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The ipRGC-driven pupil response with light exposure and refractive error in children

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Abstract

Purpose: The intrinsically photosensitive retinal ganglion cells (ipRGCs) signal environmental light, control pupil size and entrain circadian rhythm. There is speculation that ipRGCs may be involved in the protective effects of light exposure in myopia. Here, the ipRGC-driven pupil response was evaluated in children and examined with light exposure and refractive error.

Methods: Children ages 5 to 15 years participated. Subjects wore an actigraph device prior to the lab visit for objective measures of light exposure and sleep. For pupillometry, the left eye was dilated and presented with stimuli, and the consensual pupil response was measured in the right eye. Pupil measurements were preceded by five minutes dark adaptation. In Experiment 1 ($n = 14$), 1 second (s) long wavelength light (“red,” 651 nm, 167 cd/m²) and 10 increasing intensities of 1 s short wavelength light (“blue,” 456 nm, 0.167 to 167 cd/m²) were presented with a 60 s interstimulus interval. A piecewise 2-segment regression was fit to the stimulus response function to determine the functional melanopsin threshold. Pupil responses were analysed with light exposure over the previous 24 hours. For Experiment 2 ($n = 42$), three 1 s red and three 1 s blue alternating stimuli were presented with a 60 s interstimulus interval. Following an additional 5-minute dark adaptation, the experiment was repeated. Pupil metrics included peak constriction, the 6 s and 30 s post-illumination response (PIPR), early and late area under the curve (AUC). Following pupil measurements, cycloplegic refractive error and axial length were measured.

Results: For Experiment 1, PIPR metrics demonstrated a graded response to increasing intensity blue stimuli, with a mean functional melanopsin threshold of 6.2 ± 4.5 cd/m² (range: 0.84–16.7 cd/m²). The 6 s PIPR and early AUC were associated with 24-hour light exposure for high intensity stimuli (33.3 and 83.3 cd/m², $P < 0.005$ for both). For Experiment 2, there were no associations between pupil metrics and refractive error. The 6 s PIPR and early AUC to blue stimuli were significantly increased for Trial 2 compared to Trial 1.

Conclusions: The ipRGC-driven pupil responses in children were robust and similar to responses previously measured in an adult population. The 6 s PIPR and early AUC to high intensity blue stimuli were associated with previous light exposure. There were no associations between the ipRGC-driven pupil response and refractive status in this cohort.

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Keywords

refractive error; myopia; ipRGCs; melanopsin; pupil; light exposure

Time outdoors has been linked to refractive development in children,^{1–3} with those who spend more time outside having a lower odds ratio for myopia.⁴ Myopic children have been shown to exhibit significantly lower daily light exposure than emmetropic children.⁵ Evidence from studies in chicks⁶ and rhesus monkeys⁷ shows that high ambient illumination is protective against form deprivation myopia. Elevated light levels also slow the rate of lens induced myopia in chicks,⁸ although not in primates.⁹ The mechanism by which light exposure may be protective for myopia is unknown. The neurotransmitter dopamine, which increases in the retina with increased ambient illumination,^{10, 11} has been implicated in this process.¹² Additionally, dopamine decreases in form deprivation myopia,¹⁰ and dopamine agonists inhibit experimental myopia development in animal models.^{13, 14}

There has been speculation that the intrinsically photosensitive retinal ganglion cells (ipRGCs) may also play a role in refractive development.^{15, 16} The ipRGCs are a unique class of retinal ganglion cells that are directly activated by light through the expression of the photopigment melanopsin,¹⁷ with a peak sensitivity around 482 nm.¹⁸ In addition to intrinsic activation, the ipRGCs also receive input from rod and cone photoreceptors via bipolar cells.^{19–21} ipRGCs have been described as environmental illuminance detectors, and are primarily involved in non-image forming functions, including pupil size control and circadian rhythm entrainment, through the retinohypothalamic tract to the olivary pretectal and suprachiasmatic nuclei.^{22, 23} The ipRGCs also play a role in image formation, contributing to visual detection and temporal and colour processing.^{18, 24, 25} ipRGCs have been shown to synapse reciprocally with dopaminergic amacrine cells.^{26, 27} With a known role of dopamine in refractive development, it is possible that the ipRGCs and melanopsin may also influence eye growth.

Historically, the pupillary light reflex (PLR) was thought to be controlled solely by rod and cone photoreceptors.^{28, 29} However, with the discovery of the photopigment melanopsin in ipRGCs,^{17, 30} the PLR is now known to originate from contributions from rods, cones and ipRGCs.^{31, 32} ipRGC activity can be assessed *in vivo* via aspects of the PLR. With light stimulation, initial pupil constriction is primarily attributed to rod and cone photoreceptors,^{33, 34} while the post-illumination pupil response (PIPR) following light offset is attributed to the ipRGCs.^{31, 35} Rod photoreceptors have also been shown to contribute to the early redilation phase (< 1.7 seconds) of the PIPR.³⁶ The PIPR is characterised by a sustained miosis following light offset, which is the signature of ipRGC activity.³¹ Intracellular recordings of ipRGCs show that firing continues after a light stimulus has ceased when melanopsin is activated.^{18, 22} This continued firing leads to the sustained pupil constriction after stimulus offset which can be quantified to assess ipRGC activity.

