) and of the myopic group was -4.3 ± 1.6 D (-1.5 to -7.8 D, $n = 31$). Mean axial length of the emmetropic group was 23.6 ± 0.8 mm (22.3 to 25.3 mm) and of the myopic group was 25.3 ± 0.9 mm (23.2 to 26.9 mm).

Light Exposure, Sleep, and Melatonin

For all subjects, time outdoors during daylight hours over the previous week was 104.8 ± 46.6 minutes per day (range 22 to 249 minutes). The subject who reported spending 249 minutes per day outdoors was identified as an outlier and not included in analyses. For the subset of subjects who wore the Actiwatch device ($n = 24$), natural log daily light exposure was 14.0 ± 0.66 lux (range 12.5 to 15.2 lux). For morning melatonin concentration, 43 subjects were included in the analysis; 7 subjects did not provide adequate saliva samples. Mean melatonin concentration, measured in the morning prior to pupil measurements, was 6.9 ± 3.5 pg/mL (range 0.84 to 17.8 pg/mL); two subjects with the highest values were identified as outliers and not included in regression analyses.

Increased time outdoors during the previous week associated with higher morning melatonin concentration ($R^2 = 0.23$, $F = 11.6$, $df = 38$, $P = 0.0016$, Figure 2A). For the subset of subjects with objective light exposure data, cumulative white light exposure was binned into four time slices, 1 day, 3 days, 5 days and 7 days, with Bonferroni corrected significance level of $P = 0.013$. Morning melatonin concentration was significantly associated with white light exposure over the previous 7 days, ($R^2 = 0.29$, $F = 8.12$, $df = 20$, $P = 0.0099$, Figure 2B), but not the previous 5 days ($P = 0.02$), 3 days ($P = 0.05$) or 1 day ($P = 0.51$). Morning melatonin concentration was also significantly associated with red and blue light exposure over the previous 7 days (see Appendix Figure A1, available at [LWW insert link]). However, red and blue light exposure were highly correlated with white light exposure ($R^2 = 0.97$, $P < 0.0001$ for both, Figure 3); therefore, red and blue were not considered independent variables. There were no significant refractive error group differences in time outdoors, light exposure, or morning melatonin.

Mean PSQI score for all subjects was 5.2 ± 2.7 (range 1 to 15). The subject with a score of 15 was identified as an outlier and excluded from analyses. PSQI scores were not normally distributed, so comparisons were made using the Mann-Whitney test. Mean PSQI was significantly lower for emmetropic subjects (4.2 ± 2.3) than myopic subjects (5.6 ± 2.2 , $t_{stat} = 2.2$, $P = 0.036$), indicating poor sleep quality in the myopic group (PSQI > 5). To understand if poor sleep quality was driven by the high myopes in the group, as previously reported, the myopic group was divided into low myopes (> -0.50 to -4.75 , $n = 21$) and high myopes (> -4.75 , $n = 10$). However, there were no significant differences in the PSQI between the two groups ($t_{stat} = 1.2$, $P = 0.22$). Regression analysis for PSQI sleep score and morning melatonin showed there was no relationship between these factors ($R^2 = 0.007$, $df = 39$, $P = 0.61$). For the subset of subjects who wore the Actiwatch for one week, mean daily

sleep duration between refractive error groups was not significantly different ($P = 0.09$), with emmetropes sleeping 457.2 ± 38.1 minutes per night, and myopes sleeping 421.7 ± 43.1 minutes per night. Sleep efficiency was not significantly different between emmetropes ($83.3 \pm 7.9\%$) and myopes ($85.3 \pm 4.3\%$, $P = 0.50$).

