Binocular Summation in Postillumination Pupil Response Driven by Melanopsin-Containing Retinal Ganglion Cells

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PURPOSE. To investigate how melanopsin-mediated intrinsically photosensitive retinal ganglion cell (ipRGC) signals are integrated binocularly using chromatic pupillometry. We hypothesized that if the melanopsin system is summative, there will be a greater postillumination pupillary response (PIPR) under binocular conditions after viewing bright blue light.

METHODS. Pupillary responses in 10 visually normal participants were recorded with an eye tracker following full-field stimulation of red (long wavelength) and blue (short wavelength) light of equal intensity (dim: 0.1 cd [candela]/m², bright: 60 cd/m²) and duration (400 ms). Individual monocular (left eye) pupil responses were measured first, followed by binocular responses. Each participant repeated the same protocol on 3 separate days, at similar times of day. PIPR was recorded for bright red and blue conditions only, whereas maximum pupillary constriction (MPC) was measured under both bright and dim conditions during red and blue light stimulation.

RESULTS. Bright blue light stimulation induced greater PIPR under binocular than monocular viewing conditions ($F_{(1,9)} = 79.52$, $P < 0.001$). Bright red light stimulation induced minimal PIPR and showed no significant difference between viewing conditions post Bonferroni correction ($F_{(1,9)} = 5.49$, $P = 0.04$). MPC was greater during binocular than monocular viewing conditions for all light stimuli, but was greatest following blue compared to red light stimulation.

CONCLUSIONS. A larger PIPR was induced using a binocular than a monocular full-field stimulus of equal intensity and duration, demonstrating that melanopsin-mediated ipRGC signals are summated binocularly. This study expands our current understanding of the melanopsin system and may be used as an additional marker to stratify diseases according to their etiologies.

Keywords: melanopsin, binocular, monocular, PIPR, summation

Since the discovery of the third photoreceptor type, the melanopsin-containing intrinsically photosensitive retinal ganglion cell (ipRGC), great strides have been made in understanding both the mechanisms and functional properties underlying retinal signaling.[1] The presence of melanopsin, an opsin-based photopigment, allows the ipRGCs to transduce light information directly (intrinsic photoactivity) in addition to their secondary relay role as conduits for rod- and cone-mediated signaling (extrinsic activity).[2–5] Among these three types of photoreceptors that mediate the pupil light reflex (PLR), the rod system transduces light under scotopic conditions and saturates quickly after activation. At higher illumination levels, cone responses begin to dominate but eventually adapt.[6] As illumination increases further, intrinsic melanopsin-mediated photoactivity becomes increasingly influential in mediating the PLR.[7] Melanopsin phototransduction has a peak spectral sensitivity at ~480 nm (short-wavelength blue light), a higher activation threshold, a longer latency to fire action potentials, and a poor temporal resolution.[3,8–10] Upon melanopsin activation, ipRGCs exhibit a prolonged firing pattern that persists post stimulus offset.[3,8,9] The ability to integrate both intrinsic and extrinsic signals allows ipRGCs to capture the full range of ambient irradiance information pertinent to human vision.[9]

Human ipRGC photoactivity can be assessed in vivo using chromatic pupillometry, a technique whereby constituent PLR components are measured based on each photoreceptor's spectral sensitivity and its dynamics. In general, using a brief flash stimulus, immediately upon stimulus onset, the PLR consists of a rapid constriction phase predominantly mediated by rod/cone activity that quickly returns to baseline.[11,12] If melanopsin is activated, however, it results in a sustained constriction phase post stimulus offset,[11–13] known as the postillumination pupillary response (PIPR). This sustained response is unaffected by pharmacologic inactivation of rod/cone inputs in nonhuman primates,[13] and can be temporally isolated in the laboratory by recording the PLR following a bright blue stimulus and measuring the PIPR over a time interval well past stimulus offset.[11,14]

The crossing of visual signals at the level of the midbrain allows monocular stimulation to elicit a simultaneous and balanced pupillary response in both eyes, known as the consensual light response.[15,16] Accordingly, in recent literature, retina-driven pupil response dynamics of the human eye have been evaluated as follows: (1) stimulation of a dilated eye and PLR recording of the fellow eye, (2) stimulation of an undilated eye and PLR recording of the fellow eye or (3) stimulation and PLR recording of the same eye.[11–14,17–32] The presence of binocular summation of the PLR has been well established and evaluated across rod-dominated scotopic and cone-dominated photopic luminance ranges.[33–36] Nonhuman primate models have also been used to investigate the characteristics and neural substrates of the PLR in greater detail,[37–40] and similarly report the presence of binocular summation.[37,40] All of these studies, however, largely predate the discovery of melanopsin and have not examined the sustained melanopsin-mediated pupillary responses.

In more recent reports, Lei and colleagues[11,14] demonstrated an increasingly larger PIPR with an increase in retinal stimulation area from hemifield to central-field to full-field stimulation,[11,14] indicating summation effects across a single retina. This retinal spatial summation was also demonstrated by Park and McAnany,[27] who found that increasing stimulus area elicited a greater sustained response under high melanopic illuminance conditions. Given that binocular viewing stimulates a greater total retinal area, this should result in a greater response when both retinas are stimulated simultaneously.

To expand our current understanding of spatial summation in the ipRGC pathway, we investigated whether monocular melanopsin-mediated ipRGC signals are summated postchiasmally using a binocular full-field chromatic pupillometry paradigm in visually normal observers. If the melanopsin system is binocularly summative, we hypothesize that there will be an overall greater response amplitude in PIPR when both eyes are stimulated simultaneously under bright blue (short-wavelength) light conditions, but not during bright red (long-wavelength) light stimulation.