The PLR has been studied extensively in humans from pre-term to adult ages,^{37–40} although pupil parameters specific to ipRGCs have not yet been reported in children. It is well known that baseline pupil size decreases with age in adulthood.^{41, 42} Daluwatte, *et al* examined various pupil parameters in children ages 6 to 17 years, and found that baseline pupil

diameter increased from 6 to 12 years old, and pupil constriction to light stimuli increased from 6 to 8 years old.³⁸ In adults age 21 to 70 years, Adhikari, *et al* analysed the PLR relative to baseline pupil size and showed that both the transient PLR and the ipRGC-driven PIPR were independent of age for the population tested.⁴² Based on the literature, the PLR (including the PIPR) in children would be expected to be similar to adults.

Previous studies have shown that the ipRGC-driven pupil response in adults is not associated with refractive error.^{42, 43} However, accumulating evidence suggesting that increased light exposure is protective of myopia in children warrants further investigation evaluating the association of ipRGCs with refractive error and light exposure in a younger cohort. To date, few studies have evaluated the ipRGC-driven pupil response in children. The ipRGC-mediated PIPR has been previously reported in four children (two with autosomal dominant retinitis pigmentosa,⁴⁴ one with Leber congenital amaurosis,⁴⁵ and one control). In both of the previous studies, the children's data were grouped with adult data, and, therefore, it is unknown whether children's PIPR responses are consistent with previously published data in adults. More recently, investigators assessed chromatic pupillography in children, and included rod, cone and ipRGC activating stimuli.⁴⁶ Results showed that in children ages 3 to 17, a significant PIPR was elicited following a high intensity short wavelength stimulus compared to a long wavelength stimulus, and that there were no differences in the response across the age range tested. However, only one trial of one stimulus intensity was tested for each subject, and the PIPR was relatively small; the authors suggested that the protocol was suboptimal for the stimulation of melanopsin.⁴⁶

The goals of this study were twofold. First, we sought to test a clinical protocol for evaluating the ipRGC-driven pupil response in children and compare results to those in adults. To this aim, we investigated pupil responses over a range of stimulus intensities and with multiple trials to determine optimal conditions for eliciting melanopsin-driven pupil responses in young subjects. Second, we examined ipRGC-driven pupil metrics with light exposure and refractive error in children.

Methods

Children ages 5 to 15 years were recruited for this study. Parents provided written permission and children provided assent. The study was approved by the institutional review board at the University of Houston and procedures followed the tenets of the Declaration of Helsinki. All lab visits occurred between 9:00 am to 11:00 am to minimise potential effects of circadian variation.⁴⁷ 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. Best-corrected visual acuity for all subjects was 20/25 (6/7.5, 0.10 logMAR) or better. No subjects had ocular pathology such as cataracts or glaucoma. No subjects were taking prescription medications known to affect pupil size or sleep aids such as melatonin.

For pupillometry, the left eye was dilated with 2.5% phenylephrine (<https://paragonbiotech.com>) and 1% tropicamide (<https://www.henryschein.com>). With this drug combination, a previous study demonstrated that 50% of subjects with dark irides reached a

6 mm pupil within 12 minutes, and pupil reactivity was eliminated in 90% of subjects within 20 minutes.⁴⁸ After a 20-minute dilation period, pupillometry was performed. Light pulses were presented to the left eye, and the consensual pupil response was recorded in the right eye, as described previously.⁴⁹ Stimuli were presented using the LED-driven Ganzfeld ColorBurst system (<http://diagnosysllc.com>) centred 10 mm in front of the left eye, providing an approximately 140 degree field of view. Long wavelength “red” pulses (651 nm, half-max width 25 nm, using the Spectroradiometer CS1W (<http://sensing.konicaminolta.us>)) were presented to stimulate a primarily rod/cone-driven pupil response, and short wavelength “blue” pulses (456 nm, half-max width 20 nm) were presented to stimulate a combined rod/cone and ipRGC-driven pupil response. The instrument was programmed in luminance (cd/m^2), and subsequently, irradiance ($\text{photons/cm}^2/\text{s}$) was calculated for each stimulus from power measurements using an optical power meter (<https://www.newport.com>) and melanopic alpha-opic lux was calculated using the toolbox provided by Lucas, *et al.*⁵⁰

The right pupil was recorded at 60 Hz using a frame mounted binocular ViewPoint EyeTracker with infrared illumination (<http://www.arringtonresearch.com>). The infrared LED light source has a lambda max of 943 nm with a half-max width of 46 nm, Ocean Optics Spectrometer (<https://oceanoptics.com>). For each subject, the camera was calibrated by first focusing at the pupil plane, then capturing an image of a 5 mm artificial pupil. During the experiments, the subjects fixated with the right eye on a red laser spot directed at the wall at a distance of 4 metres.

