Significant Correlations between Focal Photopic Negative Response and Focal Visual Sensitivity and Ganglion Cell Complex Thickness in Glaucomatous Eyes

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ABSTRACT

Purpose: To determine whether there are significant correlations between the focal photopic negative response (PhNR) and the focal visual sensitivity and the ganglion cell complex (GCC) thickness in glaucomatous eyes.

Design: Single-center observational study.

Methods: Fifty-two eyes of 52 patients (71.4±9.42 years) with clinically diagnosed open angle glaucoma were studied. Thirty-six age-matched normal subjects served as controls. The focal PhNR of the focal macular electroretinograms (fmERGs) were elicited by a 15° circular or a superior semicircular or an inferior semicircular stimulus centered on the fovea. The thickness of the GCC was measured in the corresponding retinal areas in the spectral-domain optical coherence tomographic images. The visual sensitivities (dB) were measured by microperimetry from the retinal area where the fmERGs were elicited and were converted to liner values (1/Lambert).

Results: The focal PhNR amplitudes were significantly correlated with the visual sensitivities of the full-circle and the superior and inferior semicircular responses (R=0.532, 0.530 and 0.526, respectively; $P<0.0001$). The GCC thickness was correlated with the visual sensitivities in the same areas with stronger correlations (R=0.700, 0.759 and 0.650, respectively; $P<0.0001$). The focal PhNR amplitudes

were proportionally reduced with the thinning of the GCC thickness (R=0.494, 0.518 and 0.511, respectively; *P*<0.0001).

Conclusions: The significant correlations between the focal PhNR amplitudes and the focal visual sensitivities and the GCC thickness indicate that they may be good biomarkers to track the changes in the physiology and anatomy of the macular area in glaucomatous eyes.

INTRODUCTION

The electroretinogram (ERG) is the sum of electrical responses of the different retinal cells elicited by light stimulation. The photopic negative response (PhNR) is a component of the full-field cone ERGs, and it originates from the electrical activity of the retinal ganglion cells (RGCs) and their axons of the entire retina [1]. It has been found that the amplitude of the PhNR is reduced in eyes with various diseases affecting the inner retina and the optic nerve including glaucoma [2-15].

The focal macular ERGs (fmERGs) were first recorded by Miyake et al, and they originate from the electrical activity of the neurons in a small area of the macula [16, 17]. Experimental and clinical evidence have shown that the PhNR of the fmERGs, designated as the focal PhNR, originates from the neural activities of the RGCs of only the focal area of the retina stimulated [12, 18-23]. Analyses of the focal PhNRs have allowed clinicians and researchers to assess the physiological normality of the RGCs and their axons in the area stimulated by the light stimulus.

Glaucoma is an ocular disease characterized by a degeneration of the RGCs and their axons [24, 25], and it has been shown that the focal PhNR can be used to assess the function of the macular area in patients with glaucoma [18, 26]. We have

reported that the focal PhNR amplitude had a better sensitivity than the full-field PhNR to discriminate eyes with open angle glaucoma (OAG) especially at the early stages [27, 28].

A curvilinear relationship was found between the amplitude of the focal PhNR and the visual sensitivity (dB) determined by standard automated perimetry (SAP) [18]. The focal PhNR amplitude was markedly reduced with a slight reduction of the visual sensitivity (dB) at the early stage of glaucoma while the reduction of the focal PhNR amplitude was minimal with a large reduction of the visual sensitivity (dB) at a more advanced stage.

The visual sensitivity (dB) is generally expressed as a logarithmic value. Therefore, when the visual sensitivity (dB) is converted to a linear value as 1/Lambert, the focal PhNR amplitude is linearly correlated with the visual sensitivity expressed as 1/Lambert. One of the limitations of our earlier study was that the retinal area where the fmERGs were recorded did not completely correspond to the area where the visual sensitivities were measured.

