Test–Retest Reliability of Hemifield, Central-Field, and Full-Field Chromatic Pupillometry for Assessing the Function of Melanopsin-Containing Retinal Ganglion Cells

Shaobo Lei,[1] Herbert C. Goltz,[1,2] Manokaraananthan Chandrakumar,[1] and Agnes M. F. Wong[1–3]

[1]Program in Neurosciences and Mental Health, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada
[2]Department of Ophthalmology and Vision Sciences, University of Toronto, Toronto, Ontario, Canada
[3]Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada

Correspondence: Agnes M.F. Wong, Department of Ophthalmology and Vision Sciences, The Hospital for Sick Children, 555 University Avenue, Toronto, ON, Canada M5G 1X8; agnes.wong@sickkids.ca.

Submitted: October 27, 2014
Accepted: January 12, 2015

Citation: Lei S, Goltz HC, Chandrakumar M, Wong AMF. Test–retest reliability of hemifield, central-field, and full-field chromatic pupillometry for assessing the function of melanopsin-containing retinal ganglion cells. *Invest Ophthalmol Vis Sci*. 2015;56:1267–1273. DOI:10.1167/iovs.14-15945

Purpose. We evaluated the test–retest reliability of current methods of inducing the melanopsin-driven postillumination pupil response (PIPR) under hemifield, central-field, and full-field stimulation conditions.

Methods. Pupil response was recorded with an eye tracker in 10 visually normal participants. Light stimuli were presented using a Ganzfeld screen with a custom-built device that allows specific regions of the retina to be stimulated. Blue light stimulation at 400 cd/m^2 intensity was presented for 400 ms to the lower and upper halves of the central 30° fields (hemifields), central 30° field (central-field), and full-field to induce PIPR. Red light full-field stimulation also was presented with the same intensity and duration as a control condition. Test–retest reliability of the PIPR measures was assessed by calculating the intraclass correlation coefficient (ICC) of six repetitions for lower and upper hemifield stimulation, and three repetitions for central-field and full-field stimulation.

Results. Hemifield, central-field, and full-field blue light stimulation induced increasingly greater PIPR in ascending order, while full-field red light stimulation induced no PIPR. Mean lower and upper hemifield PIPR was highly symmetric. Mean ICC of blue light PIPR was 0.87 for lower hemifield, 0.88 for upper hemifield, 0.95 for central-field, and 0.94 for full-field stimulation.

Conclusions. We validated a new and repeatable method to measure PIPR induced by hemifield, central-field, and full-field light stimulation. Good PIPR measurement reliability was obtained under all conditions. This practical and reliable protocol will facilitate the clinical application of PIPR testing in different disease populations.

Keywords: pupil light reflex, pupillometry, test–retest repeatability, melanopsin, retinal ganglion cells

The melanopsin-containing intrinsically photosensitive retinal ganglion cells (ipRGCs) are a specialized subset of photoreceptor cells in the retina.[1] Melanopsin is an opsin-based photopigment that allows ipRGCs to absorb light energy directly and initiate the process of phototransduction,[2] such that the ipRGCs are capable of generating and discharging depolarizing action potentials in response to light exposure with or without synaptic input from rods or cones.[3–6] The ipRGCs give rise to a non–image-forming visual pathway that detects environmental light irradiance independent of conscious perception, and provide afferent signals for the pupillary light reflex and circadian rhythm photoentrainment.[4,7–9] The ipRGC's photoactivity can be assessed in vivo using the chromatic pupillometry technique. While rapid-phase pupil constriction following the onset of light stimuli represents the rod/cone-driven extrinsic photo activity, isolated intrinsic photoactivity of ipRGC can be assessed by measuring the sustained pupil constriction after the offset of high intensity blue light stimulation.[10–12] This so-called postillumination pupil response (PIPR) remains largely unchanged after pharmaceutical blockage of synaptic input from the rods and cones, and is believed to be a consequence of melanopsin-driven prolonged firing of ipRGCs.[13]

While measuring PIPR using chromatic pupillometry holds promise as an objective means of assessing melanopsin-containing retinal ganglion cell function independent of photoreceptors,[12,14] consensus has not yet been reached on a standardized clinical protocol. Originally, PIPR was induced with prolonged exposure to high intensity short wavelength light (duration ranging from a few seconds to a few minutes),[10,13–18] which is not practical as a clinical test. Recently, we described a protocol that can induce a large PIPR with a full-field blue light flash of only a few hundred milliseconds, and we found that PIPR is a function of stimulus intensity, duration, and retinal area stimulated.[11] While stimulus intensity and duration can be adjusted easily in most of the previously described chromatic pupillometry systems, adjusting the stimulus area/location has not been investigated to our knowledge. Such a methodology may potentially have important clinical implication in diseases with localized inner retina damage such as glaucoma, branch

