Effect of Stimulus Intensity and Visual Field Location on Rod- and Cone-Mediated Pupil Response to Focal Light Stimuli

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PURPOSE. To assess the effect of stimulus intensity on rod- and cone-mediated pupil light reflex (PLR) to small stimuli presented at central and peripheral visual field (VF) locations.

METHODS. The PLR to small ($0.43°$) chromatic stimuli was tested in the right eye of healthy subjects. Blue (485 ± 20 nm) and red (625 ± 15 nm) stimuli were presented at incremental light intensities ($0.5–3.75$ log cd/m^2) at peripheral ($21.21°$) and central ($4.24°$) VF locations using a chromatic pupilloperimeter under mesopic or blue light adaptation conditions. The percentage of pupil contraction (PPC), maximal pupil contraction velocity (MCV), latency of MCV (LMCV) and the ratio of central to peripheral responses for PPC (Q_{PPC} value) were determined.

RESULTS. Under mesopic light adaptation conditions, the mean PPC recorded in response to red stimuli was lower than blue stimuli in all VF locations and light intensities, and the Q_{PPC} values were higher in response to red compared with blue light stimuli across the light intensity range tested. With blue background light, the pupil responses for red and blue light stimuli were approximately the same in the peripheral VF. LMCV was nearly constant in all VF locations for blue and red stimuli, respectively.

CONCLUSIONS. The chromatic pupilloperimeter enables the assessment of rod- and cone-contribution to the PLR in different VF locations. The optimal light intensities determined here for the assessment of focal activation of the two photoreceptor systems may be used for clinical evaluation of photoreceptor health.

Keywords: rod, cone, pupillary light reflex, chromatic pupilloperimetry, perimetry

The pupil light reflex (PLR) controls the pupil contraction and dilation in response to light. It is predominantly mediated by a subset of intrinsically photosensitive retinal ganglion cells (ipRGCs) that contain the photopigment melanopsin.[1–3] The ipRGCs integrate light information obtained by activation of the intrinsic melanopsin photopigment and extrinsic synaptic inputs received from rod and cone photoreceptors to control the PLR.[4] The different spectral sensitivity, light intensity thresholds, and cell number of the three photoreceptor systems enable the assessment of their relative contributions to the PLR. This has been examined by manipulating the characteristics of PLR in response to large-field ($\geq 45°$) flash red and blue light stimuli under different adaptation conditions.[5–10] These studies have demonstrated that the PLR obtained in response to low-intensity blue light under dark adaptation mainly reflects rod activity, as predicted by the large number of rods in the retina and their spectral sensitivity in the blue-light range. The pupil contraction in response to red light under blue light adaptation is mainly mediated by a cone-driven response, as predicted by the lower number of cones in the retina and the high spectral sensitivity of M- and L-cones to red light.[4] The pupil response to blue light at high intensity under dark adaptation is mediated primarily

from direct, intrinsic activation of ipRGCs, correlating with their peak spectral sensitivity at 482nm and lower sensitivity to light compared to the rods and cones.[11,12]

Since the PLR is objective and noninvasive, measuring the PLR induced by chromatic light may present a significant clinical value. Indeed, these and subsequent studies showed that the PLR for chromatic light may potentially be used for diagnosis and monitoring disease progression as well as determination of treatment efficacy and safety in retinal and optic nerve diseases.[5,7–10,13–16]

Recently, Park et al.[17] characterized the PLR mediated by the different photoreceptor classes when using smaller stimulus sizes ($4–16°$) presented in the center of the visual field (VF) over a large range of light intensities. Chibel et al.[18] provided evidence that the function of rods and cones at different locations in the central ($16.2°$) VF can be assessed using smaller (Goldmann size III, $0.43°$) red and blue light stimuli at 1000 and 200 cd/m^2, respectively, under mesopic (0.05 cd/m^2 uniform white light) adaptation. Thus, healthy participants showed higher percentage of pupil contraction (PPC) and maximal pupil contraction velocity (MCV) in response to red stimuli presented in central versus peripheral VF test points. Retinitis pigmentosa (RP) patients presented diminished PPC and MCV

in response to blue light compared to the control group, in VF areas that were outside the patients' chromatic dark-adapted Goldmann visual field (CDA-GVF) isopters for the blue light. Relatively milder PLR defects were recorded in the patients in response to red light stimuli in VF areas outside their CDA-GVF isopters for the red light. Based on the findings that higher PLR for red light was recorded in central versus peripheral VF test points and that pathology of RP is characterized by loss of rod function that precedes the loss of cone function, these published results suggested that the transient PLR recorded in response to small blue light stimuli was mainly rod-mediated whereas the transient PLR recorded in response to small red light stimuli was mainly cone-mediated. Furthermore, this proof of concept study demonstrated the feasibility of discriminating rod- and cone-mediated PLR in different locations in the central VF between healthy subjects and patients.

The aim of the present study was to examine the effect of stimulus intensity and VF location on rod- and cone-mediated PLR and to determine the optimal conditions for assessing the function of each photoreceptor class in central and peripheral locations of a 24° VF. Specifically, three parameters of the pupil response were examined: PPC, MCV, and the latency of MCV (LMCV). PPC directly reflects the PLR. MCV was chosen for analysis as previous studies demonstrated that RP patients presented with a more pronounced defect in MCV than in PPC.[18] Furthermore, in a study comparing the pupillary response for white light stimuli presented either as a central 10° circular or a peripheral 30–60 degree annular stimulus, Ortube et al.[19] demonstrated that the relative difference of the MCV between the central and peripheral stimuli was significantly lower in diabetic retinopathy patients compared with controls, suggesting that MCV may have a diagnostic potential for retinal pathologies. The LMCV parameter was found to be relatively constant throughout the 16.2° visual field in healthy subjects.[18] High variability in LMCV between different VF locations was observed in RP patients, correlating with vision loss, and ROC analysis demonstrated the usefulness of this parameter for RP diagnosis with high specificity and sensitivity.[18] The present study was aimed at characterization of these three pupil response parameters at different light intensities at central and peripheral targets of a 24° VF for in depth characterization of the rod and cone photoreceptor mediated PLR in healthy subjects and as a model for future clinical testing of retinal and optic nerve pathologies.