Experiment 1

Experiment 1 was carried out to determine the functional melanopsin threshold and assess correlations between pupil metrics and light exposure ($n = 14$). For the purposes of this investigation, the functional melanopsin threshold is the short wavelength light intensity in which a sustained PIPR can confidently be distinguished from the long wavelength pupil response. Subjects wore an Actiwatch Spectrum actigraph device (<http://www.usa.philips.com>) continuously for one week prior to pupil measurements for objective measurement of light exposure, activity and sleep. The light sensor in the Actiwatch consists of colour-sensitive photodiodes to measure illuminance of white light in lux (range 0.1–200,000 lux), and the irradiance of the red, green, and blue components in microwatts per square centimetre ($\mu\text{W/cm}^2$, wavelength range 400–700 nm). Average daily white light exposure (log lux, base 10), blue light exposure (log $\mu\text{W/cm}^2$), red light exposure (log $\mu\text{W/cm}^2$) and time outdoors for the 24 hours before the lab visit were derived from the Actiwatch, and correlated to pupil metrics. Additionally, activity and sleep data were evaluated to ensure that subjects had normal sleep/wake patterns. Actiwatch data were analysed using the device software, Philips Actiware 6.0.8 (www.actigraph.com).⁵¹ Time outdoors per day was calculated as minutes spent exposed to greater than 1000 lux.^{51, 52}

For pupillometry, following the 20-minute dilation period, subjects dark-adapted for 5 minutes (< 0.1 lux). Baseline pupil size was recorded for 10 seconds (s), then a 1 s red stimulus (167 cd/m^2) and 10 increasing intensities of a 1 s blue stimulus (0.167 to 167 cd/m^2)

in 1/3 log unit steps) were presented to the dilated left eye with a 60 s interstimulus interval, as the consensual pupil response was recorded in the right eye.

Following pupillometry, the right eye was dilated. Cycloplegic autorefraction) was measured for each eye using the WAM-5000 autorefractor (<http://www.grandseiko.com>), and spherical equivalent refractive error (SER) was calculated as an average of five measurements. Biometry was measured using the Lenstar biometer (<https://www.haag-streit.com>), and axial length was calculated for each eye as an average of five measurements.

Experiment 2

Experiment 2 was carried out to assess the influence of refractive error and intrasession repeatability ($n = 42$). Intrasession comparisons were carried out to begin to understand the optimal number of trials that should be included in a children's clinical testing protocol. The subject's left eye was dilated, and the subject underwent a five-minute dark adaptation period as described above. Baseline pupil diameter was recorded for 10 seconds, then alternating 1 s red (33 cd/m^2) and 1 s blue (16.7 cd/m^2) stimuli were presented with a 60 s interstimulus interval, for a total of six pulses of light, resulting in three stimulations each for red and blue stimuli. These stimulus intensities were chosen because the findings in Experiment 1 showed that 33 cd/m^2 red and 16.7 cd/m^2 blue stimuli elicited approximately equal pupil constriction, the blue intensity was above the functional melanopsin threshold, avoided saturation of the response, and subjects were able to tolerate the stimulus. Pupil metrics for the three red stimuli were averaged, and those for the three blue stimuli were also averaged. Following Trial 1, subjects again dark adapted for five minutes, and the experiment was repeated in Trial 2. The right eye was then dilated, and refraction and axial length were measured, as described above.

Data analysis

Pupil data were analysed offline using a custom written MATLAB program (<https://www.mathworks.com>), as described previously.⁴³ Data were filtered to remove blinks and artefacts, which were identified as intervals of pupil-aspect ratio outside of six standard deviations of the mean pupil aspect ratio during stable pupil tracking. The use of a 6-standard deviation threshold excluded spurious data while retaining usable, reliable data. Pupil metrics were calculated to describe photoreceptor activity (Table 1). Representative responses are shown for one subject with pupil metrics indicated in Figure 1. Baseline pupil diameter was calculated as the average pupil diameter over 10 s before the first stimulus. Peak pupil constriction was calculated for each stimulation as the maximum pupil constriction following light onset, and presented as per cent of baseline pupil diameter. Peak pupil constriction primarily represents rod and cone contributions, but may also receive some melanopsin influence.³⁴ ipRGC activity was assessed through four metrics of the PIPR, as described in the literature.^{42, 53} The 6 s and 30 s PIPR were calculated as the mean pupil diameter (per cent of baseline) averaged over 6–7 s and 30–31 s, respectively, after 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, respectively, after stimulus offset. The areas were computed as the trapezoidal approximation of the integral of 100% minus the per cent pupil

diameter (i.e the difference between the pupil and baseline) for the respective intervals (unitless). For AUC metrics, missing data points due to filtering were interpolated linearly.

For Experiment 1, a stimulus response function was generated for the 6 s PIPR to increasing intensities of blue stimuli. The function was fit with a piecewise 2-segment linear regression, SigmaPlot 10.0, (<https://systatsoftware.com>), and the break point was taken as the functional melanopsin threshold, i.e. when a significant sustained pupil constriction was evident compared to the red stimulus. For each blue stimulus intensity above the melanopsin activation threshold, the 6 s PIPR and early AUC were analysed with log white light, blue light and red light exposure (over the previous 24 hours) using linear regressions. Significance level was set at 0.0125 following Bonferroni correction for multiple comparisons.

For Experiment 2, average pupil metrics for the red and blue stimuli for Trial 1 were compared to pupil metrics for Trial 2 using a two-tailed paired t-test to assess intrasession differences. Additionally, pupil metrics were compared between non-myopic and myopic subjects using a Mann-Whitney U Test for unequal sample sizes. Differences with a p value < 0.05 were considered significant.