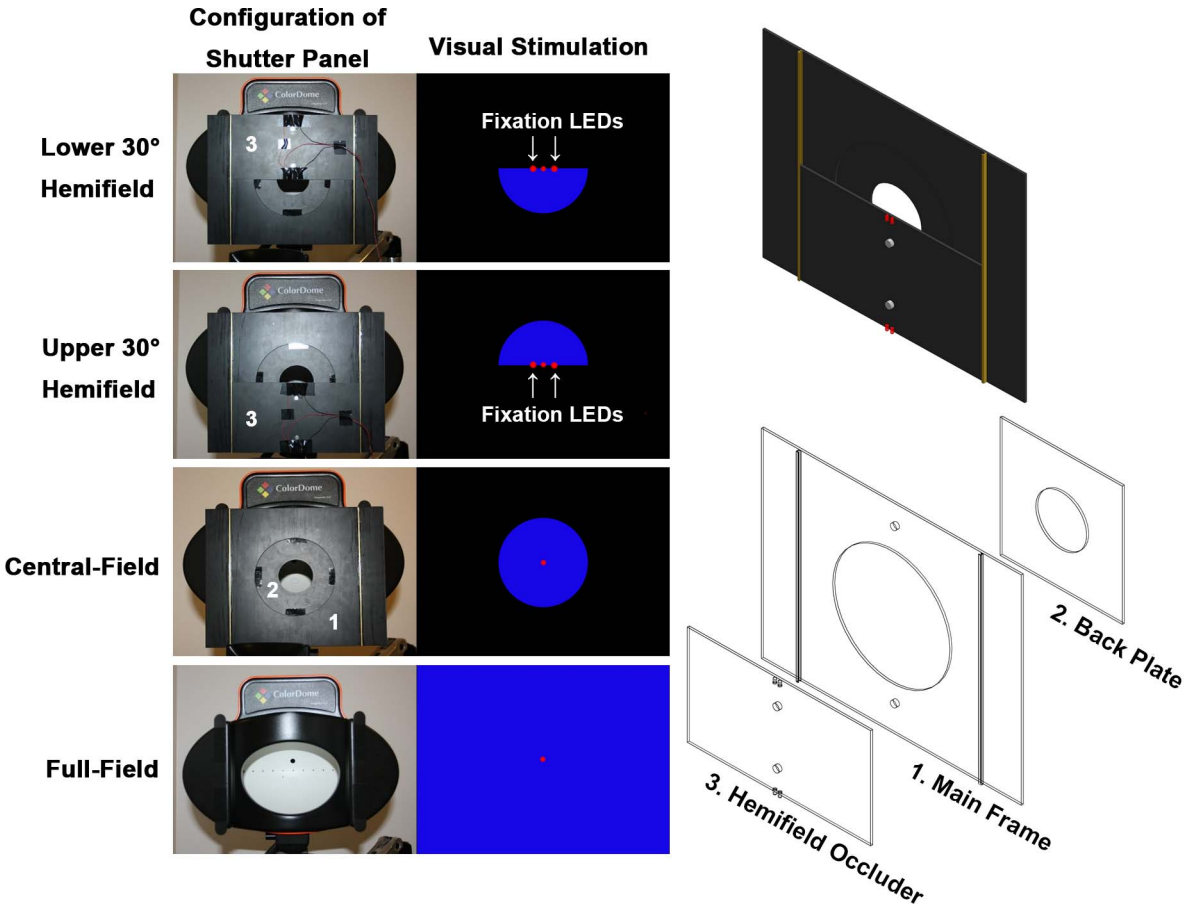

FIGURE 1. The shutter panel device. The shutter panel comprises three components: the main frame, back plate with 50 mm diameter opening, and hemifield occluder with two horizontally-aligned red LEDs on the upper and lower edges. When hemifield stimulation is presented, the shutter panel is attached to the opening of Ganzfeld dome. The participant is instructed to align the center fixation LED in the back of the Ganzfeld dome in the middle between the two horizontally-aligned LEDs on the edge of the hemifield occluder. The illumination of the occluder LEDs is controlled by a toggle switch. For full-field stimulation, the shutter device is removed and the forehead of the subject rests against the edge of the Ganzfeld dome.

retina vein occlusion, maculopathy, and anterior ischemic optic neuropathy, among others. The first goal of this study was to develop a PIPR testing method in which stimulation area/location can be adjusted to present hemifield, central-field, and full-field stimulation. The second goal was to evaluate the test–retest reliability of hemifield, central-field, and full-field PIPR in visually normal subjects.

METHODS

Participants

A total of 10 visually-normal subjects participated in the study (six females, mean age 30 years, age range 19–56 years). The experiments were conducted monocularly, with the right eye being stimulated and recorded. The left eye was patched. The study was approved by the Research Ethics Board at The Hospital for Sick Children. All the procedures adhered to the guidelines of the Declaration of Helsinki. Informed consent was obtained from each participant.

Apparatus

The configuration of our chromatic pupillometry system and the method to present full-field stimulation has been described previously.[11] An additional shutter panel was custom-built to present central-field and hemifield stimuli. The shutter panel has a back plate with a round opening of 50 mm in diameter (the size of the opening can be changed by changing the back plate), which resulted in 30° central field stimulation when positioned 95 mm away from the subject's eye. A removable rectangular hemifield occluder can be inserted in the front of the shutter panel to block the upper or lower half of the 30° central field (Fig. 1). To ensure the hemifield stimulation was aligned accurately, two red mini LED lights (3 mm in diameter) were installed on the upper and lower edge of the hemifield occluder. For example, to stimulate the upper hemifield, the hemifield occluder was positioned at the bottom of the panel to block the lower half of the central opening. Two red LED lights on the upper edge of the hemifield occluder flanked the central fixation red LED light in the Ganzfeld screen. The subject was instructed to align all three lights while maintaining fixation on the central one, thereby ensuring that only half of the 30° central field was stimulated.