Pupil Measurements

Following 5 minutes dark adaptation, baseline pupil diameter of undilated right eyes was 6.1 ± 0.8 mm (range 3.7 to 7.5 mm), which was not significantly different between the emmetropic (6.10 ± 0.71 mm) and myopic (6.08 ± 0.86 mm) groups ($P = 0.95$). For long wavelength stimulation, pupils re-dilated rapidly following light offset. For short wavelength stimulation, pupils re-dilated at a slower rate following light offset. These dynamics are evident by the statistically significantly smaller values for the 6 s and 30 s PIPR and larger values for the early and late AUC for 1 s and 5 s to short wavelength stimulation compared to long wavelength stimulation (paired t-test, $P < 0.0001$; Table 3), except for the 30 s PIPR to 1 s stimulation, which was not significantly different between long and short wavelength stimulations ($P = 0.10$). All metrics were significantly greater (i.e. lower PIPR values and higher AUC values) for 5 s stimulation versus 1 s stimulation of the same wavelength ($P < 0.01$ for all), except for the 30 s PIPR and late AUC to long wavelength light ($P = 0.09$ and 0.90 , respectively).

Trends suggested that the 6 s PIPR to 1 s short wavelength stimulation was associated with white light exposure over the previous day ($R^2 = 0.18$, $df = 23$, $F = 6.7$, $P = 0.05$, Figure 4A) and blue light exposure over the previous day ($R^2 = 0.21$, $df = 20$, $F = 5.22$, $P = 0.03$, Figure 4C). However, these relationships were not significant following Bonferroni correction to a significance level of $p = 0.006$. There was no relationship between red light exposure over the previous day and the 6 s PIPR ($R^2 = 0.07$, $df = 21$, $F = 1.70$, $P = 0.21$, Figure 4E). There were no associations between the 6 s PIPR to 5 s short wavelength stimulation and light exposure (Figures 5B, 5D, 5F). Additionally, there were no associations between the 30 s PIPR, early AUC or late AUC to 1 or 5 s short wavelength stimulation and light exposure (see Appendix Figures A2, A3, A4, available at [LWW insert link]). Pupil metrics to 1 s and 5 s long wavelength stimulation were not associated with light exposure. Pupil metrics were not associated with objectively measured white light exposure over the previous 3, 5 or 7 days (data not shown).

Pupil metrics were not significantly different between emmetropic and myopic subjects, shown for the 1 s stimulus in Figure 5A and 5 s stimulus in Figure 5B. While the 6 s PIPR to a 5 s short wavelength stimulus was modestly increased for myopes (i.e. lower PIPR value, $t_{stat} = -2.0$, $df = 48$, $P = 0.05$), the difference was not significant after Bonferroni correction.

DISCUSSION

A primary outcome of this study was that objectively measured habitual light exposure was associated with melatonin concentration. Additionally, our results showed that myopic subjects exhibited poor sleep quality in comparison to emmetropic subjects. Small and inconsistent associations between the ipRGC driven pupil response, light exposure and refractive error were not significant.

A primary role for ipRGCs is photic regulation of melatonin release from the pineal gland.³⁷ We demonstrated that systemic morning melatonin concentration was associated with mean time spent outdoors and light exposure over the previous 7 days. However, morning melatonin concentration was not associated with mean light exposure over the previous 1 to 5 days, suggesting that the luminance integration period for melatonin is 5 to 7 days. A potential mechanism for increased morning melatonin with increased habitual light exposure is a phase shift in circadian time. A limitation of the current study is that melatonin was only measured at one time point. Melatonin is known to undergo a diurnal rhythm, in which the systemic levels increase at nighttime, and decrease in the morning.³⁸ Measuring melatonin at multiple time points throughout a twenty-four hour period would address whether a phase shift occurs with increased light exposure. Our results are consistent with a recent study reporting higher morning melatonin levels with increased daytime light exposure in the elderly.³⁹ While the age of the current subjects is younger, sampling methodology was similar between studies. Another recent study showed that melatonin levels were higher in summer compared to winter;⁴⁰ summer has a longer photoperiod and, hence, likely greater light exposure. Previous studies evaluated brief exposure to bright light on systemic melatonin. Lewy, et al, demonstrated an immediate suppression of melatonin during bright light at nighttime that returned to normal values after darkness was resumed.⁴¹ Owen and Arendt showed that light suppression of melatonin was more effective in the latter part of the night.⁴² These studies suggest that time of day of light exposure plays an important role in how melatonin is affected. Because of the variability in when our subjects received high light doses over the observational week, we were unable to draw conclusions based on time of day.