METHODS

Participants

All participants were screened clinically, including tests of visual acuity (Early Treatment Diabetic Retinopathy Study [ETDRS] Chart), color vision (Mollon-Reffin Minimal Colour Vision Test), refractive error, ocular motility, slit-lamp examination, and a nondilated fundus exam. Ten visually normal participants were included in the study (4 females, mean age 23 ± 2.6 years, age range 20–29 years). Informed consent was obtained from each participant. The study was approved by the Research Ethics Board at The Hospital for Sick Children, Toronto, Canada. All study protocols adhered to the guidelines of the Declaration of Helsinki.

Experimental Conditions and Procedure

Overview. The entire experiment was repeated three times on separate days to produce three trials per participant. Because of the known dependency of the melanopsin response on circadian rhythms,[25] all recordings were done during the day and each recording session was completed at a similar time for each participant. All three trials were done within a 1-month period of the first testing session, ranging from 1 to 17 days between sessions. Each session took approximately 1.5 hours to complete. All luminance values were converted to melanopic illuminance (melanopic lux) and log quanta (log$_{10}$

photons/cm^2/s) using the Irradiance Toolbox proposed by Lucas et al.[41]

Apparatus and Stimuli. Prior to the experiment, participants were situated in a quiet and dimly lit room for 10 minutes (<1 cd [candela]/m^2 as measured with a Gossen MAVOLUX 5032 C Meter, Nurnberg, Germany, equivalent to <12.33 log quanta). Each participant was fitted with a binocular spectacle frame-mounted eye tracker (Arrington Research, Scottsdale, AZ, USA) that used near-infrared (940 nm) illuminating diodes and small infrared cameras to track pupil size at a sample rate of 60 Hz. Participants were instructed to rest their head on a chin rest and fixate on a central light-emitting diode (LED) fixation target presented in the Ganzfeld stimulator (Espion V5 system with the Color-Dome LED full-field stimulator; Diagnosys LLC, Lowell, MA, USA). The cameras were adjusted so that the iris was centered and occupied approximately 80% of the camera display on the recording interface. A region of interest was set to exclude eyelashes, and the image thresholds were set to produce the most stable pupil tracking. The position of the pupils was monitored in real time to ensure that each participant maintained central fixation for the duration of the recording. During the experiment, the pupillometry recordings were conducted in the dark (0 cd/m^2 as measured with a Gossen MAVOLUX 5032 C Meter, equivalent to 0 log quanta).

In a previous study using the same experimental setup and light sources, measurable PIPR emerged at 3.16 cd/m^2 (13.02 log quanta) with a 1-second stimulus duration, and increased with increasing light intensity until 400 cd/m^2 (15.12 log quanta).[11] We therefore chose a luminance of 0.1 cd/m^2 (for red, i.e., long wavelength: 11.38 log quanta, and for blue, i.e., short wavelength: 11.60 long quanta) as the dim condition because no measurable PIPR is expected. Lei and colleagues[11] also reported no significant difference in monocular PIPR responses following 75, 100, 150, or 200 cd/m^2 (i.e., 14.39, 14.52, 14.69, 14.82 log quanta, respectively) full-field blue stimulations and monocular PIPR responses following 400 cd/m^2 (15.12 log quanta) center-field blue stimulation, suggesting a possible saturation effect. Therefore, we chose a luminance of 60 cd/m^2 (for red, i.e., long wavelength: 14.09 log quanta, and for blue, i.e., short wavelength: 14.29 log quanta) as our bright stimulus to avoid saturation of the melanopsin system under binocular viewing conditions. The light sources used in this study are described in detail in Table 1.

Procedure. Each of the three testing sessions consisted of two experimental blocks based on viewing condition: monocular and binocular. During the first experimental block, monocular responses from the left eye were tested. A closed-loop paradigm was used, in which the eye that was stimulated was also the eye that was recorded, while the nontested fellow eye was occluded with an eye patch. For all conditions, stimulus flashes were 400 ms in duration, which has been reported to be more comfortable for participants and generates more consistent retinal stimulation (less blinking).[11] In order to stabilize pupil size for the subsequent baseline recording period, participants first began with a 1-minute interval of dark adaptation, during which participants were instructed to close their eyes. At 10 seconds post trial onset, participants received one flash trial of dim red light followed by one flash trial of dim blue light in the same recording period. Pupillary responses following each dim flash trial were recorded from 5 seconds prior to light stimulus onset (baseline measure) until 15 seconds after stimulus offset (see Fig. 1a for more information). Past studies have shown this recording period is adequate to capture the full pupillary response trajectory under these stimulus conditions.[11,42] After a 1-minute rest period, the bright flash trials were tested. The bright experimental condition was initiated with 10 seconds of dim amber light.

TABLE 1. Stimuli Characteristics for Light Sources Used

Stimulus	Peak λ, nm	Full Width Half Max	Luminance, cd/m^2	Melanopic Illuminance, α-Opic Lux	Log Quanta, $\log_{10}[1/cm^2/s]$
Dim red, long λ	638	17	0.1	0.00	11.38
Dim blue, short λ	465	32	0.1	1.08	11.60
Dim amber, medium λ	598	17	0.1	0.00	10.83
Bright red, long λ	635	22	60	0.12	14.09
Bright blue, short λ	470	31	60	568.93	14.29

This was used to ensure similar starting retinal adaptation states across all bright trials and to ensure precisely 2 minutes of dark adaptation (where participants were instructed to close their eyes) prior to each bright flash. At 10 seconds after trial onset, participants received a bright red flash. Pupillary responses were recorded from 5 seconds prior to stimulus onset until 40 seconds after stimulus offset. Participants were then given a 1-minute rest, and the bright experimental condition was repeated again with a bright blue light flash, followed by a 40-second recording period post stimulus offset (see Fig. 1b for more information).

