Comparisons of Photopic Negative Responses Elicited by Different Conditions from Glaucomatous Eyes

Authors: Yuji Hara, Shigeki Machida, Satoshi Ebihara, Masahiko Ishizuka, Atushi Tada, Tomoharu Nishimura

Affiliation: Department of Ophthalmology, Dokkyo Medical University Saitama Medical Center, 2-1-50 Minamikoshiagya, Koshigaya, Saitama, 343-8555, JAPAN

Corresponding author: Shigeki Machida, MD, PhD

Department of Ophthalmology, Dokkyo Medical University Saitama Medical Center

2-1-50 Minamikoshiagya, Koshigaya, Saitama, 343-8555, JAPAN

E-mail: machidas@dokkyomed.ac.jp

Tel: +81 48 965 1111

Fax: +81 48 965 1127

Running title: PhNR obtained by different recording conditions in OAG

Figures: 6

Tables: 3

Words: 3,839

Acknowledgements/Disclosures

Funding/Support: This study was supported by JSPS KAKENHI Grant Number 18K09420 (SM). We thank to Dr. Duco Hamasaki for editing the manuscript.

Financial Disclosure: none

Competing Interest: none declared.

ABSTRACT

Purpose: To compare the clinical significance of the photopic negative response (PhNRs) elicited by different stimuli from glaucomatous eyes.

Methods: Eighty-four eyes of 84 patients with open angle glaucoma (OAG) and 40 eyes of 40 normal subjects were studied. Cone electroretinograms (ERGs) were elicited by white stimuli on a white background (W/W) or red stimuli on a blue background (R/B). The luminance of the stimuli was 0.5, 1.0, 2.0 or 3.0 cd-s/m², and that of the background light was 10 cd/m². The first and second troughs of the ERGs that appeared following the b-wave were designated as PhNR1 and PhNR2, respectively. The thickness of the circumpapillary retinal nerve fiber layer (cpRNFL) was measured by spectral-domain optical coherence tomography. The mean deviation (MD) was determined by standard automated perimetry. The area under the receiver operating characteristic curves (AUCs) were created to determine the diagnostic ability of the PhNRs elicited by the different stimulus conditions.

Results: The correlation coefficients of the amplitudes of the PhNR1 elicited by W/W stimuli to the MDs and cpRNFL thickness were generally stronger, and the regression lines were steeper than that for the amplitudes of the PhNR1 elicited by R/B stimuli. In contrast, the correlation coefficients of the amplitudes of the PhNR2 elicited by R/B

stimuli to the MDs and cpRNFL thickness were generally stronger, and the regression lines were steeper than the amplitudes of the PhNR2 elicited by W/W stimuli. With both types of stimuli, the slopes of the regression lines became steeper when the ERG recorded with higher stimulus intensities. The AUCs were significantly larger for the PhNR2 elicited by the R/B stimuli at 3.0 cd-s/m² than for PhNR1 and PhNR2 elicited by W/W stimuli at the same intensity when the PhNRs were used for diagnosing advanced glaucoma.

Conclusion: The PhNR1 and PhNR2 elicited by the W/W and R/B stimuli are suitable measures to assess the function of the RGCs in eyes with OAG. The PhNR2 elicited by R/B stimuli at higher stimulus intensities is most effective in detecting functional and structural changes of the RGCs with the highest diagnostic ability in discriminating advanced glaucoma.

Key words: photopic negative response, retinal ganglion cell, glaucoma, cone ERG, cone

INTRODUCTION

Full-field cone electroretinograms (cone ERGs) have been used to evaluate the cone-driven functions in the retina of animals and patients. The photopic negative response (PhNR) of the cone ERGs originates from the activities of the retinal ganglion cells (RGCs) and their axons [1, 2]. Thus, the PhNRs have been used to evaluate the function of the RGCs in patients with glaucoma [3-8], optic nerve diseases [9-16], ischemic retinal diseases [17-19], and surgical insults [20-23].

Viswanathan et al used monochromatic red stimuli on a monochromatic blue background (R/B) produced by light-emitting diodes (LEDs) to elicit the PhNRs. They found that the PhNRs originated from the spiking RGCs and their axons in monkeys [1, 2]. Rangaswamy et al demonstrated that the pharmacologically-isolated responses driven by the RGCs were more dominant in the cone ERGs elicited by R/B stimuli than with white stimuli on a white background (W/W) in monkey eyes when low and middle intensities stimuli were used [24]. This suggested that larger PhNRs can be elicited by R/B stimuli than by the W/W in a selected range of stimulus intensities. However, broadband R/B stimuli produced by xenon flashes using color filters has been reported to not bring about this benefit [25].