Consistent with the literature, pupil re-dilation was slower following short wavelength stimuli compared to long wavelength stimuli, as indicated by the smaller 6 s PIPR and larger early and late AUC values for short wavelength stimuli. Our results showed trends that increased light exposure over the previous day was associated with the amplitude of the PIPR to a 1 s short wavelength light stimulus, as measured at one consistent time point in the morning, suggesting increased morning ipRGC activity, or a shift in circadian phase, with increased habitual light exposure. While this finding did not reach statistical significance following correction for multiple comparisons, the trend is in accordance with a previous study that showed slowed pupillary redilation (i.e. increased ipRGC activity) to 0.1 Hz flashing stimuli with greater habitual light exposure.²⁸ Further studies with a larger sample size should be carried out to address the significance of these findings. It is of interest to note that, here, the observed trends were evident for both white and blue habitual light exposure, but not for red light exposure, providing further evidence that a driving input for the ipRGCs is short wavelength light. Other studies evaluated circadian changes in the ipRGC driven pupil response over 24 hours in controlled lighting environments, and found that pupil responses demonstrated circadian modulation that was under the influence of an endogenous circadian clock.^{29, 30} Our results suggest that, while relative pupil function undergoes circadian modulation independent of external light cues, the absolute values may be influenced by previous light exposure which could explain variability between subjects.

Pupil size is influenced by several factors in addition to ipRGC activity, including age, lethargy and autonomic input.⁴³ While intrinsic contributions to iris musculature cannot be

eliminated, efforts were taken in our protocol to minimize input, including using a controlled dark environment, presenting a fixation target that with minimal stimulus for accommodation, and performing measurements at a consistent time of day (i.e. morning). Pupil diameters were normalized to each subject's baseline pupil diameter following 5 minutes of dark adaptation to account for variations in baseline pupil size.⁴⁴ With normalization to baseline pupil size, Zele showed that ipRGC-driven PIPR was independent of age.²⁷ On the other hand, Herbst, et al, demonstrated that some metrics of the ipRGC-driven pupil response were associated with age, and attributed this to increased scatter by the lens with age.⁴⁴ Our results also found no relationship between the PIPR and age; however, our population included a limited age range up to 40 years.

Light is a potent cue for circadian rhythm entrainment, and studies show that phototransduction through the ipRGCs plays an important role in setting the circadian clock.⁴⁵ Studies in chicks,⁴⁶ tree shrews⁴⁷ and rhesus monkeys⁴⁸ have shown that a diurnal light:dark photoperiod is essential for normal refractive development. Li, et al, showed that at least 4 hours of darkness is required in chicks for normal emmetropization.⁴⁹ Additionally, high ambient light levels decreases experimental myopia in chicks.^{50, 51} Results from these animal studies indicating that illumination level and periodic light:dark cycles are critical for normal refractive development suggest that intrinsic circadian clock and dopamine/melatonin pathway play a role in eye growth through the ipRGCs.⁵²

Previous studies have shown that, in an adult population, objectively measured outdoor time is not significantly related to refractive error in adult populations.³² However, evidence suggests that time outdoors is protective for myopia in children.^{5, 53} To date, the relationship between the ipRGC-driven pupil response and light exposure has not been measured in children. The protective effects of outdoor light against myopia may be due to a variety of factors. High intensity light exposure experienced outdoors increases retinal dopamine and also directly stimulates the ipRGCs, which have synaptic connections to dopaminergic amacrine cells.¹⁵ Dopamine has been shown to regulate mRNA expression of melanopsin in ipRGCs.⁵⁴ Decreased dopamine has been shown to induce myopia in mice,⁵⁵ while dopamine agonists, such as apomorphine, inhibit experimental and spontaneous myopia in chicks and guinea pigs.^{56, 57} Another possible protective mechanism of light on myopia include increases in vitamin D.⁵⁸ Optical factors may play a role as well, such as a decrease in aberrations due to a smaller pupil size with high intensity light. Additionally, outdoor scenes provide an infinite viewing distance and little requirement for accommodation.