During the second experimental block, the entire experimental procedure was repeated binocularly. Between monocular and binocular experimental blocks, a 15-minute break was given in an ambient hospital light environment (50–250 cd/m^2 as measured with a Gossen MAVOLUX 5032 C Meter, equivalent to 13.61–14.31 log quanta) for three reasons: to ensure the melanopsin system had enough time to regenerate fully,[28] to allow the eyes to reach an equal retinal adaptation state following the monocular viewing (i.e., the eye under cover was dark adapting during monocular block), and to maintain wakefulness and task engagement.[43] The exact conditions were repeated in each of the three sessions.

Data Processing and Analysis. Eye tracker data were analyzed offline using a custom-written script (MATLAB; MathWorks, Inc., Natick, MA, USA). To remove blink artifacts, a median (window width of 0.5 second) and low-pass (fourth-order, zero-phase Butterworth) filter with a cutoff frequency of 5 Hz were used, as described by Lei and colleagues.[11] A visual inspection of filtered data was done in a graphical user interface (GUI) in order to detect artifacts and ensure data quality.

To account for individual variability in pupil size, the pupil data were normalized to a 5-second baseline period before each stimulus onset (i.e., normalized pupil size = absolute pupil size/baseline pupil size). Given the well-established consensual response of the pupillary light reflex and the symmetry in pupil responses between the two eyes in normal participants,[15,16,36] we report only the left eye data; there was no difference between left and right eye data for all participants.

Two variables were measured for this experiment. One was maximum pupil constriction (MPC)—the smallest pupil size post stimulus presentation. MPC represents pupillary dynamics driven mainly by rods and cones. MPC was calculated for all stimulus conditions used in this study (dim red, dim blue, bright red, and bright blue). The other was PIPR—the mean pupillary constriction over a 15-second interval (10–25 seconds post light stimulation). PIPR has been validated in the literature as an index of melanopsin photoactivity.[11,14] PIPR was calculated only for the bright blue and red conditions, because dim light conditions have been shown to cause only a transient pupil response that returns to baseline quickly, such that the response is indistinguishable from noise in the poststimulus interval where PIPR is calculated.[11] See melanopic illuminance in Table 1 for more information. The
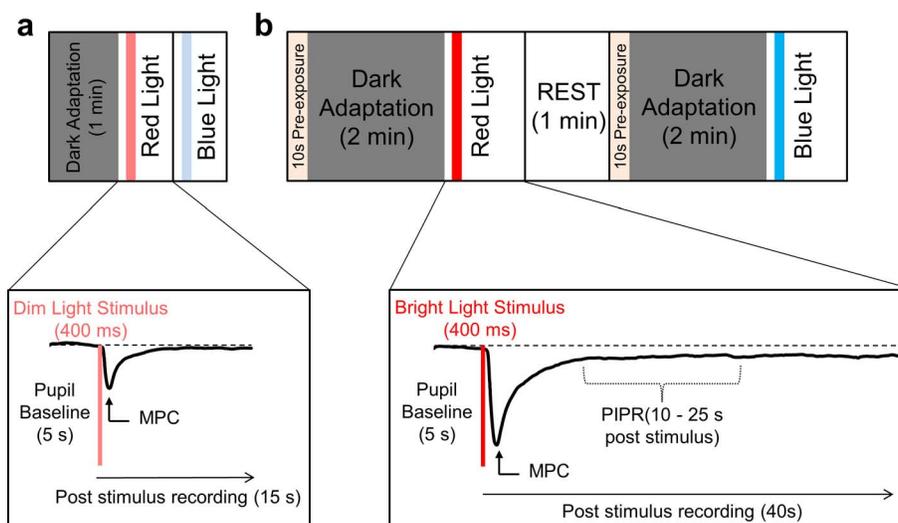

FIGURE 1. (a) Stimulation paradigm for the dim (0.1 cd/m^2) viewing condition block. Participants were dark adapted for 1 minute and pupil responses were recorded 5 seconds before stimulation and 15 seconds following a 400-ms flash of red light. This was repeated with a blue flash of same duration and intensity. (b) Stimulation paradigm for bright (60 cd/m^2) viewing condition block. Subjects first received 10 seconds of dim amber light (0.1 cd/m^2) light as pre-exposure to ensure similar starting points. Subjects were then dark adapted for 2 minutes and pupil responses were recorded for 40 seconds following a 400-ms flash of red light. This was repeated with a blue flash of the same duration and intensity.

TABLE 2. Average Intersession Variability

Pupil Measure	Stimulus Luminance	Red, Long λ		Blue, Short λ	
		Monocular	Binocular	Monocular	Binocular
MPC	Dim	0.019 ± 0.02	0.013 ± 0.009	0.025 ± 0.03	0.017 ± 0.01
	Bright	0.022 ± 0.018	0.017 ± 0.012	0.02 ± 0.018	0.017 ± 0.013
PIPR	Bright	0.02 ± 0.018	0.019 ± 0.013	0.028 ± 0.021	0.029 ± 0.018

intersession variability was calculated using a method similar to that of Koozekanani et al.,[44] and ranged from 0.013 to 0.029 (see Table 2). For the largest difference (i.e., 0.029), the variability is ~7% of the mean pupil measurement.