Experimental Conditions and Procedure

Before the experiment, subjects were exposed to an indoor hospital environment with ambient lighting levels ranging from 80 to 400 lux for at least 2 hours. During the pupillometry recordings, participants were seated in a darkened room (0 lux) with their head rested on a chinrest. The Ganzfeld was positioned 95 mm away from the participant's eyes (measured from the shutter panel) during the presentation of blue light

Table 1. PIPR Values of Each Test Trial From the Five Stimulation Conditions

	Lower Hemifield	Upper Hemifield	Central-Field	Full-Field Blue	Full-Field Red
Test 1	0.92 ± 0.05	0.90 ± 0.05	0.84 ± 0.08	0.64 ± 0.12	0.98 ± 0.03
Test 2	0.91 ± 0.06	0.88 ± 0.08	0.83 ± 0.10	0.67 ± 0.10	1.00 ± 0.03
Test 3	0.90 ± 0.06	0.92 ± 0.03	0.82 ± 0.10	0.68 ± 0.08	0.98 ± 0.02
Test 4	0.90 ± 0.04	0.90 ± 0.06	N/A	N/A	N/A
Test 5	0.89 ± 0.06	0.90 ± 0.06	N/A	N/A	N/A
Test 6	0.92 ± 0.04	0.90 ± 0.05	N/A	N/A	N/A
ANOVA	$F_{(2.04,\ 18.36)} = 1.15$ $P = 0.339$	$F_{(2.63,\ 23.71)} = 1.07$ $P = 0.375$	$F_{(1.50,\ 13.49)} = 1.06$ $P = 0.355$	$F_{(1.63,\ 14.64)} = 2.26$ $P = 0.146$	$F_{(2.00,\ 17.97)} = 0.96$ $P = 0.402$

(467 ± 17 nm) for lower hemifield, upper hemifield, and 30° central-field stimulation. The order of the three conditions was randomized. The shutter panel then was removed and the subject was repositioned closer to the Ganzfeld screen, with the forehead touching the upper edge of the Ganzfeld screen opening, then full-field red (640 ± 10 nm) and blue stimulation were presented. Experimental trials always were initiated with 10 seconds of dim amber light (590 ± 7 nm, 0.3 cd/m^2) pre-exposure (to ensure precisely 90 seconds of dark adaptation before each light stimulation), followed by 90 seconds of dark adaptation, which then was followed by red or blue light stimulation (400 cd/m^2, 400 ms) in darkness. Pupillary response was recorded in real-time at 60 Hz, starting from 5 seconds before the onset of light stimulation until 35 seconds after its offset. The overhead room lighting (200 lux) was turned on afterwards and the subjects were allowed to take a short break (30 seconds to 2 minutes) before starting another trial to prevent carry-over effects and fatigue. Each condition was repeated three times in experiment session 1. Within one month of the first session, a second session was done during which all subjects were tested with lower and upper hemifield stimulation only, with each condition repeated 3 times in randomized order. All experiments were conducted during the day between 8 AM and 2 PM.

Data Analysis

Data from the eye tracker were analyzed offline using a custom-written script (MatLab; MathWorks, Inc., Natick, MA, USA). Data were filtered and inspected as described previously.[11] The filtered data then were normalized to the baseline pupil size calculated from the mean pupil size during a 5-second period before the onset of each stimulus (i.e., normalized pupil size = absolute pupil size/baseline pupil size). Two parameters were measured: (1) PIPR was the mean pupil size over a 20-second interval from 10 to 30 seconds after the offset of light stimulation. Our previous work found that the cone-driven pupil responses subsided within 10 seconds after the offset of light stimuli, so this measurement is expected to represent "pure" melanopsin activity. (2) Maximal Pupil Constriction (MPC) was the smallest pupil size following light stimulation. This parameter primarily represents rapid phase pupil constriction driven by rods and cones, but may receive melanopsin influence under certain conditions.[19] For both parameters, a smaller value represented greater pupil constriction.

Statistical analyses were performed using SPSS 19.0 (IBM Corporation, Armonk, NY, USA). Differences in mean PIPR and MPC were compared using 1-way repeated measures ANOVAs separately among five different testing conditions: (1) lower hemifield (lower half of the central 30° field) stimulation using blue light (400 ms, 400 cd/m^2), (2) upper hemifield (upper half of the central 30° field) stimulation using blue light (400 ms, 400 cd/m^2), (3) central-field (both halves of the central 30° field) stimulation using blue light (400 ms, 400 cd/m^2), (4) full-field stimulation using blue light (400 ms, 400 cd/m^2), and (5) full-field stimulation using red light (400 ms, 400 cd/m^2). Within each condition, means of each repeated test also were compared using 1-way repeated measures ANOVA. All post hoc pairwise comparisons were adjusted for multiple comparisons using the Bonferroni method. A value of $P < 0.05$ was considered statistically significant.

The hemifield PIPR and MPC measurements of the two recording sessions (session 1, tests 1–3 versus session 2, tests 4–6) were compared using paired sample *t*-tests and Pearson correlation.