Results

Experiment 1

Mean subject age was 9.0 ± 1.8 years ($\pm$ SD, range: 5.7 to 12.4 years). Mean SER was $+0.12 \pm 0.8$ D ($+1.6$ to -1.4 D), and mean axial length was 23.0 ± 0.5 mm (21.9 to 23.8 mm). The group was composed of 11 emmetropic and 3 myopic subjects. Because of the narrow refractive error range, these data were not analysed with respect to refractive error. According to actigraphy, subjects spent an average of 73.1 ± 39.9 minutes outdoors per day over the previous week. Average daily white light exposure was 5.83 ± 0.7 log lux, blue light exposure was 4.43 ± 0.7 log $\mu\text{W}/\text{cm}^2$, and red light exposure was 4.56 ± 0.6 log $\mu\text{W}/\text{cm}^2$. Light exposure data were normally distributed (Shapiro-Wilk test) and no values were found to be outliers (Tukey outlier detection). All subjects demonstrated normal sleep/wake patterns, with 8.6 ± 0.6 hours of sleep per day.

Pupil metrics for all subjects ($n = 14$) for red and blue stimuli are shown in Table 2, along with the stimulus luminance (cd/m^2), measured corneal irradiance ($\text{photons}/\text{cm}^2/\text{s}$), and calculated melanopsin alpha-opic lux⁵⁰ for each stimulus. The relative pupil diameter traces for all 11 stimuli (1 red, and 10 increasing intensities of blue) for a representative subject are shown in Figure 2. For all subjects, relative peak pupil constriction with blue stimulus intensity is shown in Figure 3, and PIPR metrics are shown in Figures 4A-D. The mean functional melanopsin threshold, determined as the break point of the 2-line fit to the stimulus intensity versus 6 s PIPR, was 6.2 ± 4.5 cd/m^2 (range 0.84–16.7 cd/m^2).

Linear regression analysis was used to assess the relationships between the 6 s PIPR and early AUC to 1 s blue stimuli for the four highest intensities (16.7, 33.3, 83.3 and 167 cd/m^2) with objectively measured light exposure over the previous 24 hours. For these analyses, one subject was omitted because the Actiwatch had an error during the experiment

and did not collect data. These four stimulus intensities were examined because they were above the melanopsin threshold for all subjects. The 6 s PIPR and early AUC for 33.3 and 83.3 cd/m² to 1 s blue stimuli were significantly associated with white light exposure (shown for 6 s PIPR in Figure 5). Similarly, these metrics were also associated with blue and red light exposure. However, blue and red light exposure were highly correlated with white light exposure and not considered independent measures ($R^2 = 0.99$, $P < 0.001$ for both, Supplemental Figure 1). Results for red and blue light exposure can be seen in the supplemental material (Supplemental Figures 2 and 3).

Experiment 2

Of the 42 children that participated in Experiment 2, data from five children (ages 5–6) were excluded because the children could maintain fixation during the stimulus presentation. For the remaining 37 children, mean subject age was 10.44 ± 2.5 years (5.5 to 15.7 years). Mean SER was $+0.23 \pm 1.74$ D (+4.57 to -4.19 D), and mean axial length was 23.15 ± 0.78 mm (21.17 to 25.58 mm). Baseline pupil size for all subjects was 6.46 ± 0.77 mm. Subjects were classified as non-myopic (> -0.50 D, $n = 26$) or myopic (≤ -0.50 D, $n = 11$).

Mean relative pupil diameter for 1 s red and 1 s blue stimuli are shown in Figure 6 for Trials 1 and 2, with the 95% confidence interval ($n = 37$). For all subjects, baseline pupil diameter following five minutes of dark adaptation was not significantly different between Trial 1 (6.46 ± 0.77 mm) and Trial 2 (6.54 ± 0.70 mm, $P = 0.81$). Maximum pupil constriction was significantly greater for 1 s blue stimuli compared to 1 s red stimuli, i.e. the relative pupil diameter was smaller for blue stimuli (0.49 ± 0.05) compared to red stimuli (0.56 ± 0.05 , $P < 0.001$, Figure 7A). Maximum pupil constriction following a 1 s red stimulus was significantly greater for Trial 2 (0.53 ± 0.06) compared to Trial 1 (0.56 ± 0.05 , $P = 0.006$). Similarly, maximum pupil constriction following a 1 s blue stimulus was significantly greater for Trial 2 (0.45 ± 0.04) compared to Trial 1 (0.49 ± 0.05 , $P < 0.001$).

For post-illumination pupil metrics, the 6 s PIPR following a 1 s red stimulus was similar for Trials 1 and 2 ($P = 0.74$, Figure 7B). However, the 6 s PIPR following a 1 s blue stimulus was significantly greater (i.e., smaller value), for Trial 2 (0.69 ± 0.09) than for Trial 1 (0.74 ± 0.10 , $P = 0.03$). Similarly, the early AUC following a 1 s red stimulus was similar for Trials 1 and 2 ($P = 0.40$), while the early AUC following a 1 s blue stimulus was significantly greater for Trial 2 (3.69 ± 0.68) than for Trial 1 (3.21 ± 0.80 , $P = 0.001$, Figure 7C). The late post-illumination pupil metrics, including the 30 s PIPR and late AUC, were not significantly different for red or blue stimuli between Trials 1 and 2 ($P > 0.05$ for all).