Statistical analyses of the pupil metrics were computed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA). The dataset contained no outliers, as defined as studentized residuals greater than ±3. The data passed both normality (Shapiro-Wilk test, $P > 0.05$) and equal variance (Levene's test, $P > 0.05$) analyses. Differences in MPC and PIPR were compared using separate 2-way repeated measures ANOVAs with two factors: viewing condition (two levels: monocular and binocular) and stimulus color (two levels: red and blue). In the presence of a significant interaction in the 2-way ANOVA, simple effects were run, and the data were Bonferroni corrected to adjust for multiple comparisons. Accordingly, statistical significance was set at $P < 0.0125$.

RESULTS

Dim Light Stimulation

Figures 2a and 2b show the mean PLRs for 10 visually normal participants (with three trials per participant) recorded during monocular and binocular viewing conditions and in response to dim red and blue light stimulation. The induced PLRs were characterized by a transient constriction phase followed by a rapid return to baseline. Descriptive statistics (mean ± SD) are included in Table 3.

With regard to MPC, overall, responses were significantly greater for binocular than monocular viewing ($F_{(1,9)} = 20.29$, $P = 0.001$, partial $\eta^2 = 0.69$), a mean difference of 0.04 (95% confidence interval [CI]: 0.02–0.06). There was also a main effect of stimulus color, where MPC responses were greater following blue light stimulation ($F_{(1,9)} = 390.52$, $P < 0.001$, $\eta^2 = 0.98$), a mean difference of 0.1 (95% CI: 0.09–0.11). There was no significant interaction between viewing condition and stimulus color ($F_{(1,9)} = 0.029$, $P = 0.87$, $\eta^2 = 0.003$; Fig. 2c).

Bright Light Stimulation

Figures 3a and 3b show the mean PLRs for 10 visually normal participants (with three trials per participant) recorded during monocular and binocular viewing conditions in response to bright red and blue light stimulation. Overall, the induced PLRs following red light stimulation were characterized by a rapid constriction phase followed by a rapid return to near baseline levels. In contrast, blue light stimulation elicited a sustained pupillary constriction during the recording period.

Maximum Pupillary Constriction. As can be seen in Figure 3c, there was a significant interaction between stimulus color and viewing condition for mean MPC values ($F_{(1,9)} = 12.78$, $P = 0.006$, partial $\eta^2 = 0.59$), and thus simple main effects were run and corrected for multiple comparisons. Mean MPC values following red light stimulation were statistically significantly greater under binocular ($\bar{x} = 0.36$, $\sigma = 0.06$) than

monocular ($\bar{x} = 0.33$, $\sigma = 0.06$) viewing conditions ($F_{(1,9)} = 20.13$, $P = 0.002$, partial $\eta^2 = 0.69$), a mean difference of 0.04 (95% CI: 0.02–0.05). The same pattern was found during blue light stimulation—binocular responses ($\bar{x} = 0.57$, $\sigma = 0.05$) were statistically significantly greater than monocular ones ($\bar{x} = 0.48$, $\sigma = 0.06$) ($F_{(1,9)} = 54.80$, $P < 0.001$, partial $\eta^2 = 0.86$), as well, with a mean difference of 0.09 (95% CI: 0.06–0.11).

When comparing MPC values recorded between the two stimulus wavelengths during binocular viewing, a greater MPC was induced following blue ($\bar{x} = 0.57$, $\sigma = 0.05$) than red ($\bar{x} = 0.36$, $\sigma = 0.06$) light stimulation ($F_{(1,9)} = 96.89$, $P < 0.001$, partial $\eta^2 = 0.92$), a mean difference of 0.21 (95% CI: 0.16–0.25). Similarly, during monocular viewing, there was also greater MPC following blue ($\bar{x} = 0.48$, $\sigma = 0.06$) than red ($\bar{x} = 0.33$, $\sigma = 0.06$) light stimulation ($F_{(1,9)} = 75.81$, $P < 0.001$, partial $\eta^2 = 0.89$), a mean difference of 0.16 (95% CI: 0.12–0.20).

Postillumination Pupillary Response. As can be seen in Figure 3d, there was a significant interaction between stimulus color and viewing condition ($F_{(1,9)} = 175.25$, $P < 0.001$, partial $\eta^2 = 0.95$); thus simple main effects were run and corrected for multiple comparisons. Although a trend is evident, mean PIPR responses measured following red light stimulation were not significantly different for binocular ($\bar{x} = 0.07$, $\sigma = 0.02$) compared to monocular ($\bar{x} = 0.04$, $\sigma = 0.03$) viewing conditions ($F_{(1,9)} = 5.49$, $P = 0.04$, partial $\eta^2 = 0.38$), a mean difference of 0.03 (95% CI: 0.001–0.06). Following blue light stimulation, however, binocular ($\bar{x} = 0.40$, $\sigma = 0.08$) responses were significantly greater than monocular ($\bar{x} = 0.27$, $\sigma = 0.08$) responses ($F_{(1,9)} = 79.52$, $P < 0.001$, partial $\eta^2 = 0.90$), a mean difference of 0.13 (95% CI: 0.1–0.2).