The International Society for Clinical Electrophysiology and Vision (ISCEV) Standard has recommended that the cone ERGs should be elicited by W/W stimuli [26]. Recently, the ISCEV published an extended protocol for the PhNR in which R/B stimuli were recommended to elicit the PhNRs [27]. There have been at least two studies that compared the clinical significance of the PhNR recorded under different stimulus conditions from normal and diseased eyes [5, 8]. Sustar et al and Barejee et al compared the diagnostic ability of the PhNR elicited by R/B to those elicited by W/W stimuli in discriminating eyes with open-angle glaucoma (OAG) from normal eyes. They found that the PhNRs elicited by R/B stimuli had higher sensitivity and better specificity than the PhNRs elicited by W/W stimuli in discriminating OAG. The amplitudes of the PhNRs elicited by R/B were more strongly correlated with the visual field defects and OCT findings. In both studies, the PhNR amplitudes were measured as the trough after the i-wave [28]. However, in some reports, the PhNR amplitude was measured as the trough before the i-wave when the cone ERGs were elicited by W/W stimuli because the PhNRs were more prominent before the i-wave under this stimulus combination [9, 11, 17, 18, 20]. In addition, the clinical significance of the PhNRs elicited by a single stimulus intensity has been evaluated [5, 8]. These earlier findings indicated that the

techniques of eliciting and measuring the PhNRs can affect the amplitudes of the PhNRs. However, the most effective techniques have not been definitively determined.

Thus, the aims of this study were twofold; first, to compare the clinical significance of the PhNRs elicited by W/W to those elicited by R/B stimuli of different intensities in eyes with OAG; and second, to determine a suitable method for measuring the PhNR elicited by the two stimulus conditions.

METHODS

Patients

Eighty-four eyes of 84 patients with open angle glaucoma (OAG) were studied. The patients were being treated in the Glaucoma Unit of the Dokkyo Medical University Saitama Medical Center, and their ages ranged from 32 to 89 years with a mean ± standard deviation of 73.8 ± 9.28 years. The diagnosis of OAG was based on the presence of a glaucomatous optic disc associated with visual field defects measured by static visual field perimetry. According to the diagnostic criterion for minimal abnormality in the visual field [30], the visual field defect was determined to be glaucomatous when it met one of three criteria: 1, the pattern deviation plot showed a cluster of three or

more non-edge points that had sensitivities lower sensitivity than that in 5% of the normal population (P <0.05) and one of the points had a sensitivity that was lower than 1% of population (P <0.01); 2, the value of the corrected pattern standard deviation was lower than that of 5% of the normal visual field (P<0.05); and 3, the Glaucoma Hemifield Test indicated that the field was outside the normal limits. In all glaucomatous eyes, the intraocular pressure was controlled under 21 mmHg by means of anti-glaucoma eye drops at the time of the ERG recordings. We included eyes with worse visual field defects in patients with bilateral OAG for analysis in this study.

Forty eyes of 40 normal volunteers, ranging in age from 31 to 81 years with a mean of 62.0 ± 13.1 years were also studied.

This research was conducted in accordance with the Institutional Guidelines of Dokkyo Medical University, and the procedures conformed to the tenets of the Declaration of Helsinki. An informed consent was obtained from all subjects after a full explanation of the nature of the experiments.

Recording Cone ERGs

The pupils were dilated to approximately 8 mm in diameter by topical 0.5% tropicamide and 0.5% phenylephrine HCl. The electrical signals were picked-up by a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Laboratories, Iowa City, IA, USA). A chlorided silver electrode was placed on the left ear lobe as the ground electrode.

The stimuli and background lights were presented in a ganzfeld dome, and the stimulus and recording systems of the UTAS Visual Testing System with SunBurst Ganzfeld (LKC Technologies, Inc., Gaithersburg, MD, USA) were used. The cone ERGs were elicited by red stimuli (λ_{max} = 627 nm, half-amplitude bandwidth = 20 nm) on a blue background (λ_{max} = 470 nm, half-amplitude bandwidth = 25 nm). The responses were digitally bandpass filtered from 0.5 to 500 Hz, and 15 to 30 response were averaged with an inter-stimulus interval of 1 second. The cone ERGs were elicited by either R/B or W/W stimuli which were photopically matched by measuring intensities of the stimuli and background lights with a photometer (IL1700, International Light Technologies, Inc. Peabody, MA, USA). The stimulus and background lights were produced by LEDs. The intensity of the stimuli was photopically matched to 0.5, 1.0, 2.0, or 3.0 photopic cd-s/m². The intensity of the background light was photopically matched to 10 photopic cd/m² which is the intensity recommended by the ISCEV extended protocol for the

PhNR [27]. The duration of the stimulus was 2 msec.

The b-wave amplitude was measured from the first trough to the peak of the following positive wave (Figure 1A). The i-wave was defined as the first positive wave after the b-wave [28]. The amplitudes of the PhNR1 and PhNR2 were measured from the baseline to the troughs just before and after the i-wave, respectively. The implicit times of the PhNR1 and PhNR2 were measure from the stimulus light onset to the troughs of each wave recorded with stimulus intensity of 3.0 cd-s/m^2 in normal subjects. Since deflection of the baseline by blinking strongly affects the configuration of the PhNR, we discarded recordings with baseline deflections.

Optical Coherence Tomography (OCT)

The circumpapillary retinal nerve fiber layer (cpRNFL) thickness was measured at 512 × 128 points around the optic nerve head using circular scans of 1.73 mm in radius by spectral-domain OCT (SD-OCT, RS-3000 Advance, Nidek Co. LTD, Gamagori, Aichi, Japan). The averaged cpRNFL thickness was used for the statistical analyses. We only included OCT images with good quality in this study.