Microperimetry (MP) measures the visual sensitivity of focal areas of the retina while

99 the ocular fundus is being monitored. MP uses an auto-tracking system which
100 maintains the stimuli on a specific retinal area. An earlier study has demonstrated
101 that the visual sensitivity measured by standard automated perimetry (SAP) and MP
102 were significantly correlated with the ganglion cell complex (GCC) thickness
103 measured by spectral-domain optical coherence tomography (SD-OCT) in
104 glaucomatous eyes [29]. However, it has not been determined whether the
105 amplitudes of the focal PhNR were significantly correlated with the focal visual
106 sensitivities determined by MP.
107
108 Earlier we determined the correlations between the focal PhNR amplitude with the
109 visual sensitivity obtained by SAP [18] and the GCC thickness obtained by OCT [22]
110 in patients with glaucoma. Because these investigations were done on different
111 patients using different ERG recording conditions for each study, we were not able to
112 compare correlation coefficients obtained by these studies directly. Thus, the
113 purpose of this study was twofold; to determine whether there is a significant
114 correlation between the amplitude of the focal PhNR and the focal visual sensitivity
115 and the thickness of the GCC, and to determine the differences in these correlations.
116 We shall show that the amplitude of the focal PhNR was significantly correlated with
117 the focal visual sensitivity (1/Lambert) and the GCC thickness, and the GCC

thickness had a stronger correlation with the visual sensitivity (1/Lambert) than the focal PhNR amplitude.

METHODS

Patients

Fifty-two eyes of 52 patients (mean age: 71.4 ± 9.42 years; range 45 to 88 years) with OAG were studied. The diagnosis of OAG was made by the presence of a glaucomatous optic disc associated with visual field defects determined by SAP. Thirty-six eyes of 36 normal controls (age 70.0 ± 8.60 years, range 43 to 85 years) were studied in the same way.

This research was approved by the Institutional Review Board of Dokkyo Medical University and conducted accordance with the Institutional Guidelines, 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 experiments.

Focal ERG Recordings

The pupils were maximally dilated to approximately 8 mm diameter with a topical

mixture of 0.5 tropicamide and 0.5% phenylephrine HCL. After topical anesthesia by 4% lidocaine and 0.4% oxybuprocaine HCL, a Burian-Allen bipolar contact lens electrode (Hansen Ophthalmic Laboratories, Iowa City, IA, USA) was inserted into conjunctival sac. A chlorided silver electrode was placed on the left ear lobe as the ground electrode. The stimulus system was integrated into an infrared fundus camera (ER-80, Kowa Company, Ltd., Aichi, Japan), and the stimulus was circular (dotted circle in Figure 1a), or a superior semicircular or an inferior semicircular stimulus with a diameter of 15-degree (semicircles with black solid line in Figure 1a). The circular stimulus was centered on the fovea and the edge of the semicircular stimulus was set on the foves, and the position was confirmed by viewing the ocular fundus on the monitor of the fundus camera. The intensity of stimulus and background lights was 30 cd/m^2 and 1.5 cd/m^2, respectively. The stimulus duration was 16.6 msec. All subjects were instructed to fixate a point in the center of the visual field during the fmERG recordings. The fixation was monitored through the image of the infrared funds camera on a monitor.

The ERG responses were digitally bandpassed from 5 to 200 Hz (PuREC, Mayo Corporation, Inazawa, Aichi, Japan), and approximately 300 responses were averaged. The rate of stimulation was 4.85 Hz, and the overall recording time for one

response was around 1.5 minutes. The focal PhNR amplitude was measured from the baseline to the negative trough at 70 msec after the stimulus onset as reported (Figure 2b) [18, 22].

Standard Automated Perimetry (SAP)

The Humphrey Visual Field Analyzer (Model 750, Humphrey Instruments, San Leandro, CA, USA) was used for the SAP. The SITA Standard strategy was applied to program 30-2, and the measurements of the visual sensitivity were made after at least 3 minutes of adaptation to the background light.

The mean deviation (MD) was defined as the mean of the differences between the measured sensitivity and normal values of age-matched controls which was embedded in the program. Thus, the MDs represented the reduction of the visual sensitivities over the whole visual field. We classified patients with glaucomatous visual fields into three stages; early (MD >-6 dB), intermediate (-6 dB $\geq$ MD $\geq$ -12 dB), and advanced (MD <-12 dB) stages of glaucoma. The glaucoma patients were divided by their MDs into those with early (n = 24), intermediate (n = 12), and advanced (n = 16) glaucoma.