METHODS

Participants

The Sheba Medical Center Institutional Review Board (IRB)/Ethics Committee approval was obtained for this trial. The study was conducted according to the tenets of the Declaration of Helsinki and was registered at www.clinicaltrials.gov (Registration No. NCT02014389). Informed written consent was obtained from all participants. For the pupilloperimetry testing in mesopic conditions, 14 healthy volunteers were included, 7 males and 7 females with mean age of 31 ± 6 years; (mean $\pm$ SD), range, 24–43 years. For the pupilloperimetry testing under blue light adaptation conditions, 5 healthy volunteers were included, 4 females and 1 male with mean age of 36 ± 8 years; (mean $\pm$ SD), range, 27–46 years. Inclusion criteria were normal eye examination, best-corrected visual acuity (BCVA) of 20/20, normal color vision, no history of past or present ocular disease, no use of any topical or systemic medications that could adversely influence efferent pupil movements, and normal 24-2 Swedish Interactive

Threshold Algorithm (SITA), developed for the Humphrey standard perimeter (Humphrey Field Analyser II, SITA 24-2; Carl Zeiss Meditec, Inc., Jena, Germany).

Light Stimuli

The light stimuli were presented by a chromatic pupilloperimeter, comprised of a Ganzfeld dome apparatus placed 330 mm from the patient's eye (Fig. 1A). The study was performed in a dim lit room (0.04 cd/m^2). The stimuli were presented in the subject's right eye, and the PLR was recorded from the same eye with the subject's left eye occluded. Participants were asked to fixate on a white light (6 cd/m^2) at the center of the dome (marked with a white arrow in Fig. 1A). For background light generation, a light-emitting diode (LED) was placed inside the dome, above the forehead rest. For mesopic background conditions, a uniform, white background light at intensity of 0.04 cd/m^2 was used. For suppression of rod activity, the test was performed with a blue background light (6 cd/m^2), by covering the LED with a blue filter (e-color+ E079 "Just Blue", transmission 8%; Rosco Laboratories, Stamford, CT, USA). The intensity of the blue background light was chosen based on published work by Park et al.,[7,17] demonstrating efficient suppression of rod-mediated pupil response. Following 2-minute adaptation for either mesopic or blue light background conditions, small chromatic light stimuli (Goldmann size III, 0.43°) were presented from LEDs in eight VF test points within the 30° VF: four central VF test points (4.24 degrees), and four peripheral VF test points (21.21 degrees; Fig. 1B). The PLR was recorded at 10 light intensities, in 8 locations and 2 wavelengths. Subjects were tested in 10 runs. In each run, a single light intensity was tested in the following sequence of VF test targets (Fig. 1B): (A) central-nasal-superior (CNS); (B) peripheral-nasal-superior (PNS); (C) central-temporal-superior (CTS); (D) peripheral-temporal-superior (PTS); (E) central-nasal-inferior (CNI); (F) peripheral-nasal-inferior (PNI); (G) central-temporal-inferior (CTI); and (H) peripheral-temporal-inferior (PTI). The PLR for red light (625 $\pm$ 15 nm) was tested first at each of these VF locations, followed by testing the PLR for blue light (485 $\pm$ 20 nm) at the same light intensity using the same sequence of VF test targets (CNS – PNS – CTS – PTS – CNI – PNI – CTI – PTI). The 10 runs were at the following sequence of light intensities: 0.5, 1, 1.5, 2, 2.5, 2.75, 3, 3.25, 3.5, 3.75 log cd/m^2. There was a 2-minute break between runs. Stimulus duration was 1 second, and the inter-stimulus interval was 4 seconds. Light intensities were determined by measurement with LS-100 luminance meter (Konica Minolta Sensing, Ramsey, NJ, USA).

The stimulus light intensities tested were well below the recommendations of outlined in IEC 62471 on photobiological safety of lamps and lamp systems, and ICNIRP Guidelines on limits of exposure to incoherent visible and infrared radiation.