168 **Visual field analysis**

169 The Humphrey Visual Field Analyzer (Model 750, Humphrey Instruments, San Leandro,
170 CA, USA) was used for the static visual field analysis. The SITA Standard strategy was
171 applied to program 30-2. The mean deviation (MD) was defined as the mean of the
172 differences between the measured sensitivity and normal values of age-matched
173 controls. The MD represents the diffuse depression of sensitivity of the visual field. From
174 the MD, we classified patients with glaucomatous visual fields into three groups; early
175 (MD > -6 dB, n = 34, -2.42 ± 1.78 dB), moderate (-6 dB ≥ MD ≥ -12 dB, n = 22; -8.40 ±
176 1.76 dB), and advanced (MD < -12 dB, n = 26, -18.5 ± 5.57 dB) defect of the visual field.
177 When the fixation loss rate is higher than 20%, the field examination was determined to
178 be unreliable and excluded from the analysis. In addition, when the false-positive or
179 false-negative error rates exceeded 33%, the visual field was not used for the analysis.
180

181 **Statistical Analyses**

182 Two-way repeated measure ANOVA was used to compare the intensity-response
183 function of the amplitudes of the PhNR elicited by the R/B and the W/W stimuli in
184 normal subjects and patients with glaucoma. In addition, post hoc tests were performed
185 following the ANOVA to determine the statistical significance between paired data at

each stimulus intensity. Pearson's coefficients of correlation were calculated to determine the strength of the correlation between the amplitude of PhNR1 and PhNR2 and the cpRNFL thickness or the MD. Analysis of covariance (ANCOVA) was performed to determine whether the differences in the slopes of the regression lines were significant. The receiver operating characteristic (ROC) curves and the area under the ROC curves (AUC) were used to describe the diagnostic ability of the PhNR measurements. These analyses were performed using Prism 5.1(GraphPad Software Inc, San Diego, CA, USA) and MedCalc® v18.11 (MedCalc Software bvba, Ostend, Belgium). The level of statistical significance was set at $P < 0.05$.

Ten eyes of 10 normal controls were tested by the ERG twice to evaluate the within-subject variability. The coefficient of variation (CV = standard deviation/mean x 100) was calculated for the PhNR amplitudes.

RESULTS

Representative cone ERGs elicited by white-on-white (W/W) and red-on-blue (R/B) stimuli

Representative cone ERGs recorded from a normal subject and a patient with advanced

glaucoma elicited by W/W and R/B stimuli are shown in Figure 1B. In the cone ERGs elicited by the W/W stimuli from the normal subject, the amplitudes of the PhNR1 were larger than that of the PhNR2 for all stimulus intensities. On the other hand, the amplitudes of the PhNR2 elicited by the R/B stimuli were larger than the PhNR1 especially at the higher stimulus intensities.

In the glaucomatous eye, the amplitudes of both PhNR1 and PhNR2 were considerably smaller for both the W/W and R/B stimuli. The peaks of the troughs of the PhNR1 elicited by the R/B and the W/W stimuli were above the baseline.

Intensity-response functions of PhNR1 and PhNR2 elicited by W/W and R/B stimuli in normal and glaucomatous eyes

The amplitudes of the PhNR1 and PhNR2 recorded from normal subjects and glaucoma patients are plotted as a function of the stimulus intensities of the W/W (Figure 2A and B) and R/B stimuli (Figure 2C and D) (A and C = PhNR1; B and D= PhNR2).

With the W/W stimuli, the amplitudes of PhNR1 and PhNR2 increased significantly as the stimulus intensities increased in normal subjects (P <0.0001, Figures 2A and 2B).

On the other hand, the PhNR1 and PhNR2 amplitudes remained unchanged despite the increase of the stimulus intensities in the glaucomatous eyes. As a result, the difference in the PhNR amplitudes between the normal and glaucomatous eyes became more prominent at the higher stimulus intensities.

The amplitudes of PhNR1 elicited by R/B stimuli remained unchanged over all stimulus intensities while the amplitudes of PhNR2 significantly increased with increasing stimulus intensities in normal eyes (P <0.05, Figure 2C and 2D). The implicit times of the i-wave were faster for the R/B than for the W/W (51.2 ± 2.6 vs 53.1 ± 2.6 msec at 3.0 cd-s/m^2). Since the i-wave counteracts the PhNR, the fast i-wave more affects the PhNR1 rather than the PhNR2 amplitude, which probably prevents growth of the R/B-elicited PhNR1 amplitude with increasing stimulus intensities. In the glaucomatous eyes, the PhNR1 and PhNR2 amplitudes remained unchanged despite increasing stimulus intensities.