Microperimetry

Microperimetry was used to determine the visual sensitivities of focal areas of the macular area (Microperimeter MP-3, Nidek Co. LTD., Gamagori, Aichi, Japan). The protocol used was the macula 14-32 grid with 32 test points in the circular area centered on the fovea with a diameter of 15° that corresponded to the area where the fmERGs were elicited (Figure 1b). The stimulus size was the same as Goldman size Ⅲ (white, 200 msec) of SAP. The background luminance was set at 31.4 apostilb (10 cd/m^2), and the range of the stimuli was 0 to 34 dB. The visual sensitivity was estimated by the 4-2 fast strategy and labeled by colors with green representing high and red representing low sensitivities (Figures 1b and 2a). The visual sensitivity (dB) of the measured point was converted to 1/Lambert and then averaged. The 1/Lambert value was designated as the linear visual sensitivity.

Optical Coherence Tomography (OCT)

The OCT images were acquired with a SD-OCT (RS-3000 Advance, Nidek Co. LTD., Gamagori, Aichi, Japan). The thickness of the GCC was measured from the internal limiting membrane to the inner plexiform layer at 512 x 128 points in the posterior pole of the eye to construct the GCC map (Figure 1a). The tracking system of the OCT system reduced the effects of eye movements which allowed a more precise

measurement of the thickness at each retinal point.

We used the thickness maps for the analysis (Figure 1a). The mean thickness was determined for each quarter of an annulus with inner diameter of 1.5 mm and an outer diameter of 4.5 mm. The selected areas were marked out in squares (Figure 1b). The GCC thickness of the temporal/superior and nasal/superior quadrants and the temporal/inferior and nasal/inferior quadrants were averaged to obtain the averaged GCC thickness for the superior and inferior semicircular retinal areas, respectively, where the fmERG and MP-3 were recorded.

Statistical Analyses

Spearman's coefficients correlations were calculated to determine the correlation between the focal PhNR amplitude, GCC thickness, and linear visual sensitivity in 1/Lambert units. These analyses were performed by SPSS 27 (IBM SPSS Statistic 27, IBM, Chicago, IL, USA). The level statistical significance was set at P <0.05.

To evaluate the inter-individual variations in the normal subjects, the coefficient of variation (CV = standard deviation/mean x 100) was calculated for the amplitudes of the focal PhNR, linear visual sensitivities, and GCC thicknesses.

RESULTS

Representative Case

The GCC map, GCC chart, MP-3 values (Figure 2a), and the fmERGs (Figure 2b) from a patient with early glaucoma are shown in Figure 3. The SD-OCT image shows a thinning of the GCC in the lower parafoveal region where the visual sensitivity was depressed at some of the measured points (red and yellow circles). There was no significant differences in the amplitudes of the a- and b-waves of the fmERGs between the normal subject and glaucoma patient. However, the focal PhNR amplitudes at 70 msec elicited by the circular and inferior semicircular stimulus spots were severely depressed compared to those of the normal subject.

Correlation between focal PhNR amplitude and visual sensitivities (dB or 1/Lambert)

The amplitudes of the focal PhNR are plotted against the averaged visual sensitivities (dB) measured for the circular, superior semicircular, and inferior semicircular retinal regions (Figure 3a-c). A curvilinear relationship was found between the focal PhNR amplitude and visual sensitivity (dB), and it appeared to be a better way to illustrate the correlation than the linear relationship in all three areas.

Thus, a large reduction of the focal PhNR amplitude corresponded to a small reduction of the visual sensitivity at the early stage of glaucoma while a slight reduction of the focal PhNR amplitude corresponded to a large reduction of the visual sensitivities at a more advanced stage of glaucoma.

The visual sensitivity (dB) was converted to a linear scale (1/Lambert) and plotted against the focal PhNR amplitude in Figures 3d to 3f. The focal PhNR amplitude was significantly and linearly correlated with the linear visual sensitivity for the circular, superior semicircular, and inferior semicircular areas (R = 0.532, 0.530 and 0.526, respectively; P <0.0001).

The correlation coefficients, slopes of the regression lines, and the *P*-values representing the focal PhNR vs focal visual sensitivities, the GCC thickness vs focal visual sensitivity and the focal PhNR amplitude vs GCC thickness relationships are presented in Table 1. The correlation coefficients and slopes are presented with the 95% confidence intervals.