The mean age of the non-myopic group was 9.75 ± 2.41 years (5.52 to 14.33 years, 12F:14M) and for the myopic group was 11.71 ± 2.52 years (7.46 to 15.72 years, 8F:3M). While the age ranges overlapped, subjects in the myopic group were older than the non-myopic group ($P = 0.05$). SER for the non-myopic group, $+1.04 \pm 1.24$ D (-0.38 to +4.57 D), was significantly more positive than for the myopic group, -1.69 ± 1.16 D (-0.56 to -4.19 D, $P < 0.001$). Axial length was significantly shorter for the non-myopic group, 22.92 ± 0.71 mm (21.25 to 24.52 mm), than for the myopic group, 23.66 ± 0.78 mm (22.60 to 25.46 mm, $P = 0.007$).

Average white light exposure over the previous 24 hours, as measured using the Actiwatch Spectrum, was similar between non-myopic and myopic refractive error groups ($P = 0.33$). Additionally, baseline pupil size and all pupil metrics for both the red and blue stimuli were similar between refractive error groups ($P > 0.05$ for all). Regression analysis showed that there was no significant association between the 6 s PIPR and age ($P = 0.13$).

Discussion

The goal of this study was to systematically evaluate the ipRGC-driven pupil response in children and investigate associations with light exposure and refractive error. Results show that children demonstrate robust ipRGC-driven pupil responses. The functional melanopsin threshold was similar to that measured previously in an adult population (children: 6.2 ± 4.5 cd/m²; adults: 3.83 ± 2.58 cd/m²).⁵⁴ Additionally, pupil metrics, including peak constriction and the PIPR, were similar to those we reported using similar protocols in adults.⁴³ We demonstrated that the 6 s PIPR and early AUC in response to high intensity blue stimuli were associated with previous light exposure, which was measured objectively over the previous day. Assessment of intrasession variability showed that some pupil metrics were significantly different for repeated trials; specifically, the maximum pupil constriction to red and blue stimuli, and the 6 s PIPR and early AUC to blue stimuli all significantly increased for the second trial within a single session. In accordance with previous studies,⁵⁵ we found that baseline pupil size in children, ages 5–12 years (6.5 ± 0.8 mm) is greater than that found in our previous study in adults, ages 17–40 years (6.1 ± 0.8 mm).⁴³ There were no associations between the ipRGC-driven pupil response and refractive error or age in this cohort.

A previous study in adults sought to develop a clinical protocol for assessing rod, cone and melanopsin contributions to the human pupil response.⁵⁶ The authors concluded that a 1 s stimulus duration was optimal for eliciting a melanopsin driven pupil response, and the 6 s PIPR was a meaningful metric for describing the post-illumination response. Here, a 1 s stimulus duration was utilised, and a stimulus response function was generated using the 6 s PIPR for increasing intensities of blue stimuli from 0.16 to 167 cd/m². The functional melanopsin threshold was defined as the blue stimulus intensity at which the post-illumination pupil metrics were significantly and consistently different from those for a red stimulus, as determined by a 2-line break point in the 6 s PIPR. The 6 s PIPR metric was chosen for this calculation because it represents a linear value, whereas the early AUC represents a summed value over time. The 6 s PIPR at the blue stimulus intensity just above the break point represents approximately 90% decrease in pupil size compared to the red stimulus at 6–7 seconds after stimulus offset. Pupil responses to blue stimuli below the break point did not demonstrate a significant post-illumination sustained constriction, which is the signature of a melanopsin-driven pupil response.⁵⁷ Park, *et al* showed that the dark-adapted pupil responses to low intensity blue stimuli are driven by the rod system,⁵⁶ although melanopsin has also been shown to operate near absolute threshold.^{34, 58} With increasing intensity of the blue stimulus, a sustained pupil constriction following light offset became evident, at which point the response was likely driven through activation of melanopsin in the ipRGCs.³¹ With the 140-degree stimulus field of view used here, the functional melanopsin threshold was approximately 6.2 cd/m², which was calculated to be a corneal

irradiance of 1.6×10^{13} photons/cm²/s. It should be noted that the *in vivo* functional melanopsin threshold described here, observed from the pupil response, is likely different from the melanopsin activation threshold determined from single cell recordings. Using *in vitro* primate retina preparations, Gamlin, *et al* demonstrated intrinsic ipRGC spiking activity to a 60 second short wavelength (470 nm) pulse with a retinal illuminance of 1×10^{12} photons/cm²/s following light adaptation,¹⁸ although this would not be expected to correlate to dark adapted *in vivo* measures here. Using a similar protocol to ours, Park, *et al* demonstrated that a sustained, ipRGC-driven pupil response was evident to stimuli as low as 1 cd/m² in adult subjects,⁵⁶ and Lei, *et al* reported a detectable PIPR at approximately 3.16 cd/m².⁵⁹ Here, we report a slightly higher functional melanopsin threshold (6.2 cd/m²). Differences in threshold between studies is likely due to different criteria for determining the threshold, as all of our pupil responses to blue light were compared to high intensity red light (167 cd/m²) as opposed to matched luminance red stimulus for each blue intensity level.