Correlations between PhNR amplitudes and mean deviations (MD) of visual fields

The correlations between the amplitudes of PhNR1 and the MDs obtained by static visual field perimetry are shown in Figure 3. The amplitudes of the PhNRs elicited by

240 the R/B stimuli are shown in the left column and those elicited by W/W stimuli in the
241 right column. The PhNR1 were elicited by 0.5 (Figure 3A and B), 1.0 (Figure 3C and D),
242 2.0 (Figure 3E and F), and 3.0 cd-s/m^2 (Figure 3G and H). The correlation coefficients
243 and slopes of the regression lines are presented in Table 1.
244
245 The amplitudes of the PhNR1 elicited by both the R/B and W/W stimuli were
246 significantly smaller in eyes with a reduced MD at all intensities (P <0.0005; Figure 3).
247 The correlation coefficients between the amplitudes of the PhNR1 and the MD were
248 generally higher for the PhNR1 elicited by W/W stimuli than those elicited by the R/B
249 stimuli except for stimulus intensity of 2.0 cd-s/m^2 (Table 1).
250
251 The slopes of the regression lines representing the correlation between the PhNR
252 amplitude and MD were compared when the ERG was elicited by different stimulus
253 intensities (Table 1). With an increase of the stimulus intensity, the slopes of the
254 regression lines became steeper for both stimulus conditions (Figure 3, Table 1). For the
255 PhNR1 elicited by R/B stimuli, a significant difference was found in the slopes between
256 0.5 and 3.0 cd-s/m^2 (P <0.05). Although there was no significant difference in the slopes
257 between the R/B and W/W at each stimulus intensity, and the slopes for the amplitudes

of the PhNR1 elicited by the W/W stimuli were always steeper than those for the PhNR2 elicited by the R/B stimuli.

The amplitudes of PhNR2 are plotted against the MDs in Figure 4, and calculations of the relationship showed that there was a significant correlation between these two parameters (Table 1). The correlation coefficients were generally better for the PhNR2 elicited by the R/B stimuli than for the W/W stimuli except for the stimulus intensity of 1.0 cd-s/m^2.

With an increase in the stimulus intensity, the slopes of the regression lines became steeper for the PhNRs elicited by both the R/B and W/W stimuli (Figure 4, Table 1). For the PhNR2 elicited by the R/B stimuli, a significant difference was found in the slopes between 0.5 and 2.0 and 0.5 and 3.0 cd-s/m^2 (P <0.05). Although there was no significant difference in the slopes between the PhNRs elicited by R/B and W/W stimuli, the slopes for the R/B were always steeper than those for the responses elicited by the W/W stimuli. Note that the slopes were 0.82 for the R/B PhNR2 and 0.50 for the W/W PhNR2 at 3.0 cd-s/m^2.

Correlations between amplitudes of PhNR and thickness of cpRNFL

A plot of the amplitudes of PhNR1 as a function of the cpRNFL is shown for the PhNR1 elicited by R/B stimuli in the left column and by the W/W stimuli in the right column of Figure 5. The PhNR1 amplitudes were elicited by 0.5 (Figure 5A and 5B), 1.0 (Figure 5C and D), 2.0 (Figure 5E and F), and 3.0 cd-s/m^2 (Figure 5G and H). The correlation coefficients and slopes of regression lines are presented in Table 2.

The PhNR1 amplitudes elicited by both the R/B and W/W stimulus intensities decreased significantly with a decrease in the cpRNFL thickness (P <0.0005; Figure 5). The correlation coefficients were larger for the W/W-elicited than for the R/B-elicited PhNR1 except for the stimulus intensity of 1.0 cd-s/m^2 (Table 2).

The slopes of the regression lines representing the correlation between the PhNR amplitude and cpRNFL thickness were compared when the ERG was elicited by different stimulus intensities (Table 2). The slopes became steeper with an increase of the stimulus intensities for both stimulating conditions (Figure 5, Table 2). The slopes for the W/W were always steeper than those for the R/B over all stimulus intensities but the difference was not significant.

The amplitudes of the PhNR2 are plotted against the cpRNFL thickness in Figure 6. There was a significant correlation between these two parameters (Table 2). The correlation coefficients were always better for the PhNR2 elicited by the R/B stimuli than with the W/W stimuli. With an increase of the stimulus intensities, the slopes of the regression lines became steeper for both recording conditions (Figure 6, Table 2). Although there was no significant difference in the slopes between the R/B and W/W, the slopes for the PhNR elicited by the R/B stimuli were always steeper than those for the W/W**.** The steepest slope was obtained for the R/B PhNR2 at 3.0 cd-s/m^2, which is nearly twice larger than that at 0.5 cd-s/m^2 (0.28 vs 0.16).

ROC curves

To determine the diagnostic capability of the PhNR1 and PhNR2 elicited by the R/B and W/W stimuli in discriminating OAG from normal eyes, ROC curves were constructed for each stimulus condition. When the AUCs were calculated for all patients, they ranged from 0.78-0.81 for the R/B-elicited PhNR1, 0.78-0.82 for the W/W-elicited PhNR1, 0.77-0.83 for the R/B-elicited PhNR2, and 0.77-0.81 for the W/W-elicited PhNR2. There were no significant differences in the AUCs between the R/B- and W/W-elicited PhNR1 and