Pupil Measurement and Analysis

Pupil diameter was recorded in real time by a computerized infrared high-resolution camera (the camera pinhole is marked with a black arrow in Fig. 1A) at a frequency of 30Hz. A custom software was used to analyze the PLR parameters (Accutome, Inc., Malvern, PA, USA).[18] Automatically excluded were tests in which the subject blinked during the first 2.5 seconds (when the light stimulus was on and during the contraction phase of the PLR) and tests in which the pupil diameter was increasing during the first 0.45 seconds following light onset. These test points were retested. Pupil responses were normalized using the mean pupil diameter of the first three measurements taken at 0.03, 0.06, and 0.09 seconds following light onset.
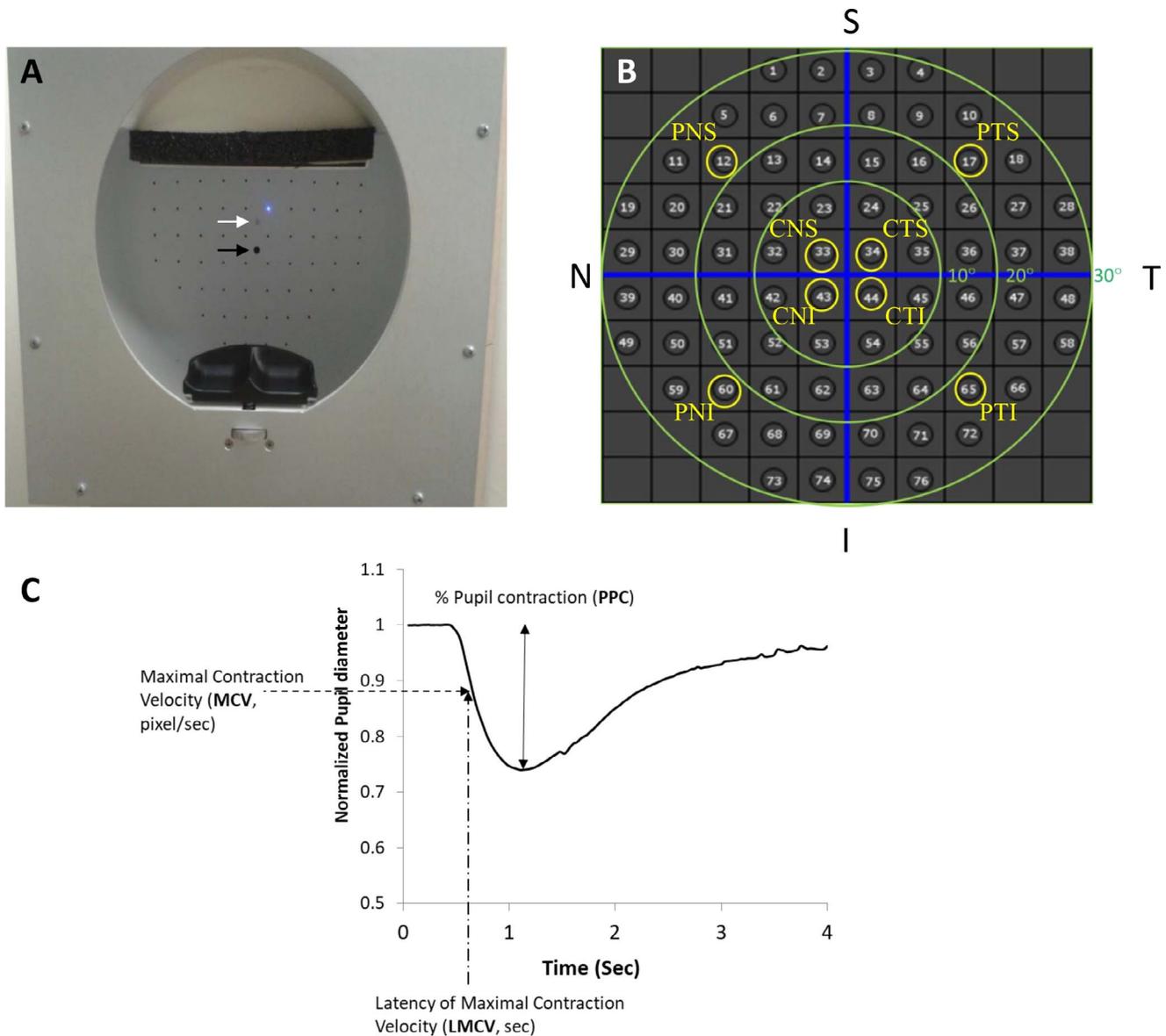
FIGURE 1. The chromatic pupilloperimeter. (A) Front view of the device. The *black arrow* points to the infra-red camera pinhole, the *white arrow* points to the fixator LED. (B) Test point locations of the chromatic pupilloperimeter. The test points used in the study are *highlighted in yellow* and were designated according to their VF location: peripheral-nasal-superior (PNS); peripheral-temporal-superior (PTS); central-nasal-superior (CNS); central-temporal-superior (CTS); central-nasal-inferior (CNI); central-temporal-inferior (CTI); peripheral-nasal-inferior (PNI); peripheral-temporal-inferior (PTI). (C) Pupil response parameters analyzed in this study.

Five parameters were calculated within the software using the change in pupil diameter over time: the initial pupil diameter (pixels); the minimum pupil diameter (pixel); the percentage of pupil contraction (PPC, percent); MCV (pixel/second); LMCV second. The PPC was determined using the following formula, as we previously described:[18]

$$PPC = \frac{Initial\ Pupil\ Diameter - Minimum\ Pupil\ Diameter}{Initial\ Pupil\ Diameter} * 100$$

(1)

The MCV was determined by calculating the maximum rate at which the pupil contracted between the initial pupil diameter measurement and the minimum pupil diameter

measurement. The LMCV was determined by calculating the time point at which the MCV occurred (Fig. 1C).[18]

To determine the variance in initial pupil diameter through the recordings, the differences between the initial pupil diameter measured during testing of the first test target in the first test run (target CNS, in response to red and blue light stimuli at 0.5 log cd/m²) and the initial pupil diameter measured during testing of the first test target in the last test run (target CNS, blue light stimulus at 3.75 log cd/m²) were determined for the 14 subjects tested under mesopic conditions. The mean initial pupil diameter measured in the first target tested in the last run in response to blue light (target CNS, blue light stimulus at 3.75 log cd/m², mean [95% confidence interval]: 6.55 mm [5.97 mm–7.12 mm]) did not

significantly differ from the initial pupil diameter measured in the first run of pupilloperimetry testing (the first test target in response to red light, target CNS, red light stimulus at 0.5 log cd/m^2, mean [95% confidence interval]: 6.96 mm [6.27 mm–7.63 mm]). The mean difference was (-0.4 mm), the 95% confidence interval was -0.8mm to +0.02mm, paired t-test, $P =$ 0.63. The mean difference between the initial pupil diameter measured in the last run in the first test target in response to blue light (target CNS, blue light stimulus at 3.75 log cd/m^2, mean and 95% confidence interval, as previously indicated), and the initial pupil diameter measured in the first run in response to blue light (target CNS, blue light stimulus at 0.5 log cd/m^2, mean [95% confidence interval]: 6.95 mm [6.38 mm–7.51 mm]) was (−0.4 mm) and the 95% confidence interval was (−0.8 mm to −0.04 mm). This difference was statistically significant (paired t-test, $P = 0.034$). Since only small differences were obtained in pupil baseline size, normalization of PPC values enabled to handle these small differences. In addition, since the light stimuli used in pupilloperimetry testing are small while the pupil size is still large enough, this change in initial pupil size is not predicted to affect the pupil light responses. To assess the relative difference of the pupillary responses between the central and peripheral stimuli, Q values were calculated for PPC recorded in response to blue and red light in different light intensities using the formula that was suggested by Ortube et al.:[19]

$$Qppc = \frac{PPCctr - PPCper}{PPCctr} \quad (2)$$

The PPCctr is the mean PPC recorded in the four central VF targets (CNS, CTS, CNI, CTI) for a given subject in a given light color and intensity; PPCper is the mean PPC recorded in the four peripheral VF targets (PNS, PTS, PNI, PTI) in that subject in response to the same light color and intensity. The PPC recorded in the central VF targets was considered the reference point based on our hypothesis that higher PPC would be recorded in the center of the VF than in peripheral VF locations in response to red light, due to the higher concentration of L-cones in the fovea.