The intrasubject coefficient of variation (CV) for PIPR and MPC were calculated for each subject, then the median and range of CV from all 10 subjects were reported for each condition. The CV is analogous to signal-to-noise ratio, and is defined in this context as the ratio of the standard deviation (SD) of the repeated measures of PIPR (or MPC) to the mean changes of pupil diameter: $CV_{(PIPR)}$ = SD/(1 − mean PIPR) and $CV_{(MPC)}$ = SD/(1 − mean MPC). The correlation between repeated tests was reported as the intraclass correlation coefficient (ICC) along with its 95% confidence interval (CI). The ICC assesses measurement reliability by comparing the variability of different measures on the same subject to the total variation across all measures and all subjects.[20–22] The formula for the ICC is: *Var (B) − Var (W)/Var (B) + Var (W),* where *Var (W)* is the pooled variance within subjects, and *Var (B)* is the variance of the measurements between subjects. Three different models of ICC were calculated for PIPR and MPC from all conditions[23]; (1) $ICC_{(1.1)}$ is a 1-way random single measure, that is measures were randomly repeated on each subject with reliability calculated from a single measurement; (2) $ICC_{(1.3)}$ is as above, but with reliability calculated by taking the average of three random measurements; and (3) $ICC_{(1.6)}$ for the hemifield conditions, is the extra three repeated measurements from the second session combined with the first three repeated measurement to calculate $ICC_{(1.6)}$ with reliability calculated by taking the average of six random measurements.

Results

Postillumination Pupil Response (PIPR)

The PIPR values for each test trial and mean pupil responses to the five stimulation conditions are shown in Table 1 and Figure 2, respectively. There were significant differences in mean PIPR among the five conditions ($F_{(1.93,\ 17.32)} = 62.68$, $P < 0.001$). Post hoc analysis revealed no statistically significant difference in PIPR between the mean of upper and lower hemifield stimulation ($P = 0.996$), the waveforms of which were largely overlapping. The hemifield PIPR measures from the two sessions (session 1, tests 1–3 versus session 2, tests 4–6) were highly correlated. There was no statistically significant difference between the hemifield PIPR from session 1 and

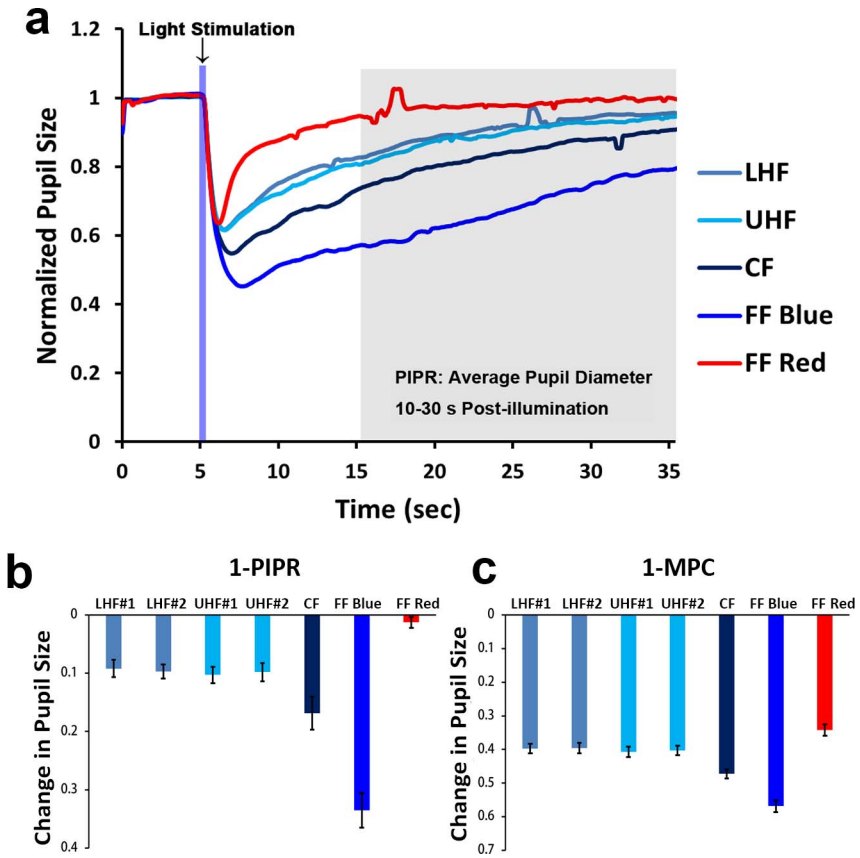

Figure 2. Mean pupil responses from 10 visually-normal subjects. Pupil diameter data were normalized to the mean of 5 seconds of baseline recording before the onset of *blue* or *red* light stimulation (400 ms, 400 cd/m^2). (**a**) Mean pupil response tracings for the five stimulation conditions. (**b**, **c**) Mean PIPR pupil size changes (1-PIPR) and mean MPC pupil size changes (1-MPC). The two sessions of hemifield recording are plotted separately, designated as "#1" and "#2." *Error bars* represent SE. LHF, lower hemifield; UHF, upper hemifield; CF, central-field; and FF, full-field.

session 2. The lower hemifield PIPR from session 1 was 0.91 ± 0.05 (mean ± SD) and 0.90 ± 0.04 from session 2 ($P = 0.55$, Pearson's $r = 0.81$). The upper hemifield PIPR from session 1 was 0.90 ± 0.04 and 0.90 ± 0.05 from session 2 ($P = 0.63$, Pearson's $r = 0.79$). Full-field blue PIPR was significantly greater than that of central-field and hemifield responses ($P < 0.001$); however, the differences between central-field PIPR and upper/lower hemifield PIPRs did not reach statistical significance ($P = 0.069$ and 0.078, respectively). Full-field red stimulation induced significantly smaller PIPR than the blue stimulation across all conditions. All P values of pairwise comparisons of PIPR are summarized in Table 2.