We evaluated four metrics specific to the PIPR, which have been utilised in many previous studies,^{35, 42, 53, 56} including the 6 and 30 s PIPR and the early and late AUC. Specifically, the 6 s PIPR and early AUC evaluate the early PIPR, while the 30 s PIPR and late AUC represent the late PIPR. The 6 and 30 s PIPR include data averaged over only a 1 s interval, while the early and late AUC include data averaged over 10 and 20 s, respectively. A previous study showed that the PIPR can be sustained for up to 83 s for a 1 s pulse and 180 s for a 30 s pulse.³⁵ We found that as blue stimulus intensity increased, the late AUC pupil metric increased, which assesses the response 10–30 seconds following light offset, suggesting that ipRGC activity was sustained longer for higher intensities. However, the 30 s PIPR was close to 1 for all intensities, suggesting minimal melanopsin activity at 30 s after stimulus offset for this stimulus paradigm.

Intrasession evaluation of pupil metrics was assessed to help determine an appropriate number of trials for each subject. Results from two trials of three red and three blue stimulations each within the same session showed that several pupil metrics increased for the second trial. For example, maximum pupil constriction increased for the second trial for both red and blue stimuli. The maximum pupil constriction is primarily driven by rod and cone inputs. Additionally, early metrics of the ipRGC-driven pupil response, the 6 s PIPR and the early AUC, increased for the second trial. Here, the 6 s PIPR to a 1 s blue stimulus was 74% in the first trial, and 69% in the second trial. This difference we found between trials, at 5% of the PIPR, could represent a clinically significant difference. The increased responses suggest that the cells increased activity through further dark adaptation or from a potentiation of the response with repeated stimulations. Potentiation of the ipRGC-driven pupil response has been previously observed using a 0.2 Hz flickering stimulus over a 2-minute interval.⁵⁴ Similarly, potentiation may also occur with repeated trials in the current stimulus paradigm. The finding that the second set of data within a session results in different pupil metrics suggests that additional trials may confound the results due to increased dark adaptation or to potentiation, and these parameters must be consistent across subjects and conditions for comparative analyses. Lei, *et al* examined the intrasession repeatability of the PIPR to red and blue stimuli (400 cd/m²) and found no significant differences between trials.⁶⁰ However, the subjects underwent a 30–120 second break in

between trials with the room lights on to avoid carry over effects. It is likely these carry over effects that are observed in our study resulting in an increased PIPR for the second trial.

One of the goals of this study was to test a protocol for assessing ipRGC pathways in children. Based on previous experiments in adults, and our findings here, we determined that an adequate clinical protocol to measure the ipRGC-driven pupil response in children includes a 5-minute dark adaptation period, six alternating 1 second red and blue stimuli with a 60-second interstimulus interval, and a blue stimulus intensity of 16.6 cd/m² (or 6.4×10^{13} photons/cm²/s), which is sufficiently above the functional melanopsin threshold. A 60-second interstimulus interval was sufficient for the pupil to return to baseline before the next stimulus. These parameters provided three responses primarily driven by the rod/cone pathway, and three responses driven by the combined rod/cone and ipRGC pathways. Additionally, the protocol was well tolerated by the subjects, and the entire protocol requires only 11 minutes following dilation. However, five of the youngest subjects, ages 5–6 years, were unable to keep their eyes open and maintain fixation during the testing and could not be included in the analysis.

In the current study, the ipRGC-driven pupil response was assessed in relationship to objectively measured light exposure. Results showed that for blue stimulus intensities, which activate the melanopsin-driven pupil response, specifically the higher intensities at 33.3 and 83.3 cd/m², the 6 s PIPR and early AUC were significantly associated with the amount of light exposure from the previous day. The relationship between the PIPR and light exposure did not reach statistical significance after Bonferroni correction for the highest intensity, 167 cd/m². Subjects reported that this stimulus intensity, at a corneal irradiance of 6.3×10^{14} photons/cm²/s, was uncomfortably bright, leading to photophobia and discomfort. The primary mediator of photophobia is the trigeminal nerve, which may in turn activate the trigemino-autonomic reflex involving Edinger-Westphal nuclei and mediating pupillary constriction.⁶¹ Therefore, we speculate that other pathways, such as that of the autonomic system, may have played a role in pupil control at the highest intensity. ipRGCs have been described as environmental irradiance detectors.²² The ipRGC-driven pupil response has been shown to vary with season, presumably due to variations in photoperiod, or available sunlight, throughout the year.⁶² Our data provide further evidence that ipRGC activity is influenced by light exposure. In accordance with previous studies in adults that evaluated similar ipRGC-driven pupil metrics, we did not find an association between the ipRGC pupil metrics and refractive error.^{42, 43, 63}

ipRGCs are spectrally tuned to short wavelength light, with a peak sensitivity at approximately 482 nm.¹⁸ Here, light exposure received one day prior to pupil measurements was measured objectively, and the red (600–700 nm) and blue (400–500 nm) components were assessed to begin to understand influences on ipRGC-driven pupil metrics. We found that both red and blue exposure were highly correlated to each other, as well as to broadband (white) light exposure ($R^2 = 0.99$). Therefore, it was not possible to independently assess the influence of spectral light exposure. Future studies in which subjects are exposed to narrow band light in a controlled laboratory environment may help to determine the influence of spectral composition on the ipRGC activity.