Statistical Analysis

Paired t-test was performed to compare between initial base line sizes at different runs. Wilcoxon Signed Ranks Test was used to compare between Q_{PPC} values for red and blue light in the different light intensities. Analyses were performed with IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY: IBM Corp.

RESULTS

### Pupilloperimetry Testing Under Mesopic Light Adaptation Conditions

The PLRs for red and blue light stimuli over a range of light intensities were recorded in 14 subjects at 4 central and 4 peripheral VF test points (test point locations are highlighted in Fig. 1B) under mesopic background light conditions. Figure 2 shows as an example of the mean normalized PLRs to red (Fig. 2A) and blue (Fig. 2B) light presented in the central nasal superior (CNS) VF test point (the VF test point location is indicated in the insert in Panel F). In both light colors, increasing the light intensity induced a monotonic increase in the PLR (Fig. 2; Supplementary Figs. S1, S2). Both red and blue light induced transient PLRs in all light intensities tested. Pupil diameter remained nearly constant during the first ~500 milliseconds following light onset. Maximal pupil contraction

was obtained within 1–1.5 seconds following light onset, and pupil diameter returned to >90% of baseline diameter within 4 seconds followings stimulus onset (Figs. 2A, 2B). The PLRs for red light at intensities lower than 2.5 log cd/m^2 were weak and the mean PPC recorded was smaller than 10% (Fig. 2A; Supplementary Figs. S1, S2). At light intensities equal to or higher than 1 log cd/m^2 the PPC recorded in response to blue light was significantly larger than the PPC recorded in response to red light presented at a similar light intensity in all VF locations (Fig. 2). For example, in test point CNS, the mean PPC recorded in response to blue light at 1.5 log cd/m^2 was 18.3% ± 9.5 % [mean ± SD, 95% CI: 12.8%–23.8%]. By contrast, the mean PPC in response to red light in the same light intensity was 3-fold lower [6.5% ± 5.2% (95% CI: 3.5%–9.5%); Fig. 2C]. Increasing the red light intensity to 2.5 log cd/m^2 resulted in a substantial pupil contraction with a mean PPC of 14.3% ± 6.9% (95% CI: 10.3%–18.3%). This response was over 2-fold lower than the mean PPC recorded in response to blue light presented at the same light intensity (30.3% ± 8.1, 95% CI: 25.6%–35.0%; Fig. 2D). Maximal PPC in response to red light (27.4 % ± 6.9 %, 95% CI: 23.1% –31.8%) was recorded when the red light was presented at the maximal light intensity used in this study (3.75 log cd/m^2). The blue light stimulus presented at 3.75 log cd/m^2 induced a nearly 1.5 fold higher PPC (40.1% ± 3.9%, 95% CI: 37.6%–42.6%; Fig. 2E). Figure 2F demonstrates a pair of pupil responses to blue and red light with nearly similar PPC, which were obtained using red light at maximal light intensity (3.75 log cd/m^2) and blue light at an 18-fold lower light intensity (2.5 log cd/m^2).

Figure 3 presents the change in the three pupil response parameters measured in representative central (CNS) and peripheral (PNS) targets as a function of log light stimulus intensity. Graphs were constructed for red light using light intensities ≥ 2.5 log cd/m^2 and for blue light using light intensities ≥ 1 log cd/m^2, since at lower light intensities there were hardly any pupil responses (Fig. 2; Supplementary Fig. S2). The mean values of the 14 subjects are presented. The PPC and MCV functions changed almost linearly in these light intensities in both colors (Figs. 3A, 3B). The thick lines connect the mean values and the dashed colored lines are the linear regression lines for these functions. The separation between blue and red lines was 1.27 log units in the central and 1.44 log units in the peripheral VF targets, respectively. Thus, in the center of the VF, the red stimulus needs to be presented at 1.27 log units higher intensity than the blue stimulus to obtain the same PPC; in peripheral VF, the red stimuli needs to be presented at 1.44 log units higher intensity than the blue stimuli to obtain the same PPC. This separation is smaller than the 1.94-log unit rod-mediated separation obtained in full field pupillometry studies under dark adaptation,[7] supporting the contribution of cones to the pupil response for red light under mesopic background conditions. Furthermore, the subjects were asked to report the hue of the stimuli and identified the red light stimuli as red, both in the central and peripheral VF targets.

Similar to the PPC, the MCV functions (Fig. 3; Panel B) increased nearly linearly with increment light intensities, with higher MCV recorded in response to blue than red light. By contrast, the LMCV functions demonstrated nearly no change in LMCV with increased light intensities, ranging from 0.6–0.8 seconds in both colors (Fig. 3; Panel C).