Correlation between GCC Thicknesses and Visual Sensitivities (dB or 1/Lambert)

The GCC thicknesses are plotted against the means of the visual sensitivity (dB) measured for the circular, superior semicircular, and inferior semicircular retinal regions in Figures 4a – 4c. There was also a curvilinear relationship between the GCC thickness and visual sensitivity (dB) in all three areas as seen in the relationships between the focal PhNR amplitude and visual sensitivity (dB).

The GCC thicknesses are plotted against the means of the linear visual sensitivity in Figures 4d to 4f. The GCC thickness was significantly and proportionately thinned with a reduction of the linear visual sensitivity for circular, superior semicircular, and inferior semicircular areas (R = 0.700, 0.759 and 0.650, respectively; *P* <0.0001).

Correlation between Focal PhNR Amplitude and GCC Thickness

The amplitudes of the focal PhNR are plotted against the mean GCC thickness of the circular, superior semicircular, and inferior semicircular retinal regions in Figures 5a to 5c. The focal PhNR amplitude was significantly correlated with the thinning of the GCC layer in the circular, superior semicircular, and inferior semicircular retinal regions (R = 0.494, 0.518 and 0.511, respectively; all *P* <0.0001).

The means, standard deviations, and coefficient of variations (CVs) of the focal

PhNR amplitude, GCC thickness, and linear visual sensitivity obtained from normal control subjects are presented in Table 2. The visual sensitivity was converted to the 1/Lambert units for the comparisons with the other linear parameters. In all retinal areas, the CVs were the highest for the focal PhNR amplitude compared to those of the GCC thickness and linear visual sensitivity. The CVs ranged from 0.37 to 0.59 for the focal PhNR amplitude and from 0.34 to 0.36 for the linear visual sensitivity while they were less than 0.1 for the GCC thickness.

One might expect that the ratio of the focal PhNR amplitude to the b-wave amplitude (focal PhNR/b-wave amplitude ratio) would reduce the CVs and improve the correlations with the linear sensitivity or GCC thickness. However, the CVs were 0.46, 0.45 and 0.55 for the circle, superior semicircle, and inferior semicircle stimuli, respectively. These values are comparable to the CVs of the focal PhNR amplitude which failed to improve the correlations with other parameters. For instance, the correlation coefficients of the focal PhNR amplitude and linear visual sensitivity ranged from 0.432 to 0.484.

DISCUSSION

The results showed that the amplitudes of the focal PhNR were significantly

correlated with the linear visual sensitivities and the thicknesses of the GCC. The results also showed that the GCC thickness had a stronger correlation with the visual sensitivity than the focal PhNR amplitude.

Conversion of Visual Sensitivity from Logarithmic to Linear Scale

The amplitudes of the focal PhNR and thickness of the GCC are both linear values while the visual sensitivity is usually expressed in logarithmic units (dB) in the clinical measurements. It has been demonstrated that the histologically determined RGC counts are strongly correlated with the SAP-determined visual sensitivity when both parameters are expressed in the same units such as linear-linear or logarithmic-logarithmic in non-human primate models and patients with glaucoma [30-33]. Hood et al were the first to apply this concept to clinical cases of glaucoma using the RNFL thickness obtained by OCT, or the amplitude of the visual evoked potentials and the visual sensitivity determined by SAP [34-36]. They found that the structure-function and function-function relationships can be explained by a simple linear model after converting the visual sensitivity (dB) to the linear visual sensitivity (1/Lambert). We successfully applied the Hood's model to the relationship between the focal PhNR amplitude and visual sensitivity determined by SAP in an earlier study [18]. In the present study, the conversion to the linear visual sensitivity allowed us to compare

the correlations between the linear biometric parameters including the focal PhNR amplitude and GCC thickness with the linear visual sensitivities.