The effect of stimulus color was also compared within each viewing condition. Under binocular viewing conditions, there was a significantly greater PIPR following blue light ($\bar{x} = 0.40$, $\sigma = 0.08$) compared to red light ($\bar{x} = 0.07$, $\sigma = 0.02$) stimulation ($F_{(1,9)} = 231.88$, $P < 0.001$, partial $\eta^2 = 0.96$), a mean difference of 0.33 (95% CI: 0.28–0.38). The same pattern was found under monocular viewing conditions, where blue light ($\bar{x} = 0.27$, $\sigma = 0.08$) induced significantly greater PIPR than that following red light stimulation ($\bar{x} = 0.04$, $\sigma = 0.03$) ($F_{(1,9)} = 114.56$, $P < 0.001$, partial $\eta^2 = 0.93$), a mean difference of 0.23 (95% CI: 0.18–0.28).

DISCUSSION

Sustained Melanopsin-Mediated Binocular Summation

Binocular summation of the PLR has been broadly investigated since the beginning of the 20th century in both humans[33–36] and animal models (macaque,[37,40] rats,[45] cats[46,47]). The presence of this phenomenon, however, has not been examined in relation to the melanopsin pathway. Consequently, this study investigated the role of the intrinsic ipRGC pathway in mediating binocular summation of the human PLR using a full-field Ganzfeld stimulation paradigm. Our major
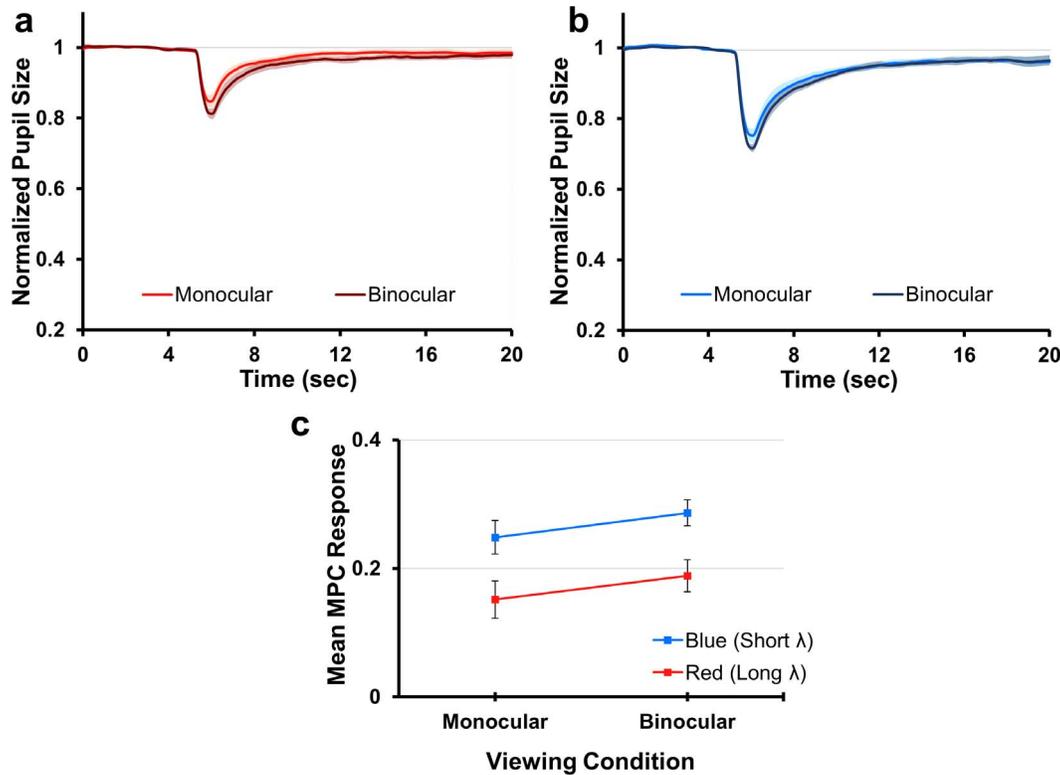
FIGURE 2. Mean normalized pupillary time courses recorded from each eye in response to 400 ms of either (**a**) red light or (**b**) blue light stimulation of 0.1 cd/m^2 under binocular and monocular viewing conditions. Data from 10 visually normal participants are included, with three trials per participant. The interaction between stimulus color (red and blue) and viewing condition (monocular and binocular) for MPC is shown in (**c**), $P = 0.87$. Overall, MPC is greater following blue than following red stimulation ($P < 0.001$) and greater under binocular than monocular viewing conditions ($P = 0.001$). *Error bands* and *error bars* represent 95% confidence intervals.

finding is that compared to full-field monocular stimulation using bright blue (high melanopic lux) light, binocular stimulation induced significantly greater PIPR at equal stimulus duration and intensity (400 ms, 60 cd/m^2). The mean pupillary time courses showed remarkably similar waveforms between the two viewing conditions, consistent with a predominant amplitude shift rather than a change in response dynamics (see Fig. 3b).