To quantitatively assess the relative difference of the pupillary responses between the central and peripheral stimuli, Q_{PPC} values were calculated in response to blue and red light in light intensities that induced a measurable pupil response for the red light (≥ 2.5 log cd/m^2) as detailed in the "Methods" section. As shown in Figure 4, the Q_{PPC} values in response for red light were higher than the Q_{PPC} values
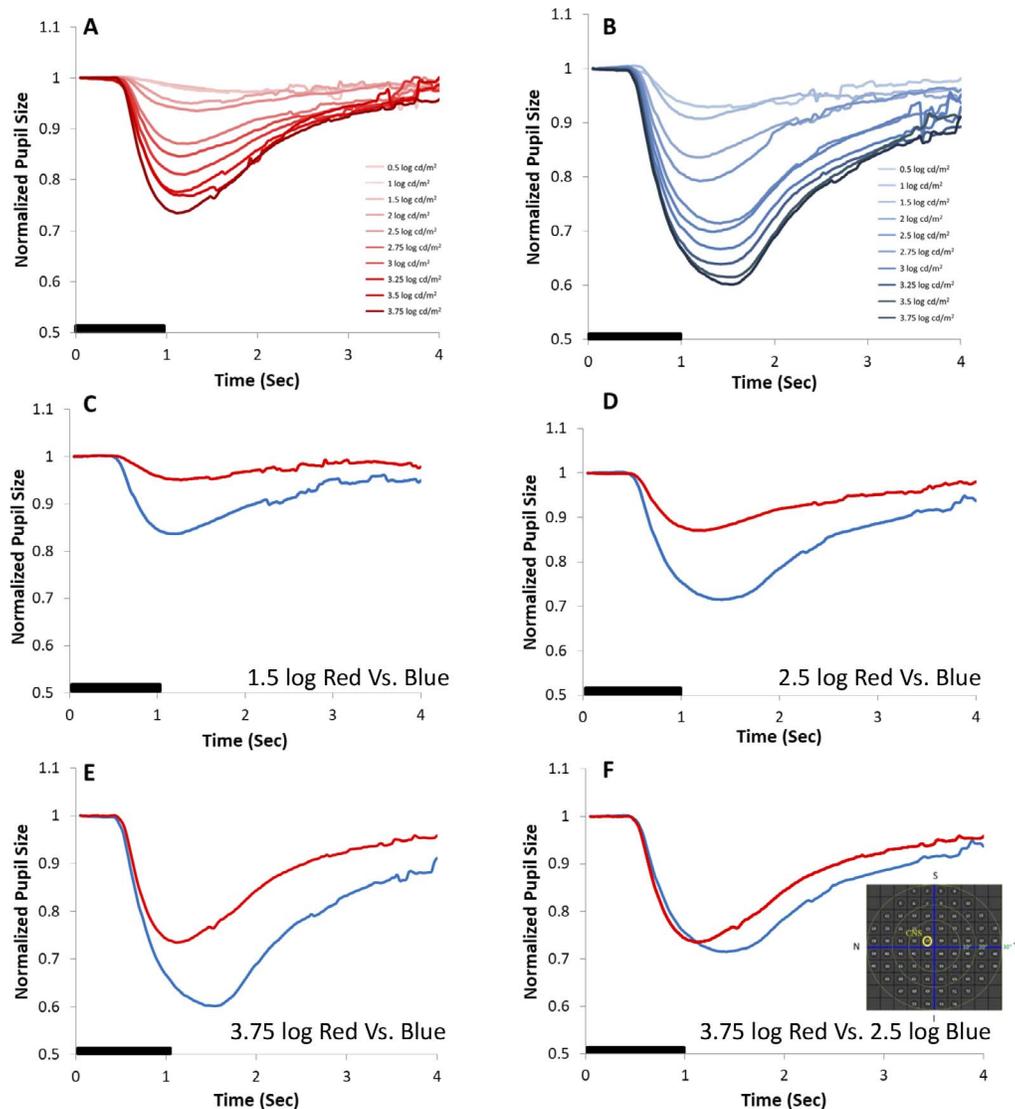
FIGURE 2. Mean pupil responses to increasing light intensities presented at a central visual field test point (CNS) under mesopic background conditions. Mean pupil responses to red (A) and blue (B) light from 14 normal subjects recorded in the central nasal superior visual field test target (CNS) in response to increasing light intensities under mesopic background conditions. (C–E) Pairs of pupil responses, from (A) and (B) to blue and red stimuli presented at (C) 1.5, (D) 2.5, and (E) 3.75 log cd/m^2. (F) Mean pupil responses to 2.5 log cd/m^2 blue versus 3.75 log cd/m^2 red stimuli. The *black bars* denote the 1-sec stimulus presentation. The insert indicates the stimulus location.

obtained in response for blue light in all light intensities. The difference in Q_{PPC} values between the red and blue was larger at light intensities lower than 3.25 log cd/m^2. In nearly all light intensities, the difference in Q_{PPC} values between the red and blue light was statistically significant. Lack of statistical significance in one of the light intensities (2.75 log cd/m^2) may have resulted from the small number of subjects.

Pupilloperimetry Testing Under Blue Light Adaptation Conditions

To further characterize the cone contribution to the PLR in response to focal light stimuli, we tested the pupillary responses on a blue light background in five subjects in the peripheral PNS and central CNS targets. Studies by Park et al.[7,17] demonstrated that under these background light conditions the rod contribution to the PLR is suppressed. In the representative peripheral VF target PNS, the pupil

responses for red and blue light stimuli presented on blue background were approximately the same across the light intensity range tested (Fig. 5; Panels A, C, E, G), suggesting that under blue background conditions the PLR in the peripheral VF for both stimuli is mainly mediated by cones.