312 PhNR2 or between the stimulus intensities.

313

314 We have reported that the R/B PhNR2 of the full-field cone ERGs elicited by stimuli with
315 high intensity and short duration had high sensitivity and specificity to discriminate eyes
316 with advanced glaucoma from normal eyes [6]. Therefore, the AUCs were obtained for
317 eyes with advanced glaucoma and compared between the R/B-elicited and W/W-
318 elicited PhNRs and between the stimulus intensities (Figure 7, Table 3). The highest
319 AUC (0.94) was observed for the R/B-elicited PhNR2 at 3.0 cd-s/m^2 (Figure 7C), which
320 was significantly higher than that for the W/W-elicited PhNR1 (0.87) (Figure 7B), R/B-
321 elicited PhNR1 (0.87) (Figure 7A), and the W/W-elicited PhNR2 (0.86) (Figure 7D) at
322 the same intensity (P <0.05). It was also higher than the AUC for the R/B-elicited
323 PhNR2 (0.84) at 0.5 cd-s/m^2 (P <0.005).

324

325 **Implicit times of PhNRs**

326 The implicit times of PhNR1 elicited by the R/B and W/W at the highest stimulus
327 intensity in normal subjects were 46.1 ± 2.6 and 44.2 ± 2.4 msec, respectively. The
328 corresponding values for the PhNR2 elicited by the R/B and W/W were 67.4 ± 3.4 and
329 66.6 ± 4.4 msec, respectively. Thus, the implicit times of PhNR2 fell in a range from 65

to 75 msec that has been reported in ISCEV extended protocol [27].

Intersession reproducibility

The CVs for the PhNR1 amplitude in normal controls were 15.1 ± 3.9 for R/B and 9.9 ± 5.8 % for W/W. The corresponding values for the PhNR2 amplitude were 16.8 ± 6.9 for R/B and 13.9 ± 11.8 for W/W, respectively. No significant difference was found between the PhNR1 and PhNR2 amplitudes or between R/B and W/W. These values are compatible with those in previous our report [4].

DISCUSSION

The reduction of the PhNR amplitudes elicited by both W/W and R/B stimuli was correlated with the decrease in the MD of the visual fields and reduction in the cpRNFL thickness. The correlations between the PhNR1 and the MDs and cpRNFL were higher for the PhNRs elicited by W/W stimuli than by the R/B stimuli. In contrast, the correlation of the amplitude of the PhNR2 to the MDs and cpRNFL thickness was higher for the PhNRs elicited by the R/B stimuli. This suggested that the W/W stimuli may be more suitable for assessing the PhNR1 and the R/B stimuli for the PhNR2 responses. The higher intensities of the stimuli used for the ERG recordings led to steeper the slopes of

348 the regression lines for both recording conditions indicating that high intensity stimuli
349 may be better for eliciting and evaluating the PhNRs. The best diagnostic ability in
350 discriminating advanced glaucoma from normal eyes was observed for the R/B-elicited
351 PhNR2 at the highest stimulus intensity.
352
353 **PhNRs elicited by W/W stimuli differ from those elicited by R/B stimuli**
354 Although the intensities of the stimuli and background lights were photopically matched,
355 the waveforms of the PhNRs were different. With the R/B stimuli, the PhNR2 amplitudes
356 became larger as the stimulus intensities increased while the PhNR1 remained
357 unchanged. Therefore, stimuli with higher intensities produced larger PhNR2 than
358 PhNR1 with R/B stimuli. On the other hand, the PhNR1 amplitudes became larger than
359 the PhNR2 amplitudes with an increase of the W/W stimulus intensities. For
360 intermediate to higher stimulus intensities, the PhNR waveforms were dominated by the
361 PhNR1 elicited by the W/W stimuli and by the PhNR2 elicited by the R/B stimuli.
362
363 It is reasonable to select the largest ERG component to assess the retinal function.
364 Therefore, the PhNR1 amplitude elicited by W/W stimuli have been used in some
365 studies [9, 11, 17, 18, 20], and the PhNR2 amplitude elicited by the R/B stimuli in other

studies [3-6, 10, 15].

Correlation of amplitude of PhNR with functional and morphological parameters

The amplitudes of PhNR1 and PhNR2 elicited by both the R/B and W/W stimuli were significantly correlated with the MD and cpRNFL thickness. These findings are consistent with previous reports [4, 5, 8, 9]. In most studies that compared the correlations of the PhNR amplitudes with the visual field or OCT findings, it was found that the amplitudes of the PhNRs elicited by R/B stimuli had higher correlation coefficients than those elicited by the W/W stimuli [5, 8]. The PhNR2 amplitude was exclusively measured and evaluated in these studies. As reported, the R/B-elicited PhNR2 had higher correlations with the MD and the cpRNFL than the W/W-elicited PhNR2. However, the W/W-elicited PhNR1 had higher correlation coefficients than the R/B-elicited PhNR1 in our study. Therefore, an advantage of the R/B stimuli over the W/W stimuli for evaluating the PhNR depends on whether PhNR1 or PhNR2 is being analyzed.