Past literature following the discovery of melanopsin has largely investigated direct or consensual pupillary responses as markers of photoreceptor activity.[11–14,17–32] Accordingly, monocular photoreceptor summation of the intrinsic pathway has been found to exhibit the following properties. First, melanopsin-mediated pupillary response amplitude is a function of retinal area stimulated.[11,14] As the stimulation area increases from hemifield to central-field to full-field stimulation, an increasingly larger PIPR is induced.[14] Second, Park and McAnany[27] found that melanopsin-mediated intraretinal responses are well accounted for by corneal flux density (CFD = stimulus luminance × retinal area stimulated), showing that greater PIPR can be induced by increasing retinal area or increasing the stimulus luminance. Consistent with these reports, our results demonstrated that additional retinal recruitment under binocular viewing conditions accounts for the reported amplitude increase in PIPR, a finding consistent with the "photon-counting" properties of the melanopsin system.[9] Similarly, in a recent study, Tsika and colleagues[48] investigated the influence of anterior ischemic optic neuropathy (AION) on intrinsic ipRGC activity. Interestingly, despite diminished monocular pupillary responses to bright blue (high melanopic lux) light relative to controls, no impairment was

evident under binocular viewing conditions. The authors suggest that in this clinical population, summation of intrinsic ipRGC signaling from the two eyes may mediate this effect. However, it is important to consider the methodological paradigm used in their study when interpreting these results. First, the high melanopic lux protocol they used was different from that of the high melanopic lux binocular protocol in terms of intensity, recording time, and number of flashes presented. Second, the PIPR was measured at 6 seconds post stimulus offset, at which point the PIPR may not have reached its full magnitude and residual cone influence may still exist.[11] Third, no direct binocular–monocular comparison was made within each group to quantify the magnitude of summation present. Nevertheless, Tsika and colleagues[48] offer intriguing circumstantial evidence suggesting ipRGC binocular summation takes place within the context of a three-part photoreceptor pathway in mediating pupillary constriction, a finding we now demonstrate explicitly in visually normal observers.

In our study, following bright red (low melanopic lux) light stimulation, a transient response with minimal PIPR was observed in both binocular and monocular viewing conditions. It has been previously shown that 10 seconds post red light illumination, the cone-driven contribution to the PLR is minimal and the pupil size returns to near baseline levels.[11] We have replicated this finding, which serves to further validate that our PIPR measure is representative of predominantly melanopsin-mediated ipRGC activity. Given that the spectral frequency of our red stimulus is at the edge of melanopsin's absorption spectrum,[8,9] red light is not an ideal stimulus to activate melanopsin to induce a sustained response
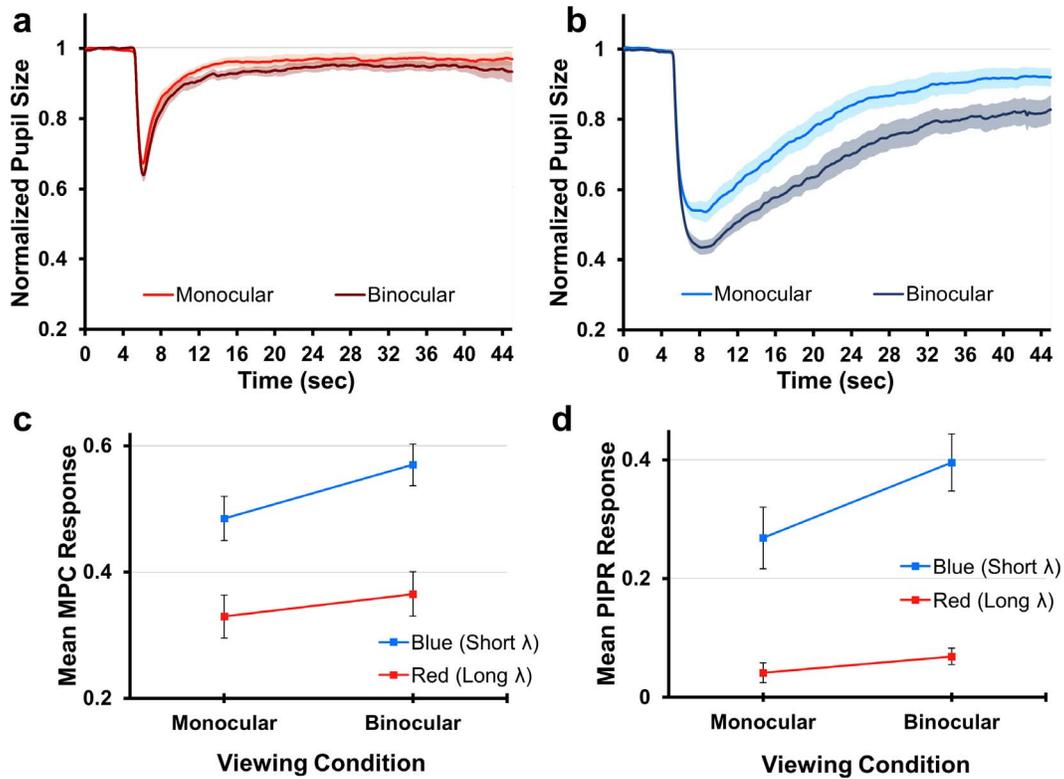
FIGURE 3. Mean normalized pupillary time courses recorded in response to 400 ms of either (a) red light or (b) blue light stimulation at 60 cd/m² intensity under binocular and monocular viewing conditions. Data from 10 visually normal participants are included, with three trials per participant. The interaction between stimulus color and viewing condition is shown in (c) for MPC ($P = 0.006$) and (d) for PIPR ($P < 0.001$). Simple main effects were run and Bonferroni corrected for multiple comparisons (significance was set at $P < 0.0125$). MPC and PIPR are greater following blue than red light stimulation under binocular (MPC: $P < 0.001$, PIPR: $P < 0.001$) and monocular (MPC: $P < 0.001$, PIPR: $P < 0.001$) viewing conditions. MPC and PIPR both show binocular summation following blue light stimulation (MPC: $P < 0.001$, PIPR: $P < 0.001$), but only MPC shows significant binocular summation following red light stimulation (MPC: $P = 0.002$, PIPR: $P = 0.04$). *Error bands* and *error bars* represent 95% confidence intervals.