In the representative central VF target CNS, the PLRs to blue and red light stimuli were approximately the same on blue background at light intensities ≤ 2 log cd/m^2 (Figs. 5B, 5D). However, at light intensities > 2 log cd/m^2 the PLRs for blue light stimuli were larger than the red stimuli (Figs. 5F, 5H). In addition, we calculated the Q_{PPC} values for the CNS and PNS test targets at light intensities that gave a substantial PLR for red light in the peripheral test target (≥ 2.5 log cd/m^2). The median Q_{PPC} value for blue light was significantly higher than the red light at 3 log cd/m^2 (0.42 vs. −0.18, $P = 0.043$). At 2.5 log cd/m^2, the median Q_{PPC} for blue light was higher than the red light but no statistical significance was obtained, most likely due to the small number of subjects (Supplementary
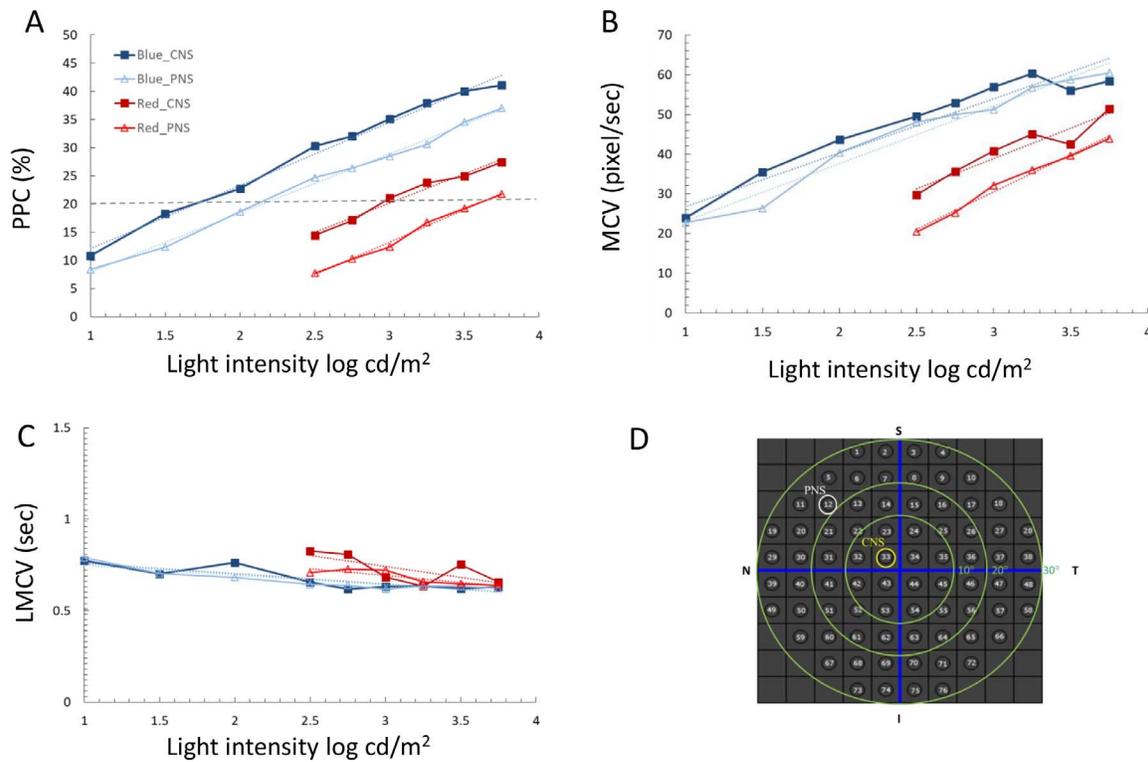
FIGURE 3. Changes in pupil response parameters as function of light color and intensity in central and peripheral VF locations. (**A**) Mean PPC, (**B**) MCV, and (**C**) LMCV recorded in 14 subjects in representative central target (CNS) and peripheral target (PNS) as indicated in panel **D**, in response to blue and red stimuli at increasing light intensities under mesopic background conditions. The *dashed lines* are the best fitting linear regression lines for each graph. The *horizontal gray dashed line* highlights the separation between the PPC functions at 20%.

Table 1). Furthermore, in the CNS test target, the pupil did not fully recover to baseline size after blue light stimulus offset at light intensities > 2 log cd/m^2 (arrows in Figs. 5F, 5H). By contrast, full recovery of pupil size was recorded within 3 seconds of red light offset at all light intensities tested. These findings may suggest a possible small melanopsin contribution to the PLR in response to blue light stimuli presented at the center of the VF at light intensities > 2 log cd/m^2.