Higher stimulus intensities elicited larger amplitudes PhNRs in normal subjects except for R/B-elicited PhNR1 while the PhNR amplitudes remained unchanged despite

increasing stimulus intensity in patients with glaucoma. As a result, the differences in the PhNR amplitudes between normal and glaucomatous eyes become more prominent at higher stimulus intensities (see Figure 2). Therefore, the slopes of the regression lines representing correlations of the PhNR amplitude with the MD or cpRNFL thickness became steeper when the ERG was recorded with the higher stimulus intensities. This indicates that the PhNR amplitudes elicited by stimuli with higher intensities decrease with a corresponding decrease in the MD or the cpRNFL thickness than those by lower intensities. In other words, the higher stimulus intensities evoke PhNRs that are more sensitive in detecting decreases of the MD and cpRNFL thickness.

The stimulus with the highest intensity of 3.0 cd-s/m^2 produced the steepest slopes. This intensity is recommended by the ISCEV Standard to record the cone ERGs [26]. Higher stimulus intensities were not evaluated in the present study because there was amplitude saturation or decrease [29]. In addition, it would evoke a blinking reaction to the flashes giving rise to artifacts that would interfere with the evaluations of the PhNR.

Abilities of PhNR amplitude to discriminate OAG from normal eyes

We could not obtain good diagnostic abilities of the PhNR amplitudes to discriminate

OAG from normal eyes. The diagnostic ability largely dependent on the degree of glaucoma. We have reported that the AUCs of the OAG were 0.748, 0.865, and 0.954 for early, intermediate and advanced glaucoma, respectively [6]. In the present study, nearly one-half of the patients had early glaucoma, which may have resulted in the relatively small AUCs.

For selected patients with advanced glaucoma, the AUCs were largest for the R/B-elicited PhNR2 at the highest stimulus intensity. This indicates that the PhNR2 at the highest intensity has a better ability in discriminating eyes with advanced glaucoma from normal eyes. This is because the regression line representing the correlation between the R/B PhNR amplitude and MDs was steepest at the highest intensity.

Limitations of this study

We have examined the responses of equally light-adapt cones rather than rods. This methodological difference should be considered when comparing our data with those of earlier studies [10]. For instance, the earlier investigators demonstrated that RGC-driven components were more dominated by the cone ERGs with the R/B than the W/W stimuli over low to middle intensities [10]. However, our results showed that the R/B-

420 elicited PhNR is better than the W/W-elicited PhNRs when it is recorded by higher
421 intensity stimuli.
422
423 Kremer et al suggested that the PhNR amplitude changes substantially with the
424 background luminance [7]. In this study, we fixed the intensity of the background light at
425 10 cd/m² which is recommended by the ISCEV extended protocol [27]. There may be
426 other suitable background intensities besides our eliciting stimulus intensities. In
427 addition, they found that the best stimulus condition was 1 cd s/m^2 for 458 nm flashes
428 on a 10 cd/m^2 591 nm background [7]. Further studies are needed to determine the
429 optimal stimulus conditions to elicit the PhNR with the best clinical significance.
430
431 We measured only cpRNFL thickness as a structural parameter in the present study.
432 Ganglion cell complex (GCC) thickness has been widely used as a structural parameter
433 for diagnosing OAG [31]. Although data is not shown, the PhNR amplitudes were
434 significantly correlated with the GCC thickness with less correlation coefficients
435 compared to corresponding values of the cpRNFL thickness. This is probably because
436 the GCC thickness only reflects retinal structure in the posterior pole while the ERG is
437 derived from the whole retina.

Conclusions

The amplitudes of the PhNR elicited by both W/W or R/B stimuli are significantly correlated with the function and morphology of the RGCs in eyes with OAG. The results suggest that the R/B-elicited ERGs are more suitable for recording the PhNR2 than the W/W stimuli, while the W/W-elicited ERGs are more suitable for eliciting the PhNR1. The PhNRs elicited by stimuli with higher intensity may be more sensitive in detecting functional and morphological loss of the RGCs. The diagnostic ability of the PhNR amplitude to discriminate advanced glaucoma from normal eyes is highest for the R/B-elicited PhNR2 recorded by the highest intensity of stimuli.

References

1. Viswanathan S, Frishman LJ, Robson JG, Harwerth RS, Smith III EL. The photopic negative response of the macaque electroretinogram: reduction by experimental glaucoma. Invest Ophthalmol Vis Sci. 1999; 40: 1124-36.
2. Viswanathan S, Frishman LJ, Robson JG. The uniform field and pattern ERG in macaques with experimental glaucoma: removal of spiking activity. Invest Ophthalmol Vis Sci. 2000; 41: 2797-810.
3. Viswanathan S, Frishman LJ, Robson JG, Walters JW. The photopic negative response of the flash electroretinogram in primary open angle glaucoma. Invest Ophthalmol Vis Sci. 2001; 42: 514-22.
4. Machida S, Gotoh Y, Toba Y, Ohtaki A, Kaneko M, Kurosaka D. Correlation between photopic negative response and retinal nerve fiber layer thickness and optic disc topography in glaucomatous eyes. Invest Ophthalmol Vis Sci. 2008; 49: 2201-7.
5. Sustar M, Cvenkel B, Brecelj J. The effect of broadband and monochromatic stimuli on the photopic negative response of the electroretinogram in normal subjects and in open-angle glaucoma patients. Doc Ophthalmol. 2009; 118: 167-77.
6. Machida S, Tamada K, Oikawa T, Gotoh Y, Nishimura T, Kaneko M, et al. Comparison of photopic negative response of full-field and focal electroretinograms

in detecting glaucomatous eyes. J Ophthalmol. 2011; 2011: pii:564131.