(see Table 1, melanopic illuminance). The red light is essentially a control condition in our experiment.

To further examine the melanopsin-mediated pupillary responses between the two viewing conditions (monocular versus binocular), we adopted a mathematical model based on forward shunting inhibition, previously used to compare binocular pupillary summation in human and nonhuman primates.[36,37,49] The forward shunting inhibition model describes pupillary responses following binocular stimulation as the combination of retinal ganglion outputs from each eye as well as their influence on one another. This interaction can be expressed by the following equation:

$$z = \frac{x}{1 + k_1 y} + \frac{y}{1 + k_2 x}$$

In this equation, x and y represent individual retinal outputs, z represents the resultant pupillary constriction, and k_1 and k_2 are constants representing the amounts of forward shunting inhibition. When one eye is occluded (i.e., zero retinal input), z reduces to x or y. Once these variables are determined, they can be used subsequently to estimate k_1 and k_2 under binocular conditions. Since our eye tracking system records pupil size in screen coordinates that are then normalized to a baseline period (i.e., normalized pupil size = absolute pupil size/baseline pupil size), we converted our data into estimated absolute pupil size (mm) to compare with existing literature. To do so, individual baseline pupil measurements were used to convert normalized values back

to screen coordinates. Measurements from an image of a ruler on the eyelid were then used to convert the screen coordinates to millimeters. Previous studies using this quantitative approach have estimated k_1 and k_2 as 0.2 in humans[36] and in the range of 0.15 to 0.2 in rhesus monkeys.[37] Albeit with different experimental design and stimulation paradigms, we found comparable k_1 and k_2 values in our study following bright blue (high melanopic lux) light stimulation ($k_1 = k_2 = 0.18$, 95% CI: 0.10–0.27, see Table 4). This suggests that binocular summation of melanopsin-mediated ipRGC signals is more complex than simple addition of monocular information. In contrast, following bright red (low melanopic lux) light stimulation, which induced minimal PIPR for both monocular and binocular viewing conditions, the k_1 and k_2 values show little summation ($k_1 = k_2 = 0.97$, 95% CI: 0.63–1.3).

A primary projection target of the ipRGC pathway is the olivary pretectal nucleus (OPN),[3,9,50] which serves as the first point of binocular integration[51] in the circuit mediating the PLR.[45,52–54] Luminance neurons within the OPN are described by tonic firing rates that increase with increasing retinal illuminance[52–54] and correspond with the dynamic range of ipRGCs.[9] In the nonhuman primate pretectum, 84% of OPN luminance neurons respond to simultaneous input from both eyes.[38] Since the OPN is the first convergence area within this circuit, the pretectum is likely where summation takes place; however, it is not clear what proportion of binocular OPN neurons receive direct input from the ipRGCs.[55] It is also worth noting that despite the large population of binocular

TABLE 3. Descriptive Statistics, Mean ± SD, of Results

Pupil Measure	Stimulus Luminance	Red, Long λ		Blue, Short λ	
		Monocular	Binocular	Monocular	Binocular
MPC	Dim	0.15 ± 0.05	0.19 ± 0.04*	0.25 ± 0.04	0.29 ± 0.03*
	Bright	0.33 ± 0.06	0.36 ± 0.06*	0.48 ± 0.06	0.57 ± 0.05†
PIPR	Bright	0.04 ± 0.03	0.07 ± 0.02	0.27 ± 0.08	0.40 ± 0.08†

* Statistically significant difference between monocular and binocular responses following either red (long λ) or blue (short λ) light stimuli, $P < 0.01$.

† Statistically significant difference between monocular and binocular responses following either red (long λ) or blue (short λ) light stimuli, $P < 0.001$.

neurons within the OPN, there is a response bias favoring the contralateral eye.[38] Subsequently, it has been suggested that further convergence at the level of the Edinger-Westphal nucleus is required to mediate equivalent responses in both eyes. We thus speculate that the Edinger-Westphal nucleus may also be involved in the summation and modulation of binocular signals.

Transient Rod/Cone-Mediated Binocular Summation

Prior to the 21st century, rods and cones were regarded as the exclusive photoreceptive cells to mediate the PLR and subsequently, past literature examining the binocular summation properties of the human PLR focused on transient rod/cone-mediated pupillary responses.[33–36] This literature utilized a large variation in methodologies to induce and quantify pupillary constriction; thus it is difficult to know the exact photoreceptor contribution to the investigated response. For example, many of these studies were not optimized to target individual photoreceptors (i.e., used luminance-modulated broad spectrum light stimulation as opposed to specific chromatic stimuli at the peak spectral sensitivities of rods and cones),[33–36] did not record the full dynamic range of the pupillary response (i.e., used photography rather than video-based pupillometry),[33–35] and varied in their pupillary measuring techniques (i.e., used paper scales positioned in the plane of the pupil[33,34]; others reported the raw data in pixel coordinates[37]). Despite these differences, all of these studies reported significant summation of the PLR when both eyes were stimulated. As expected, we also found a statistically significant difference in the transient MPC values between monocular and binocular viewing conditions across all stimulus conditions (dim red, dim blue, bright red, and bright blue). Additionally, rod-mediated responses showed greater binocular summation than cone-mediated ipRGC responses

(i.e., summation of MPC signals was greater during dim blue versus dim red light stimulation) (see Figs. 2a, 2b). In this study, we evaluated summation in the context of the current three-part photoreceptor model, demonstrating that binocular summation is a general mechanism evident in both transient and sustained components of the PLR. Our findings are consistent with those reported in the literature.