DISCUSSION

In this study, we characterized the PLR kinetics to small (Goldmann size III, 0.43°) chromatic light stimuli presented at central and peripheral VF locations with a goal to determine the optimal conditions for assessment of photoreceptor contribution to the PLR at different locations of the VF. Our results suggest that under mesopic conditions, the transient PLR for the focal blue stimuli presented at low light intensity
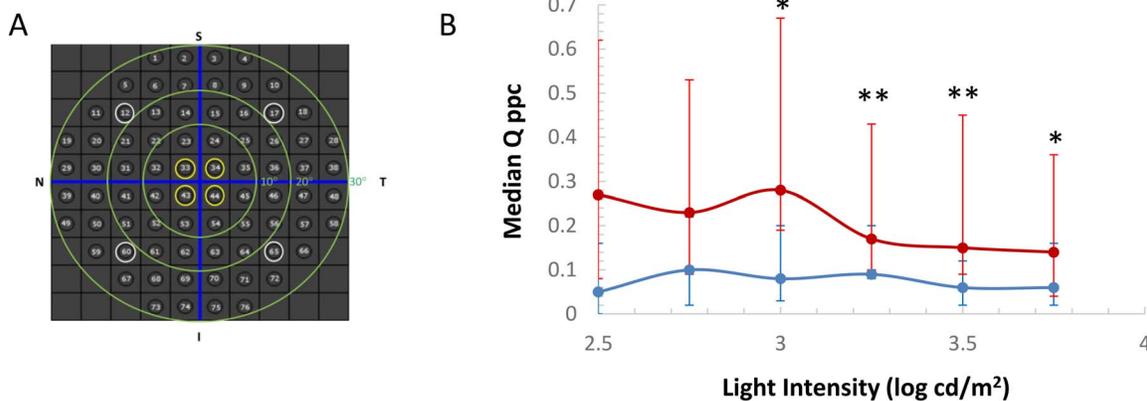
FIGURE 4. The difference in Q$_{PPC}$ values between red and blue light at increasing light intensities under mesopic background conditions. Q$_{PPC}$ values were calculated based on the PPC recorded in response to blue and red light at increasing light intensities in four central and four peripheral test targets highlighted in panel **A**, as indicated in the "Methods" section. (**B**) Wilcoxon Signed Ranks test was used to compare between the Q$_{PPC}$ values obtained in response to blue and red light in similar light intensities in the fourteen subjects under mesopic background conditions. $^*P < 0.05$; $^{**}P < 0.01$. Lower and upper whiskers represent the 25th and 75th percentiles, respectively.
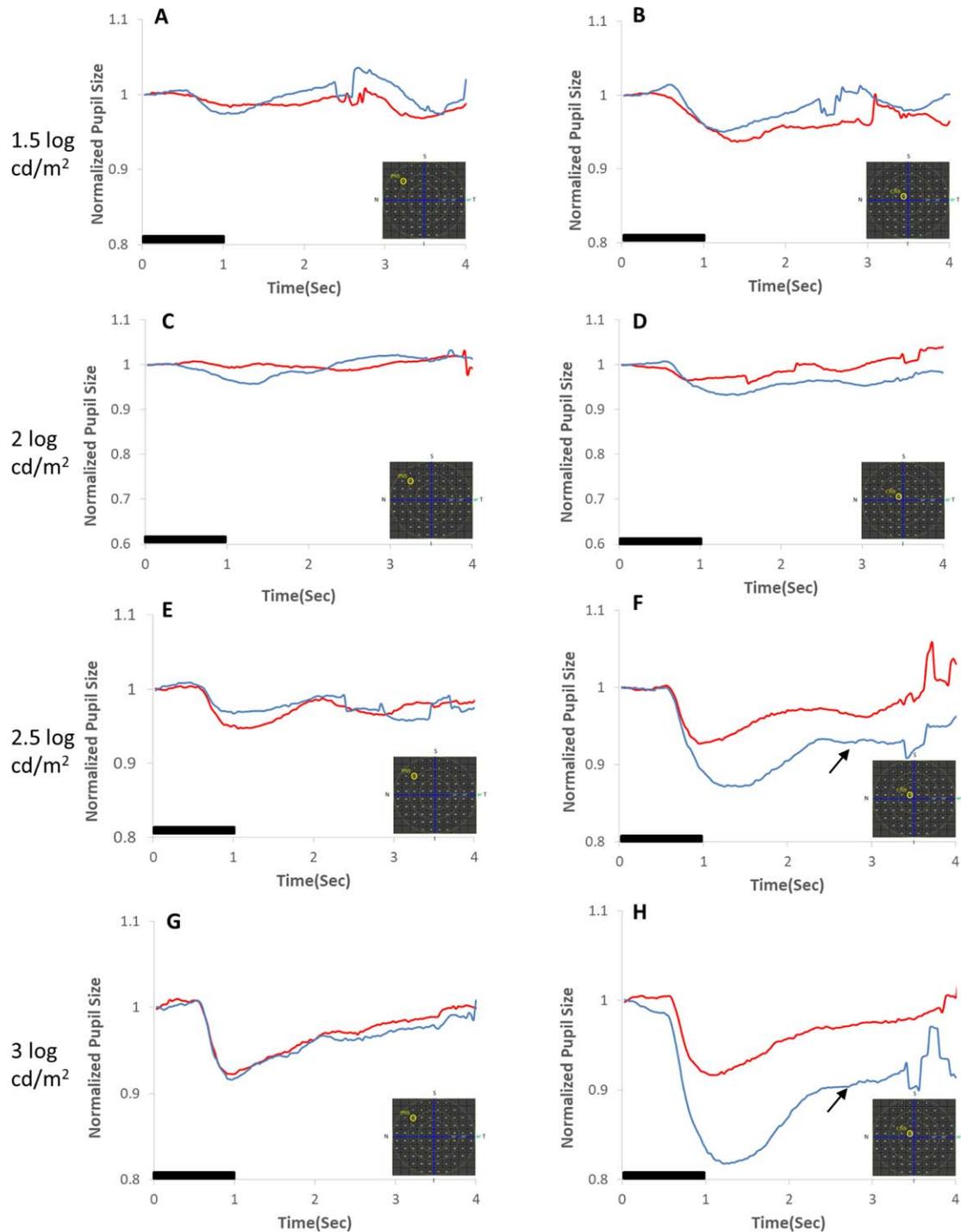
FIGURE 5. Mean pupil responses to increasing light intensities presented at a peripheral (PNS) and a central (CNS) visual field test points on a blue background. Mean pupil responses to red (*red lines*) and blue (*blue lines*) light recorded under blue background conditions in peripheral (**A**, **C**, **E**, **G**) and central (**B**, **D**, **F**, **H**) nasal superior visual field test targets (PNS and CNS, respectively, as indicated in the inserts of each graph) in response to different light intensities, as indicated on the *left*. The *black bars* denote the 1-sec stimulus presentation. The inserts indicate the stimulus location.

(≤ 2 log cd/m^2) are mainly mediated by rods, and the PLR for focal red stimuli are mainly driven by rods with a considerable contribution of cones. These conclusions are based on our data demonstrating that (1) the PPC for blue light was larger than the PPC for red light in all VF test points and across the luminance range tested; (2) substantial transient pupil contraction (PPC $\geq 10\%$) was induced using blue light at lower

light intensity than red light; (3) the separation of the PPC versus intensity linear functions between red and blue was substantially lower (1.27 and 1.44 log units, in the center and peripheral VF test points, respectively) than the rod mediated separation of 1.94 log units measured in full field pupillometry studies under dark adaptation conditions[7]; (4) the subjects identified the hue of the red stimulus as red in all VF locations;

(5) the Q_{PPC} values measured in response for the red light were larger than the Q_{PPC} values measured in response for blue light in accordance with the spatial distribution of cones and the "hill of vision" observed in visual perimetry.[18,20,21] The smaller separation of the PPC versus intensity functions between red and blue light in the central (1.27 log units) compared with the peripheral (1.44 log units) VF suggests that the relative contribution of the cones to the PLR under mesopic conditions is larger in the center of the VF than in the periphery. Our findings are supported by previous studies in patients with RP, a disease that predominantly affects the rods, demonstrating a milder defect in PLR for focal red stimuli compared with blue stimuli and nearly normal PLRs for red light stimuli presented at the center of the VF under mesopic conditions in RP patients.[18,22]