7. Kremers J, Jertila M, Link B, Pangeni G, Horn FK. Spectral characteristics of the PhNR in the full-field flash electroretinogram of normals and glaucoma patients. Doc Ophthalmol. 2012; 124: 79-90.

8. Banerjee A, Khurana M, Sachidanandam R, Sen P. Comparison between broadband and monochromatic photopic negative response in full-field electroretinogram in controls and subjects with primary open-angle glaucoma. Doc Ophthalmol. 2019; 138: 21-33.

9. Gotoh Y, Machida S, Tazawa Y. Selective loss of the photopic negative response in patients with optic nerve atrophy. Arch Ophthalmol. 2004; 122: 341-6.

10. Rangaswamy NV, Frishman LJ, Dorotheo EU, Schiffman JS, Bahrani HM, Tang RA. Photopic ERGs in patients with optic neuropathies: comparison with primate ERGs after pharmacologic blockade of inner retina. Invest Ophthalmol Vis Sci. 2004; 45: 3827-37.

11. Miyata K, Nakamura M, Kondo M, Lin J, Ueno S, Miyake Y, et al. Reduction of oscillatory potentials and photopic negative response in patients with autosomal dominant optic atrophy with OPA1 mutations. Invest Ophthalmol Vis Sci. 2007; 48: 820-4.

12. Tamada K, Machida S, Yokoyama D, Kurosaka D. Photopic negative response of full-field and focal macular electroretinograms in patients with optic nerve atrophy. Jpn J Ophthalmol. 2009; 53: 608-14.

13. Moon CH, Hwang SC, Ohn YH, Park TK. The time course of visual field recovery and changes of retinal ganglion cells after optic chiasmal decompression. Invest Ophthalmol Vis Sci. 2011; 52: 7966-73.

14. Moon CH, Hwang SC, Kim BT, Ohn YH, Park TK. Visual prognostic value of optical coherence tomography and photopic negative response in chiasmal compression. Invest Ophthalmol Vis Sci. 2011; 52: 8527-33.

15. Wang J, Cheng H, Hu YS, Tang RA, Frishman LJ. The photopic negative response of the flash electroretinogram in multiple sclerosis. Invest Ophthalmol Vis Sci. 2012; 53: 1315-23.

16. Machida S. Clinical application of photopic negative response to optic nerve and retinal diseases. J Ophthalmol. 2012: 397178.

17. Machida S, Gotoh Y, Tanaka M, Tazawa Y. Predominant loss of the photopic negative response in central retinal artery occlusion. Am J Ophthalmol. 2004; 137: 938-40.

18. Kizawa J, Machida S, Kobayashi T, Gotoh Y, Kurosaka D. Changes of oscillatory

potentials and photopic negative response in patients with early diabetic retinopathy. Jpn J Ophthalmol. 2006; 50: 367-73.

19. Chen H, Wu D, Huang S, Yan H. The photopic negative response of the flash electroretinogram in retinal vein occlusion. Doc Ophthalmol. 2006; 113: 53-9

20. Ueno S, Kondo M, Piao CH, Ikenoya K, Miyake Y, Terasaki H. Selective amplitude reduction of the PhNR after macular hole surgery: ganglion cell damage related to ICG-assisted ILM peeling and gas tamponade. Invest Ophthalmol Vis Sci. 2006; 47: 3545-49.

21. Nishimura T, Machida S, Harada T, Kurosaka D. Retinal ganglion cell function after repeated intravitreal injections of ranibizumab in patients with age-related macular degeneration. Clin Ophthalmol. 2012; 6: 1073-82.

22. Machida S, Toba Y, Nishimura T, Ohzeki T, Murai K, Kurosaka D. Comparisons of cone electroretinograms after indocyanine green-, brilliant blue G-, or triamcinolone acetonide-assisted macular hole surgery. Graefes Arch Clin Exp Ophthalmol. 2014; 252: 1423-33.

23. Yagura K, Shinoda K, Matsumoto S, Terauchi G, Watanabe E, Matsumoto H, et al. Intraoperative electroretinograms before and after core vitrectomy. PLoS One 2016; 11: e0152052.

520 24. Rangaswamy NV, Shirato S, Kaneko M, Digby BI, Robson JG, Frishman LJ. Effects
521 of spectral characteristics of ganzfeld stimuli on the photopic negative response
522 (PhNR) of the ERG. Invest Ophthalmol Vis Sci. 2007; 48: 4818-28.
523 25. Miyata K, Ueno S, Kondo M, Koyasu T, Terasaki H. Comparison of photopic
524 negative responses elicited by red and white xenon flashes in monkeys. Jpn J
525 Ophthalmol. 2008; 52: 327-30.
526 26. McCulloch DL, Marmor MF, Brigell MG, Hamilton R, Holder GE, Tzekov R, et al.
527 ISCEV standard for full-field clinical electoretinography (2015 update). Doc
528 Ophthalmol. 2015; 130: 1-12.
529 27. Frishman L, Sustar M, Kremers J, McAnany JJ, Sarossy M, Tzekov R, et al. ISCEV
530 extended protocol for the photopic negative response (PhNR) of the full-field
531 electroretinogram. Doc Ophthalmol. 2018; 136: 207-11.
532 28. Nagata M. Studies on the photopic ERG of the human retina. Jpn J Ophthalmol.
533 1963; 7: 96-124.
534 29. Joshi NR, Ly E, Viswanathan S. Intensity response function of the photopic negative
535 response (PhNR): effect of age and test-retest reliability. Doc Ophthalmol. 2017;
536 135: 1-16.
537 30. Anderson DR, Patella VM. Automated static perimetry. 2nd edition. Mosby, St.