The CV and ICC data for each condition are shown in Table 3. The first three repetitions of PIPR for the upper and lower hemifield stimulations had mean $ICC_{(1.3)}$ of 0.63 and 0.77, respectively. To test if more repetitions would increase the mean ICC, we repeated upper and lower hemifield stimulation three additional times in a second experimental session. After combining six repeated hemifield tests, the $ICC_{(1.6)}$ increased to 0.87 for the lower hemifield and 0.88 for the upper hemifield.

Maximal Pupil Constriction (MPC)

Similar to hemifield PIPR, the hemifield MPC measures from the two sessions also were highly correlated. There was no statistically significant difference between the hemifield MPCs from sessions 1 and 2. The lower hemifield MPC of session 1 was 0.60 ± 0.04 and 0.60 ± 0.05 for session 2 ($P = 0.88$, Pearson's $r = 0.85$); the upper hemifield PIPRs of session 1 was 0.59 ± 0.05 and 0.60 ± 0.04 for session 2 ($P = 0.59$, Pearson's $r = 0.82$).

Table 2. Mean PIPR and P Values for Pairwise Comparisons During the Five Testing Conditions

	Lower Hemifield PIPR = 0.91 ± 0.04	Upper Hemifield PIPR = 0.90 ± 0.04	Central-Field PIPR = 0.83 ± 0.09	Full-Field Blue PIPR = 0.66 ± 0.09
Upper hemifield PIPR = 0.90 ± 0.04	$P = 0.996$			
Central-field PIPR = 0.83 ± 0.09	$P = 0.078$	$P = 0.069$		
Full-field blue PIPR = 0.66 ± 0.09	$P < 0.001$*	$P < 0.001$*	$P < 0.001$*	
Full-field red PIPR = 0.99 ± 0.01	$P = 0.003$*	$P = 0.004$*	$P = 0.004$*	$P < 0.001$*

Mean PIPR ± SD from 10 individual participants' data obtained by averaging six repeated tests from each hemifield condition, and three repetitions for central-field and full-field conditions. P values were obtained by post hoc analysis of 1-way repeated measures ANOVA, adjusted for multiple comparisons by Bonferroni correction.

* Significant P values.

Table 3. Test–Retest Reliability of PIPR Measured During Hemifield, Central-Field, and Full-Field Stimulation

	Lower Hemifield	Upper Hemifield	Central-Field	Full-Field Blue	Full-Field Red
Median CV (range)	0.38 (0.06, 0.62)	0.30 (0.04, 0.57)	0.16 (0.02, 0.27)	0.10 (0.04, 0.23)	0.87 (−809.30, 3.36)
$ICC_{(1.1)}$ (CI 95%)	0.53 (0.14, 0.83)	0.36 (0.01, 0.74)	0.87 (0.69, 0.96)	0.84 (0.60, 0.95)	−0.26 (−0.42, 0.15)
$ICC_{(1.3)}$ (CI 95%)	0.77 (0.32, 0.94)	0.63 (0.02, 0.90)	0.95 (0.87, 0.99)	0.94 (0.83, 0.98)	−0.44 (−1.68, 0.48)
$ICC_{(1.6)}$ (CI 95%)	0.87 (0.69, 0.96)	0.88 (0.71, 0.97)	N/A	N/A	N/A

Intrasubject CV = SD/(1 − mean PIPR). This measure was calculated using six trial repetitions for hemifield conditions and three repetitions for central-field and full-field conditions. The median and range for 10 individual subjects are reported. The $ICC_{(1.1)}$ and $ICC_{(1.3)}$ were calculated from three repetitions; $ICC_{(1.6)}$ for hemifield condition was calculated by combining six repetitions across two sessions.

The MPC values for each test trial are shown in Table 4. There was a significant difference among the five conditions for mean MPC ($F_{[2.12,\ 19.11]} = 68.24$, $P < 0.001$). The differences in mean MPC in all pairwise comparisons were statistically significant except for upper hemifield versus lower hemifield ($P = 0.998$) and lower hemifield versus full-field red stimulation ($P = 0.104$). All P values for the pairwise comparisons of MPC are summarized in Table 5. The CV and ICC data for each condition are shown in Table 6. The first three repetitions of MPC for the lower and upper hemifield stimulations had mean $ICC_{(1.3)}$ of 0.92 and 0.93, respectively. After combining six repeated hemifield tests, the $ICC_{(1.6)}$ increased to 0.95 for the lower hemifield and 0.94 for the upper hemifield.

Discussion

We have designed and constructed a simple device to work with a commercially available Ganzfeld stimulator to induce hemifield, central-field, and full-field PIPR. Our device is easy to install and remove without alteration to the structure or function of the Ganzfeld stimulator itself. The test is well tolerated by the participants. With 400 cd/m^2, 400 ms blue light stimuli, we recorded differentiable PIPR and MPC from 10 visually normal subjects and found that the hemifields, 30° central-field, and full-field stimuli induced increasingly larger PIPR and MPC. These findings are consistent with our previous observation that PIPR is a function of stimulus intensity, duration, and, in particular, retinal area stimulated.[11] As we expected, mean responses of upper and lower hemifield stimulation are highly symmetric, indicating that there is no systematic bias in our hemifield stimulation apparatus nor in our normal subjects.