Given that all ipRGC signals (rod, cone, and melanopsin origin) pass through in the same subcortical regions (OPN and Edinger-Westphal nuclei), it may be assumed that every component of the pupil response should demonstrate binocular summation to the same degree. Interestingly, however, Clarke et al.[37] showed that transient responses exhibit less summation (i.e., k_1 and k_2 values of 0.3) than sustained responses (i.e., 0.15–0.2, whereby higher k_1 and k_2 values represent less binocular summation). They suggested that this effect is mediated by differences in temporal processing of the visual system,[37] citing a psychophysical study examining the perception of flicker with frequency and phase.[56] It is unclear, however, whether the perceptual and pupillary motor responses can be compared directly, as they are mediated by two different pathways.

In agreement with Clarke et al.,[37] we also found greater values for transient pupillary responses (i.e., dim and bright, red and blue MPCs, mean k_1 and k_2 ranging from 0.23 to 0.57) than for sustained pupillary responses (i.e., bright blue PIPR, mean k_1 and k_2 of 0.18) (see Table 4). In our study, MPC reflects predominantly rod- and cone-mediated activity manifested as transient maximal pupillary constriction upon brief flashes of 400-ms duration (which were shorter than the time it took for the pupil to reach MPC; e.g., the fastest response time was found to be 0.93 second for monocular dim red). In contrast, bright blue-evoked PIPR is driven by sustained ipRGC firing that provides a signal of longer duration to the subcortical integration areas. Our findings suggest that binocular summation may be dependent upon the temporal processing properties (transient versus sustained) of different visual subsystems for different stimuli.

It should be noted that although our study found comparable k_1 and k_2 values for blue-evoked PIPR (i.e., 0.18) with that reported by Clarke et al.[37] for sustained responses (i.e., 0.15–0.2), our estimated values for MPC (i.e., 0.23–0.57) are different from that reported by Clarke et al.[37] for transient responses (i.e., 0.3). This may stem from differences in the stimuli used between the studies—we used a brief flash of red or blue light, while Clarke et al.[37] used a steady-state green light. Although not designed specifically to investigate a binocular summation model in detail, our current study sheds new light on the complexity of binocular summation. Further studies are warranted to investigate this interesting phenomenon.

TABLE 4. Individual k_1 and k_2 for All Experimental Conditions

Pupil Measure	Stimulus	Mean, k_1 and k_2	95% Confidence Interval	
			Lower Bound	Upper Bound
MPC	Dim red	0.57	0.46	0.68
	Dim blue	0.47	0.39	0.52
	Bright red	0.39	0.33	0.44
	Bright blue	0.23	0.19	0.27
PIPR	Bright red	0.97	0.63	1.30
	Bright blue	0.18	0.10	0.27

Implications

The PLR pathway can be described as a sensorimotor servomechanism. It is a feedback loop that uses the irradiance levels in the environment to adjust pupillary diameter to control the level of retinal illumination.[57] Of the three photoreceptive components of the PLR, melanopsin-mediated phototransduction is optimized to continuously code environmentally relevant short-wavelength ambient stimuli (i.e., blue light).[58,59] Ham and colleagues[60] previously reported that short-wavelength blue light has a greater potential to cause acute photic damage to the retina in rhesus monkeys than long wavelengths. This damage was not simply caused by thermal injury but possibly due to a photochemical mechanism. Given the inverse relationship between energy and wavelength, the ipRGC system may serve to detect stimuli that may be potentially damaging to the eyes.[60] In fact, macular pigment (composed of isomeric carotenoids that filter blue light[61]) has been shown to attenuate photophobia, a phenomenon of perceptual light hypersensitivity[62-64] mediated by the ipRGC pathway.[65,66] Macular pigment has thus been classified as a physiological protective mechanism against acute light-induced retinal damage.[62,64] Pupillary constriction is another photoprotective mechanism, whereby high-irradiance blue stimulations elicit greater and more sustained pupillary constriction than red (low melanopic lux) stimulation, a property that we now extend to the binocular system as well. We[67] recently reported a wavelength-dependent monocular versus binocular difference in perceptual light-induced discomfort in visually normal participants using a chromatically controlled psychophysical paradigm. Using blue (high melanopic lux) light stimulation, we demonstrated significantly greater visual discomfort following binocular than monocular viewing conditions, but no equivalent result following red (low melanopic lux) light stimulation. We now confirm that melanopsin-mediated signals are summated across the two retinas as demonstrated by the increased PIPR response. More importantly, we show that the sustained nature of this summation throughout our experiment is unlikely to arise from the strictly image-forming photoreceptor pathways. Together, these studies suggest that light-induced discomfort in visually normal observers is a perceptually summated phenomenon tied to the melanopsin response. How this applies to clinical conditions remains to be investigated.

In summary, while spatial summation responses have been described within a single retina, we present the first evidence of binocular summation in the melanopsin-mediated ipRGC system in visually healthy human participants. These results have implications for our understanding of the melanopsin pathway beyond the level of the retinal ganglion cells, and this information may be useful in our understanding of photophobia.

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Corrected October 29, 2018: In the second and fourth paragraphs of the Transient Rod/Cone-Mediated Binocular Summation section, the constants κ_1 and κ_2 were changed to k_1 and k_2 to be consistent with the rest of the article.

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