Louis,121-90,1999.

31. Tan O, Chopra V, Lu AT, Schuman JS, Ishikawa H, Wollstein G, et al. Detection of macular ganglion cell loss in glaucoma by fourier-domain optical coherence tomography. Ophthalmology 2009; 116: 2305-14.

Figure legends

Figure 1: Representative cone ERGs elicited by white stimuli on a white background (W/W) to show how the amplitude of the PhNR was measured (A). The PhNR1 amplitude is measured from the baseline to the first trough following the b-wave. The PhNR2 amplitude is measured from the baseline to the trough after the i-wave. Representative cone ERGs elicited by W/W and by red stimuli on blue background (R/B) stimuli from a normal and a glaucomatous eye with moderate defect of the visual field (B). The amplitudes of both the PhNR1 and PhNR2 elicited by W/W and R/B stimuli are smaller in the glaucomatous eye. ERG: electroretinogram; PhNR: photopic negative response; OAG: open angle glaucoma

Figure 2: PhNR1 (A and C) and PhNR2 amplitudes (B and D) elicited by R/B or W/W stimuli are plotted against the stimulus intensity for normal and glaucomatous eyes. Open and solid symbols represent normal and glaucomatous eyes (square: early, triangle: intermediate and circle: advanced glaucoma), respectively. PhNR: photopic negative response; R/B: red stimuli on a blue background; W/W: white stimuli on a white background; Error bars: standard errors.

Figure 3: Correlation of the R/B-elicited PhNR1 (A, C, E, G) and the W/W-elicited PhNR1 (B, D, F, H) to the mean deviation (MD) obtained by standard automated perimetry. The stimulus intensities were 0.5 (A and B), 1.0 (C and D), 2.0 (E and F), and 3.0 (G and H) cd-s/m^2. The filled and open circles represent the glaucomatous and normal eyes, respectively. R/B: red on a blue background; PhNR: photopic negative response; W/W: white stimuli on a white background; OAG: open angle glaucoma

Figure 4: Correlation of the R/B-elicited PhNR2 (A, C, E, G) and W/W-elicited PhNR2 (B, D, F, H) with the mean deviation (MD) obtained by standard automated perimetry. The stimulus intensities were 0.5 (A and B), 1.0 (C and D), 2.0 (E and F) and 3.0 (G and H) cd-s/m^2. Filled and open circles represent glaucomatous and normal eyes, respectively. R/B: red stimuli on a blue background; PhNR: photopic negative response; W/W: white stimuli on a white background; OAG: open angle glaucoma

Figure 5: Correlation of the R/B-elicited PhNR1 (A, C, E, G) and W/W-elicited PhNR1 (B, D, F, H) with the circumpapillary retinal nerve fiber layer (cpRNFL) thickness obtained by spectral-domain optical coherence tomography. The stimulus intensities were 0.5 (A and B), 1.0 (C and D), 2.0 (E and F) and 3.0 (G and H) cd-s/m^2. Filled and

open circles represent glaucomatous and normal eyes, respectively. R/B: red stimuli on a blue background; PhNR: photopic negative response; W/W: white stimuli on a white background; OAG: open angle glaucoma

Figure 6: Correlation of the R/B-elicited PhNR2 (A, C, E, G) and W/W-elicited PhNR2 (B, D, F, H) with the circumpapillary retinal nerve fiber layer (cpRNFL) thickness obtained by spectral-domain optical coherence tomography. The stimulus intensities were 0.5 (A and B), 1.0 (C and D), 2.0 (E and F) and 3.0 (G and H) cd-s/m^2. Filled and open circles represent glaucomatous and normal eyes, respectively. R/B: red stimuli on a blue background; PhNR: photopic negative response; W/W: white stimuli on a white background; OAG: open angle glaucoma

Figure 7: Receiver operating characteristic (ROC) curves for the R/B PhNR1 (A), W/W PhNR1 (B), R/B PhNR2 (C) and W/W PhNR2 (D) in discriminating eyes with open angle glaucoma from normal eyes. The stimulus intensities were 0.5 (white), 1.0 (light gray), 2.0 (dark gray) and 3.0 cd-s/m^2 (black). The R/B PhNR2 elicited by 3.0 cd-s/m^2 had the highest value of the area under the ROC curve. R/B: red stimuli on a blue background; PhNR: photopic negative response; W/W: white stimuli on a white background.

599

600