A major function of the ipRGCs is circadian rhythm entrainment. Studies have found that circadian rhythm may play a role in refractive development. For example, in chicks, brief periods of light exposure during the night,⁶⁴ constant light,⁶⁵ or constant dark⁶⁶ result in disruptions in eye growth regulation with subsequent refractive errors ensuing. In Experiment 2 in the current study, as well as previous studies in adults,^{42, 43} associations between the ipRGC-driven pupil response and refractive error were not found. In Experiment 1, relationships between the ipRGC-driven pupil response and light exposure were demonstrated when utilising high intensity stimuli ($> 33.3 \text{ cd/m}^2$). Previous studies show that myopic children tend to have less light exposure compared to emmetropic children.⁵ It is possible that the lack of differences in pupil responses between refractive error groups was due to differences in light exposure. However, there were no significant differences in light exposure over the previous 24 hours between non-myopic and myopic groups. Additionally, the pupil response to the stimulus intensity used in Experiment 2 (16.6 cd/m^2) was not shown to be correlated with previous light exposure in Experiment 1. Another potential confounding factor is the age difference between the two refractive error groups (non-myopes: 9.75 years vs myopes: 11.71 years). Future studies with children utilising objective measures of light exposure and a higher stimulus intensity may help to clarify the complex relationship between light exposure, ipRGCs, and refractive error.

In conclusion, this study provides the first systematic evaluation of the ipRGC-driven pupil response in children. While baseline pupil size was larger than in adult populations, parameters of the PLR were similar to those previously reported in adults. Assessment of intrasession repeatability showed that responses increased for repeated trials, suggesting increased sensitivity with further dark adaptation or potentiation of the response. ipRGC-driven pupil metrics to high intensity blue light were associated with previous light exposure, while no associations were evident between pupil metrics and refractive error.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Representative pupil diameter traces for one subject (age 9 years) showing pupil metrics, including relative baseline pupil diameter, peak constriction, 6 s and 30 s post illumination pupil response (PIPR), and early and late area under the curve (AUC). Black trace indicates the stimulus.

Figure 2.

Representative pupil traces for one subject (age 9 years) to a 1 s red (167 cd/m^2) and 10 increasing intensities of 1 s blue stimuli from 0.16 to 167 cd/m^2 . The stimulus is shown in black.

Figure 3. Relative peak pupil constriction for all subjects ($n = 14$, mean $\pm$ SE) for increasing intensities of blue stimuli from 0.16 to 167 cd/m^2 .

Figure 4.

A) 6 s post illumination response (PIPR); B) 30 s PIPR; C) early area under the curve (AUC); and D) late AUC for all subjects ($n = 14$, mean $\pm$ SE) for increasing intensities of 1 s blue stimuli from 0.16 to 167 cd/m². Functional melanopsin threshold, as calculated from the break point of the 2 line fit to the 6 S PIPR (6.2 cd/m²), is indicated with the asterisk (*).

Figure 5.

Linear regression analysis for white light exposure and the 6 s PIPR for all subjects ($n = 13$) to 1 s blue stimulation for (A) 16.6 cd/m²; (B) 33.3 cd/m²; (C) 83.3 cd/m²; and (D) 167 cd/m². * indicates significance at an alpha level of < 0.05 ; ** indicates significance at a Bonferroni corrected alpha level of < 0.0125 .

Figure 6.

Mean relative pupil diameter for 1 s red and 1 s blue stimuli for Trial 1 and Trial 2 ($n = 37$). Dark red and blue traces represent Trial 1, light red and blue traces represent Trial 2, and the stimulus is shown in black. The 95% confidence intervals are shown in light grey (Trial 1) and dark grey (Trial 2).

Figure 7.

Pupil metrics to 1 s red (33.3 cd/m^2) and 1 s blue (16.7 cd/m^2) for trial 1 (solid bars) and trial 2 (open bars). A) Relative maximum pupil constriction; B) 6 s and 30 s PIPR; C) early and late AUC. $n = 37$, * indicates significance at an alpha level of < 0.05 .

Table 1:

Pupil metrics utilized to quantify photoreceptor contributions

Metric	Definition	Unit	Primarily attributed to
Baseline pupil diameter	Dark adapted 10 s pre-stimulus pupil diameter	mm, defined as 1	Rod/cone and ipRGC activity
Peak constriction	Maximum pupil constriction	ratio to baseline pupil diameter	Rod/cone activity
6 s PIPR	Mean pupil diameter 6–7 s after stimulus offset	ratio to baseline pupil diameter	ipRGC activity
30 s PIPR	Mean pupil diameter 30–31 s after stimulus offset	ratio to baseline pupil diameter	ipRGC activity
Early AUC	Integral of 100% minus the interpolated % pupil diameter, 0–10 s after stimulus offset	unitless	ipRGC activity
Late AUC	Integral of 100% minus the interpolated % pupil diameter, 10–30 s after stimulus offset	unitless	ipRGC activity

Table 2:

Pupil metrics (mean $\pm$ SE) following 1 s red and increasing intensity of blue stimuli, including peak constriction, 6 s post-illumination pupil response (PIPR), 30 s PIPR, early area under the curve (AUC), and late AUC. Stimuli are presented in photopic luminance (cd/m^2), photons ($\text{photons/cm}^2/\text{s}$), and melanopic alpha-optic lux. Values are relative to baseline pupil diameter. Stimulus intensities below the dashed line are considered to be above the functional melanopsin activation threshold.