Here, myopic subjects reported poorer sleep quality than emmetropic subjects using the PSQI. These results are in accordance with previous reports that myopic children and young adults have poor sleep quality and shorter duration compared to emmetropes.^{21, 22} Several potential mechanisms may link behavioral and circadian aspects of sleep with refractive error. Myopia has been shown to be associated with education,¹ which may be related to longer hours studying and later bed times. Circadian rhythms influenced by light exposure subserving sleep/wake patterns may also play a role in myopia development. Previous studies have shown that law students with less than 5.6 hours of darkness may progress in myopia at a faster rate than those with more hours of darkness.⁵⁹ In animal studies, disruptions in normal light dark patterns also induce refractive errors.⁶⁰

A recent study reported higher morning melatonin in myopic subjects compared to emmetropic subjects.²⁵ Similar trends were observed here; however, differences did not reach significance. The previous study measured serum melatonin, while in this study, melatonin was assessed via a salivary assay, which may have more variability in melatonin quantification. A higher morning melatonin concentration would contribute to subjectively decreased daytime wakefulness and alertness, and is consistent with the finding of poorer scores in myopic subjects from the Pittsburgh Sleep Quality Index reported here.

In conclusion, we demonstrated that systemic melatonin is influenced by light exposure over the previous 7 days. Metrics of the ipRGC-driven pupil response showed small and inconsistent associations with light exposure and refractive error. Myopic subjects exhibited poor sleep quality compared to emmetropic subjects. Results suggest that complex relationships exist between light exposure, ipRGCs and with potential downstream effects on refractive error and sleep.

Acknowledgments

Presented in part at the 2016 annual American Academy of Optometry meeting (Anaheim, CA), received award to KA for best student presentation

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APPENDICES

Appendix Figure A1.

show the correlation between morning melatonin (pg/ml) and objectively measured (A) mean blue light exposure ($\mu\text{W}/\text{cm}^2$) and (B) mean red light exposure ($\mu\text{W}/\text{cm}^2$) over the previous 7 days ($n = 24$); open symbols represent outliers and are not included in regression analysis; * indicates significance at $P = 0.05$

Appendix Figure A2.

30 s PIPR with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

Appendix Figure A3.

Early AUC with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

Appendix Figure A4.

Late AUC with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

Figure 1.

(A) Experimental protocol (B) Pupil stimulation protocol. Dark adaptation (5 m) was followed by 1 s and 5 s long wavelength stimulation, with 60 s pupil following the stimulus. The protocol was then carried out with short wavelength stimuli.

Figure 2.

(A) Correlation between time spent outdoors over the previous week and morning melatonin ($n = 43$, $P = 0.0016$), and (B) objectively measured mean white light exposure over the 7 days and morning melatonin ($n = 24$, $P = 0.0099$), open symbols represent outliers and are not included in regression analysis, * indicates significance at $P < 0.05$

Figure 3.

Correlations between objectively measured white light exposure (lux) with red and blue light exposure ($\mu\text{W}/\text{cm}^2$, red and blue symbols, respectively), * indicates significance at $P < 0.05$

Figure 4.

Six-second PIPR with objectively measured white light exposure (A, B), blue light exposure (C, D), and red light exposure (E, F) over the previous 1 day for 1 s (left panel) and 5 s (right panel) long (red symbols) and short (blue symbols) wavelength stimuli; open symbols represent outliers and were not included in regression analysis

Figure 5.

Normalized pupil diameter for a (A) 1 s pulse and (B) 5 s pulse for emmetropes (black traces, $n = 19$) and myopes ($n = 31$), overlaid for comparison. Pupil metrics include baseline, peak constriction, 6 s PIPR, 30 s PIPR, early AUC, and late AUC. Shaded regions represent the 95% confidence intervals. Stimulus is shown in yellow.