In this study, we used ICC as our assessment measure of test–retest reliability. The ICC describes how closely a set of repeated measurements resemble each other. It quantifies the direction (±) and the strength of the relation between test–retest scores by estimating their linear relation, yielding a value between +1 and −1.[24] It has been suggested that, as a general rule, a value of over 0.90 is considered high, between 0.80 and 0.90 as moderate, and under 0.80 as low reliability for using an instrument for individual decision-making.[21,22] Herbst et al[10] described a custom-built chromatic pupillometry system using 20 seconds of continuous bright blue light (300 cd/m^2) to induce PIPR, but the area of stimulation was not specified. After analyzing two repeated measurements of PIPR, they reported an ICC of 0.80. In this study, for full-field stimulation, we achieved single measure PIPR reliability of 0.84 and excellent reliability of 0.94 after averaging three measures. Similarly, for 30° central-field stimulation, we obtained single measure PIPR reliability of 0.87 and excellent reliability of 0.95 after averaging three measures. The better reliability we observed may be attributed to the properties of our testing method. First, the duration of the light stimuli we used was substantially shorter (400 ms versus 20 seconds), which minimizes the effect of eye blinking and squinting, resulting in more consistent light exposure between trials. Second, the intensity of the light stimuli we used was higher (400 vs. 300 cd/m^2), which induced a larger and more reliable PIPR.

For hemifield stimulation, however, not only is the PIPR amplitude smaller, the test–retest reliability also is substantially lower than that during central-field and full-field stimulation. Given the fixed intensity and duration of stimuli we used, as well as the smaller area of stimulation, the PIPR amplitude from either hemifield is expected to be smaller than that from central-field or full-field stimulation. The smaller amplitude of pupil constriction during the 10- to 30-second postillumination interval following hemifield stimulation makes it more susceptible to changes induced by loss of mental engagement and other natural fluctuations of pupil contractility,[25–27] which may lead to a reduced signal-to-noise ratio. To test whether using more repeated measures would achieve an acceptable test–retest reliability, we added three more trials for upper and lower hemifield stimulation. The ICC for six averaged measures increased to 0.87 (from 0.63 for three measures) for lower hemifield and to 0.88 (from 0.77 for three measures) for upper hemifield, which is considered moderate reliability and generally is acceptable for clinical use.[21,22] These findings emphasized the need to use the mean of multiple trials as the index when the PIPR values are expected to be small.

Measuring PIPR as an index of menolopsin-driven ipRGC activity is a promising new tool to assess inner retinal function

Table 4. MPC Values of Each Test Trial From the Five Stimulation Conditions

	Lower Hemifield	Upper Hemifield	Central-Field	Full-Field Blue	Full-Field Red
Test 1	0.61 ± 0.04	0.59 ± 0.05	0.53 ± 0.05	0.42 ± 0.06	0.67 ± 0.06
Test 2	0.61 ± 0.05	0.59 ± 0.06	0.54 ± 0.05	0.44 ± 0.06	0.65 ± 0.06
Test 3	0.60 ± 0.05	0.60 ± 0.05	0.52 ± 0.05	0.44 ± 0.07	0.66 ± 0.05
Test 4	0.60 ± 0.06	0.60 ± 0.04	N/A	N/A	N/A
Test 5	0.60 ± 0.05	0.60 ± 0.05	N/A	N/A	N/A
Test 6	0.61 ± 0.06	0.60 ± 0.05	N/A	N/A	N/A
ANOVA	$F_{(2.64,\ 23.75)} = 0.92$, $P = 0.436$	$F_{(2.35,\ 21.11)} = 0.37$, $P = 0.725$	$F_{(1.73,\ 15.57)} = 2.25$, $P = 0.144$	$F_{(1.26,\ 11.30)} = 0.77$, $P = 0.427$	$F_{(1.45,\ 13.07)} = 2.26$, $P = 0.152$

N/A, not applicable.

Table 5. Mean MPC and *P* Values for Pairwise Comparisons During the Five Testing Conditions

	Lower Hemifield MPC = 0.60 ± 0.05	Upper Hemifield MPC = 0.60 ± 0.04	Central-Field MPC = 0.33 ± 0.04	Full-Field Blue MPC = 0.43 ± 0.06
Upper hemifield MPC = 0.60 ± 0.04	$P = 0.998$			
Central-field MPC = 0.33 ± 0.04	$P = 0.001$*	$P = 0.001$*		
Full-field blue MPC = 0.43 ± 0.06	$P < 0.001$*	$P = 0.001$*	$P < 0.001$*	
Full-field red MPC = 0.66 ± 0.05	$P = 0.104$	$P = 0.042$*	$P = 0.001$*	$P < 0.001$*

Mean MPC ± SD from 10 individual participants' data obtained by averaging six repeated tests from individual hemifield conditions and three repetitions for central-field and full-field conditions. The *P* values were obtained by post hoc analysis of 1-way repeated measures ANOVA, adjusted for multiple comparisons by Bonferroni correction.

* Significant *P* values.