Stimulus	Corneal Irradiance	α -optic lux	Peak Constriction	6 s PIPR	30 s PIPR	Early AUC	Late AUC
red 167 cd/m^2	4.8×10^{14} photons/ cm^2/s	0.51	0.56 ± 0.08	0.90 ± 0.06	0.98 ± 0.04	1.53 ± 0.43	0.33 ± 0.55
blue 0.16 cd/m^2	4.4×10^{11} photons/ cm^2/s	3.99	0.61 ± 0.14	0.91 ± 0.05	0.97 ± 0.04	1.57 ± 0.52	0.62 ± 0.89
blue 0.33 cd/m^2	8.8×10^{11} photons/ cm^2/s	8.02	0.56 ± 0.07	0.91 ± 0.04	0.94 ± 0.07	1.68 ± 0.42	1.08 ± 0.68
blue 0.83 cd/m^2	2.3×10^{12} photons/ cm^2/s	20.08	0.52 ± 0.06	0.88 ± 0.06	0.95 ± 0.07	2.03 ± 0.50	0.91 ± 0.95
blue 1.67 cd/m^2	4.4×10^{12} photons/ cm^2/s	40.29	0.49 ± 0.06	0.87 ± 0.05	1.02 ± 0.17	2.08 ± 0.55	0.76 ± 1.12
blue 3.33 cd/m^2	8.8×10^{12} photons/ cm^2/s	80.37	0.49 ± 0.06	0.86 ± 0.08	0.98 ± 0.07	2.19 ± 0.93	0.88 ± 1.20
blue 8.33 cd/m^2	2.2×10^{13} photons/ cm^2/s	199.99	0.48 ± 0.05	0.80 ± 0.08	0.98 ± 0.05	2.55 ± 0.86	0.78 ± 1.22
blue 16.67 cd/m^2	6.4×10^{13} photons/ cm^2/s	581.3	0.45 ± 0.09	0.76 ± 0.08	0.95 ± 0.05	3.19 ± 0.81	1.42 ± 1.49
blue 33.3 cd/m^2	1.3×10^{14} photons/ cm^2/s	1170.99	0.41 ± 0.12	0.71 ± 0.13	0.95 ± 0.05	3.29 ± 1.19	1.95 ± 1.55
blue 83.3 cd/m^2	3.2×10^{14} photons/ cm^2/s	2916.99	0.39 ± 0.10	0.65 ± 0.12	0.95 ± 0.12	3.87 ± 0.96	2.32 ± 1.78
blue 167 cd/m^2	6.3×10^{14} photons/ cm^2/s	5737.44	0.40 ± 0.07	0.63 ± 0.12	0.97 ± 0.15	4.12 ± 0.84	2.97 ± 2.35

Table 3:

Age, spherical refraction (SER), axial length, objectively measured light exposure, and pupil metrics for non-myopic (n = 26) and myopic (n = 11) groups for experiment 2 (mean $\pm$ SD). Pupil metrics include baseline pupil size, peak constriction, 6 s post-illumination pupil response (PIPR), 30 s PIPR, early area under the curve (AUC), and late AUC. * indicates significance at an alpha level of < 0.05 .

Parameter	Non-Myopic Group	Myopic Group	P value
Age	9.75 $\pm$ 2.41 years	11.71 $\pm$ 2.52 years	*0.05
SER	+1.04 $\pm$ 1.24 D	-1.69 $\pm$ 1.16 D	* <0.001
Axial Length	22.92 $\pm$ 0.71 mm	23.66 $\pm$ 0.78	* <0.007
Log white light exposure	6.45 $\pm$ 1.45	6.99 $\pm$ 1.27	0.32
Baseline pupil size	6.51 $\pm$ 0.86 mm	6.35 $\pm$ 0.51	0.74
Red Stimulus			
Peak constriction	0.56 $\pm$ 0.04	0.54 $\pm$ 0.07	0.30
6 s PIPR	0.93 $\pm$ 0.07	0.90 $\pm$ 0.04	0.13
30 s PIPR	1.0 $\pm$ 0.08	0.97 $\pm$ 0.03	0.13
Early AUC	1.38 $\pm$ 0.54	1.63 $\pm$ 0.46	0.27
Late AUC	0.32 $\pm$ 1.47	0.87 $\pm$ 0.59	0.30
Blue Stimulus			
Peak constriction	0.50 $\pm$ 0.04	0.47 $\pm$ 0.06	0.06
6 s PIPR	0.75 $\pm$ 0.11	0.71 $\pm$ 0.09	0.41
30 s PIPR	0.98 $\pm$ 0.08	0.94 $\pm$ 0.04	0.14
Early AUC	3.08 $\pm$ 0.83	3.51 $\pm$ 0.68	0.10
Late AUC	1.19 $\pm$ 1.79	2.01 $\pm$ 0.95	0.16