Table 1

Pupil metrics utilized to quantify photoreceptor contributions.

Metric	Definition	Unit	Expected change
Baseline pupil diameter	Dark adapted 10 s pre-stimulus pupil diameter	mm, defined as 100%	
Peak constriction	Maximum pupil constriction	% of baseline pupil diameter	Smaller value indicates greater constriction
6 s PIPR	Mean pupil diameter 6–7 s after stimulus offset	% of baseline pupil diameter	Smaller value indicates greater ipRGC activity
30 s PIPR	Mean pupil diameter 30–31 s after stimulus offset	% of baseline pupil diameter	Smaller value indicates greater ipRGC activity
Early AUC	Integral of 100% minus the interpolated % pupil diameter, 0–10 s after stimulus offset	unitless	Larger value indicates greater ipRGC activity
Late AUC	Integral of 100% minus the interpolated % pupil diameter, 10–30 s after stimulus offset	unitless	Larger value indicates greater ipRGC activity

Table 2

Age, sex, spherical refraction (SER), axial length, time outdoors (mean minutes per day), white light exposure, sleep duration and efficiency, morning melatonin concentration, and PSQI scores; p value for emmetropes vs myopes;

	All subjects	Emmetropes	Myopes	P value
Age (years)	26.9 ± 6.2	27.7 ± 5.5	26.3 ± 6.6	0.50
Sex	26F:24M	9F:10M	17F:14M	1.0
SER (n = 50)	-2.5 ± 2.7 D	+0.38 ± 0.48 D	-4.3 ± 1.6 D	* < 0.005
Axial Length (mm, n = 50)	24.6 ± 1.2	23.6 ± 0.8	25.3 ± 0.9	* < 0.005
Time outdoors (minutes, n = 50)	104.8 ± 46.6	107.6 ± 42.1	103.0 ± 49.9	0.74
White light exposure (log lux, n = 24)	13.99 ± 0.66	14.09 ± 0.66	13.8 ± 0.85	0.42
Sleep duration (minutes, n = 24)	435.5 ± 43.9	457.2 ± 38.1	421.7 ± 43.1	0.09
Sleep efficiency (n = 24)	84.6 ± 5.9%	83.3 ± 7.9%	85.3 ± 4.3%	0.50
Melatonin (pg/ml, n = 43)	6.9 ± 3.5	6.5 ± 2.5	7.1 ± 4.0	0.60
PSQI (n = 50)	5.3 ± 2.7	4.2 ± 2.3	5.9 ± 2.7	* < 0.05

* indicates significance at p = 0.05.

Table 3

Pupil metrics for all subjects (n = 50) for 1 s and 5 s long wavelength (red) and short wavelength (blue) stimulations; metrics include peak constriction (% of baseline) during stimulation, 6 s and 30 s post illumination pupil response (PIPR, %), early and late area under the curve (AUC, unitless).

Stimulus	Peak constriction	6 s PIPR	30 s PIPR	Early AUC	Late AUC
1 s red	62.1 ± 5.8%	92.6 ± 2.9%	97.2 ± 3.8%	1.22 ± 0.30	0.61 ± 0.62
1 s blue	48.4 ± 10%	68.3 ± 8.8%	95.9 ± 4.9%	3.51 ± 0.75	1.98 ± 1.04
	* p < 0.001	* p < 0.001	p = 0.11	* p < 0.001	* p < 0.001
5 s red	44.8 ± 4.8%	89.5 ± 6.0%	98.5 ± 4.8%	1.70 ± 0.51	0.59 ± 0.90
5 s blue	37.3 ± 3.4%	64.4 ± 8.4%	92.4 ± 5.3%	3.84 ± 0.64	3.02 ± 1.14
	* p < 0.001	* p < 0.001	* p < 0.001	* p < 0.001	* p < 0.001

* indicates significance at p = 0.05 for red versus blue stimulations.