Table 6. Test–Retest Reliability of MPC Measured During Hemifield, Central-Field, and Full-Field Stimulation

	Lower Hemifield	Upper Hemifield	Central-Field	Full-Field Blue	Full-Field Red
Median CV (range)	0.06 (0.10, 0.04)	0.05 (0.11, 0.02)	0.05 (0.11, 0.01)	0.02 (0.04, 0.01)	0.04 (0.11, 0.01)
$ICC_{(1.1)}$ (CI 95%)	0.79 (0.51, 0.94)	0.86 (0.66, 0.96)	0.70 (0.38, 0.90)	0.76 (0.46, 0.92)	0.88 (0.70, 0.97)
$ICC_{(1.3)}$ (CI 95%)	0.92 (0.76, 0.98)	0.93 (0.85, 0.97)	0.88 (0.64, 0.97)	0.90 (0.72, 0.97)	0.90 (0.72, 0.97)
$ICC_{(1.6)}$ (CI 95%)	0.95 (0.89, 0.99)	0.94 (0.86, 0.98)	N/A	N/A	N/A

Intrasubject CV = SD/(1 − mean MPC). This measure was calculated using six trial repetitions for hemifield conditions and three repetitions for central-field and full-field conditions. The median and range for 10 individual subjects are reported. The $ICC_{(1.1)}$ and $ICC_{(1.3)}$ were calculated from three repetitions; $ICC_{(1.6)}$ for hemifield condition was calculated by combining six repetitions across two sessions.

independent of conventional photoreceptors (rods and cones). Using predominately central field focal chromatic stimulation, Park et al.,[12] and Kardon et al.[14,28] provided evidence that a clinical chromatic pupillometry protocol could assess differentially the rod and cone-driven rapid phase responses and melanopsin-driven steady state and postillumination response. The central-field PIPR testing method also has been used by other groups,[12,15–18,29,30] including investigations into diseases, such as retinitis pigmentosa,[28,29] Leber's hereditary optic neuropathy,[12] glaucoma,[15,17] as well as circadian rhythm of ipRGC activity.[30] Our previous work further refined the testing protocol of PIPR using full-field stimulation.[11] We induced consistent PIPR with a full-field stimulus of only a few hundred milliseconds. To our best knowledge, all previous studies regarding melanopsin-driven PIPR testing used either central or full-field stimulation, but within subject comparison of PIPR from different subregions of retina has not been investigated. Although multifocal "perimetry-like" pupillography technique also has been developed,[31,32] however, the induced pupil responses usually are small and transient, it is still unclear whether multifocal pupillography can adequately induce melanopsin-driven response in a consistently detectable manner. The ability of our method to induce and compare full-field, central-field, and hemifield PIPR is of considerable clinical significance. While full-field stimulation can be used to assess PIPR as an index of generalized melanopsin-driven ipRGC function across the whole retina, central-field stimulation is more appropriate in diseases confined to the posterior pole, such as maculopathy.[33] Differential hemifield responses are particularly useful in conditions where retinal ganglion cell damage is topographically asymmetric, for exampls, early glaucoma and anterior ischemic optic neuropathy.[34,35] Analyzing differential PIPR responses from full-field, central-field, and hemifield stimulation also may facilitate the localization of retinal damage and further expand the clinical use of PIPR testing.

In summary, we have described a practical method to induce full-field, central-field, and hemifield PIPR as indices of melanopsin-driven intrinsic ipRGC photoactivity. Full-field and central-field PIPR have good test–retest reliability with either a single measure or an average of multiple measures. For hemifield PIPR, however, the reliability of single measure estimates is low; accordingly, we recommended using the average of multiple (at least six) measures to attain acceptable reliability. Investigators may use this information when interpreting their PIPR test results.

Acknowledgments

Supported by the Canada Foundation for Innovation, John and Melinda Thompson Endowment Fund for Vision Neuroscience, and the Department of Ophthalmology and Vision Sciences at The Hospital for Sick Children.

Disclosure: **S. Lei**, None; **H.C. Goltz**, None; **M. Chandrakumar**, None; **A.M.F. Wong**, None

References

1. Berson DM, Dunn FA, Takao M. Phototransduction by retinal ganglion cells that set the circadian clock. *Science*. 2002;295:1070–1073.
2. Do MTH, Kang SH, Xue T, et al. Photon capture and signalling by melanopsin retinal ganglion cells. *Nature*. 2008;457:281–287.
3. Güler AD, Ecker JL, Lall GS, et al. Melanopsin cells are the principal conduits for rod–cone input to non-image-forming vision. *Nature*. 2008;453:102–105.
4. Hattar S, Liao H-W, Takao M, Berson DM, Yau K-W Melanopsin-containing retinal ganglion cells: architecture, projections, and intrinsic photosensitivity. *Science*. 2002;295:1065–1070.
5. Hattar S, Lucas RJ, Mrosovsky N, et al. Melanopsin and rod–cone photoreceptive systems account for all major accessory visual functions in mice. *Nature*. 2003;424:75–81.
6. Provencio I, Rodriguez IR, Jiang G, Hayes WP, Moreira EF, Rollag MD. A novel human opsin in the inner retina. *J Neurosci*. 2000;20:600–605.
7. Gooley JJ, Lu J, Chou TC, Scammell TE, Saper CB. Melanopsin in cells of origin of the retinohypothalamic tract. *Nature Neurosci*. 2001;4:1165–1165.