By contrast, under blue light background conditions, our results suggest that the cones are the main mediators of the PLR for focal red and blue stimuli. This conclusion is based on the findings that the PLRs for blue and red light stimuli were approximately the same across the luminance range tested in the peripheral VF, and at light intensities ≤ 2 log cd/m^2 in the central VF. At higher light intensities, larger PLRs were recorded for the blue light compared with the red light in the center of the VF. These findings are supported by the study of Park et al.[17] who obtained larger PLRs in response to blue light compared to red light using 4° stimulus size presented in the center of the VF under blue background conditions. In addition, the pupil did not fully recover to baseline size after blue light stimulus offset at light intensities > 2 log cd/m^2 in the center of the VF, suggesting a possible small melanopsin contribution to the PLR for high intensity blue stimuli in the central VF under blue background conditions. Park et al.[7] have also demonstrated a small contribution of melanopsin to the PLR for full-field blue light stimuli presented at high light intensity under rod-suppressing blue background conditions. These results are also in accordance with the studies of Joyce et al.,[23] demonstrating that the melanopsin-mediated post-illumination pupil response amplitude to blue light is lower in the peripheral retina than the central retina, as well as studies demonstrating that in the human retina the highest concentration of ipRGCs is at the parafovea and their concentration decreases with increasing eccentricity.[4,24] Furthermore, under blue background conditions, higher Q_{PPC} values were obtained for blue light compared with red light. The blue background light may have activated the S-cones that have an antagonistic effect of on L+M cone and melanopsin inputs to the PLR.[25] The high intensity blue light stimuli presented in the center of the VF on the blue background, may have activated melanopsin, masking the S-cone inhibitory effect and leading to larger PLRs in response to blue light in the central compared to peripheral VF and high Q_{PPC} values. Since the red stimulus light has low melanopsin excitation, the S-cone inhibition of L+M cone mediated PLR for red light under blue background conditions may have reduced the PLR in response to red stimulus leading to low and even negative Q_{PPC} values. These findings are supported by the topology of S-cones in the retina, as the S-cones are highly concentrated in the parafovea and constitute an average of 7%–8% of the cones at > 5 degrees eccentricity.[26,27] Future studies with retinal and optic nerve degeneration patients and using longer duration of inter-stimulus intervals and recording are predicted to shed more light on the relative contributions of melanopsin, S-, L- and M-cones to the PLR for focal chromatic stimuli presented at different VF locations. These include studies with retinitis pigmentosa (rod-cone dystrophy) patients that typically present an initial rod photoreceptor degeneration followed by loss of cones[28]; patients with cone-rod dystrophy that have a major deficit of cones that exceeds that of rods[29]; patients with enhanced s-cone syndrome that lack or have low levels of rods, M- and L-cones and have abnormally high number of S-cones[30–32]; and patients with optic nerve degeneration (glaucoma) who have reduced melanopsin-mediated PLRs.[33,34]

One of the aims of the presented study was to determine the appropriate conditions to test rod and cone function at different VF location, as a step toward development of a clinical protocol for future testing of visual pathway pathologies. Since we plan to test pupil responses to blue and red focal light stimuli at 54 or 76 VF targets (similar to the Humphrey 24-2 or 30-2 protocols, respectively), it would not be feasible to alter the background conditions during the test. Hence, we suggest that under mesopic background condition, pupillo-perimetry testing using focal blue light at 2 log cd/m^2 and red light at 3 log cd/m^2 will enable assessment of the relative contribution of rods and cones, respectively, to the pupil response at different locations of the VF. Blue light stimuli at 2 log cd/m^2 induced substantial and measurable PPC values in central and peripheral VF locations (Figs 2, 3; Supplementary Figs S1, S2), and the pupil response for the blue light stimuli at this intensity was substantially reduced in the peripheral and central VF targets using blue background light (Fig. 5, Panels C, D), suggesting that the rods significantly contribute to the pupil response for focal blue light stimuli at 2 log cd/m^2 under mesopic conditions in both central and peripheral VF locations. The red light at 3 log cd/m^2 induced substantial PPC values in the central VF and lower but measurable PPC values in the peripheral VF (Figs. 2, 3; Supplementary Figs. S1, S2), and the maximal and significant difference in Q_{PPC} values between red and blue light was obtained at this intensity (Fig. 4), suggesting that presenting red stimuli at 3 log cd/m^2 under mesopic background conditions, will enable measurement of PLRs that present a considerable contribution of cones.

In the present study, three PLR parameters were assessed: PPC, MCV, and LMCV. Higher PPC was associated with higher MCV. Both PPC and MCV increased monotonically at increment light intensities for both red and blue stimuli (Figs. 3A, 3B) and were lower in response to red light compared with blue light, suggesting that both parameters may potentially be used for assessment of rod and cone function in pathologies. This will be tested in future clinical trials with patients. At light intensities that induced a substantial pupil contraction ($>$ 10% PPC), a nearly constant LMCV was recorded, with mean LMCV ranging between 0.6–0.8 seconds, regardless of light stimulus color or VF location. The monotonic increase in MCV and PPC in response to increasing light intensities, may reflect an increase in the number of photoreceptors activated by each small stimulus. By contrast, the constant LMCV recorded may suggest a threshold of PLR latency that is not dependent on the number of activated photoreceptors. These findings are in accordance with our previous study in which a relatively constant LMCV was recorded in 16 healthy participants at 76 test points within a 16° VF in response to blue and red light at 2.3 log cd/m^2 and 3 log cd/m^2, respectively.[18]

Our study is limited by the small number of subjects and the testing of young adults only (ages 24–46). In future studies, we will characterize the PLRs of elderly healthy subjects and patients with color blindness, retinal and optic nerve degenerations at different VF locations to compile a database of PLR VF "maps" at different age groups and pathologies.

Taken together, our data suggest that using small (0.43°) blue and red light stimuli at 2 log cd/m^2 and 3 log cd/m^2, respectively, under mesopic adaptation may enable an evaluation of the contribution of rod and cone activation, respectively to the PLR at different locations of the VF.

The test was well tolerated by the subjects. In futures studies, we will use these conditions as a model to assess the different parameters of the PLR in patients with macular,

retinal, and optic nerve degenerations. Such studies may lead to development of objective assessment of VF defects and treatment efficacy.

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