8. Hannibal J, Hindersson P, Knudsen SM, Georg B, Fahrenkrug J. The photopigment melanopsin is exclusively present in pituitary adenylate cyclase-activating polypeptide-containing retinal ganglion cells of the retinohypothalamic tract. *J Neurosci*. 2002;22:RC191.
9. Hatori M, Le H, Vollmers C, et al. Inducible ablation of melanopsin-expressing retinal ganglion cells reveals their central role in non-image forming visual responses. *PloS One*. 2008;3:e2451.
10. Herbst K, Sander B, Milea D, Lund-Andersen H, Kawasaki A. Test–retest repeatability of the pupil light response to blue and red light stimuli in normal human eyes using a novel pupillometer. *Front Neurol*. 2011;2:10.
11. Lei S, Goltz HC, Chandrakumar M, Wong AM. Full-field chromatic pupillometry for the assessment of the postillumination pupil response driven by melanopsin-containing retinal ganglion cells. *Invest Ophthalmol Vis Sci*. 2014;55:4496–4503.
12. Park JC, Moura AL, Raza AS, Rhee DW, Kardon RH, Hood DC. Toward a clinical protocol for assessing rod, cone, and melanopsin contributions to the human pupil response. *Invest Ophthalmol Vis Sci*. 2011;52:6624–6635.
13. Gamlin PD, McDougal DH, Pokorny J, Smith VC, Yau K-W, Dacey DM. Human and macaque pupil responses driven by melanopsin-containing retinal ganglion cells. *Vis Res*. 2007;47:946–954.
14. Kardon R, Anderson SC, Damarjian TG, Grace EM, Stone E, Kawasaki A. Chromatic pupil responses: preferential activation of the melanopsin-mediated versus outer photoreceptor-mediated pupil light reflex. *Ophthalmology*. 2009;116:1564–1573.
15. Feigl B, Mattes D, Thomas R, Zele AJ. Intrinsically photosensitive (melanopsin) retinal ganglion cell function in glaucoma. *Invest Ophthalmol Vis Sci*. 2011;52:4362–4367.
16. Kankipati L, Girkin CA, Gamlin PD. Post-illumination pupil response in subjects without ocular disease. *Invest Ophthalmol Vis Sci*. 2010;51:2764–2769.
17. Kankipati L, Girkin CA, Gamlin PD. The post-illumination pupil response is reduced in glaucoma patients. *Invest Ophthalmol Vis Sci*. 2011;52:2287–2292.
18. Nissen C, Sander B, Lund-Andersen H. The effect of pupil size on stimulation of the melanopsin containing retinal ganglion cells, as evaluated by monochromatic pupillometry. *Front Neurol*. 2011;2:92.
19. McDougal DH, Gamlin PD. The influence of intrinsically-photosensitive retinal ganglion cells on the spectral sensitivity and response dynamics of the human pupillary light reflex. *Vision Res*. 2010;50:72–87.
20. Barrett P. Assessing the reliability of rating data. *Retrieved March*. 2001;25:2002.
21. Shrout PE, Fleiss JL. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull*. 1979;86:420.
22. Vincent W, Weir J. *Statistics in Kinesiology*, 4 ed. Champagne, IL: Human Kinetics; 1994.
23. MacLennan RN. Interrater reliability with SPSS for Windows 5.0. *Am Stat*. 1993;47:292–296.
24. Portney L, Watkins M. *Foundations of Clinical Research: Applications to Practice*. New York, NY: Appleton & Lange; 2000.
25. Kahneman D. *Attention and Effort, 1973*. Englewood Cliffs, NJ: Prentice-Hall; 1973.
26. Kahneman D, Beatty J. Pupil diameter and load on memory. *Science*. 1966;154:1583–1585.
27. McLaren JW, Erie JC, Brubaker RF. Computerized analysis of pupillograms in studies of alertness. *Invest Ophthalmol Vis Sci*. 1992;33:671–676.
28. Kardon R, Anderson SC, Damarjian TG, Grace EM, Stone E, Kawasaki A. Chromatic pupillometry in patients with retinitis pigmentosa. *Ophthalmology*. 2011;118:376–381.
29. Kawasaki A, Crippa SV, Kardon R, Leon L, Hamel C. Characterization of pupil responses to blue and red light stimuli in autosomal dominant retinitis pigmentosa due to NR2E3 mutation. *Invest Ophthalmol Vis Sci*. 2012;53:5562–5569.
30. Münch M, Léon L, Crippa SV, Kawasaki A. Circadian and wake-dependent effects on the pupil light reflex in response to narrow-bandwidth light pulses. *Invest Ophthalmol Vis Sci*. 2012;53:4546–4555.
31. Chang DS, Arora KS, Boland MV, Supakontanasan W, Friedman DS. Development and Validation of an Associative Model for the Detection of Glaucoma Using Pupillography. *Am J Ophthalmol*. 2013;156:1285–1296.
32. Maddess T, Ho Y-L, Wong SS, et al. Multifocal pupillographic perimetry with white and colored stimuli. *J Glaucoma*. 2011;20:336–343.
33. Augood CA, Vingerling JR, de Jong PT, et al. Prevalence of age-related maculopathy in older Europeans: the European Eye Study (EUREYE). *Arch Ophthalmol*. 2006;124:529–535.
34. DeLeón-Ortega J, Carroll KE, Arthur SN, Girkin CA. Correlations between retinal nerve fiber layer and visual field in eyes with nonarteritic anterior ischemic optic neuropathy. *A J Ophthalmol*. 2007;143:288–294.
35. Hart WM Jr, Becker B. The onset and evolution of glaucomatous visual field defects. *Ophthalmology*. 1982;89:268–279.

Investigative Ophthalmology & Visual Science