GCC is Better Biomarker Than Focal PhNR Amplitude to Tract Visual Sensitivity in Glaucomatous eyes

The GCC thickness map is widely used to assess anatomical changes of the RGCs in patients with glaucoma in the clinic [37]. Earlier studies have demonstrated that the visual sensitivity determined by SAP or MP were significantly correlated with the GCC thickness in patients with glaucoma [29]. We have reported that the correlation between the GCC thickness and focal PhNR amplitude in a semi-circular area with a 15 degree-diameter placed at the macula [22] was comparable to those of the present study.

In this study, the coefficients of correlation between the GCC thickness and linear visual sensitivity were higher with a range from 0.650 to 0.759) than those between the focal PhNR amplitude and linear visual sensitivity with a range from 0.526 to 0.532). The averaged CVs in normal subjects were much higher for the focal PhNR amplitude with a range from 0.37 to 0.59 than that of the GCC with a range from 0.07 to 0.08). These values indicated that the GCC thickness has less individual

variations than that of the focal PhNR amplitude (see Table 2). The difference in the correlation coefficients appears to be due to the differences in the CVs.

According to the differences of the CVs, the correlation coefficients between the GCC thickness and focal PhNR amplitude should be better than those between the linear visual sensitivity and the focal PhNR amplitude because these two functional parameters have large CVs. However, the former ones are comparable to the later ones indicating that factors other than the individual variations could be involved in determining the strength of the correlations of the focal PhNR amplitude with structural or functional parameters.

Possible Factors Affecting Coefficients of Correlations

The following factors can possibly affect the strength of the correlations of the focal PhNR amplitude with structural and functional parameters. First, the Müller cells are believed to play an important role in generating the electrical responses of the ERGs [38]. The RGCs produce spiking electrical responses while the PhNR is a slow wave. The discrepancy between the configurations of the mass and individual electrical responses of the RGCs can be explained by the glial mediation in shaping the PhNR. Experimental evidence showed that an intravitreal injection of Ba^{2+} in cats

346 blocked the K^+ current in glia cells with the subsequent elimination of the PhNR [39].
347 Tanihara and coworkers [40] reported that glial fibrillary acid protein was upregulated
348 in non-human primate models with glaucoma indicating that an impairment of the
349 Müller cells may occur in glaucomatous eyes. The Müller cell damage would directly
350 affect the focal PhNR amplitude while the visual sensitivity and GCC thickness would
351 remain unchanged as long as the RGCs were functioning. We have reported a lack
352 of significant correlations between the PhNR amplitude and RNFL thickness in the
353 early stage of traumatic optic neuropathy [3]. Thus, the glial mediation could result in
354 mismatches between the focal PhNR amplitude and other parameters. Second, the
355 MP-3 and SD-OCT devices have an auto-tracking system, while the stimulus area
356 for the focal macular ERG is manually adjusted during the recordings. This could
357 lead to non-exact correspondence of the stimuli. Third, there is a difference in the
358 nature of functional measurements between the focal PhNRs and the visual
359 sensitivities. The focal PhNR is a mass response representing activities of the RGCs
360 in a specific area of the retina, while the visual sensitivity is the threshold at a
361 specific point of the retina. In addition, the threshold and amplitude are not always
362 affected by a specific retinal disorder to the same extent [41, 42]. These factors could
363 contribute to the negative effects on the correlations of the focal PhNR amplitude
364 with other functional and structural parameters.

Comparison with our earlier studies

We have also reported that the focal PhNR amplitude was significantly correlated with the linear visual sensitivity (1/Lambert) obtained by SAP [18] and GCC thickness [22] in patients with glaucoma. However, we studied different participants using different recording conditions in these studies which makes it difficult to compare the correlation coefficients between the studies directly. In the present study, we have conducted the functional and structural assessments on the same patients which enabled us to compare the correlation coefficients between various parameters.

Limitations of Study

Several limitations of this study can be raised. First, because this study was a cross-sectional study, we could not determine how the function-function or function-structure relationship changed over time with advancing glaucoma. Second, we did not take displacements of the RGCs from the foveal field into consideration when placing measuring points of the MP-3. The displacement is important if a point-to-point comparison is made [43-45]. However, we compared the averaged values of GCC thickness and visual sensitivity in a specific area. Third, the measurements of

the focal PhNR amplitude is still controversial. We have been measuring the focal PhNR amplitude at 70 msec after the stimulus onset because in normal subjects the average implicit time of the focal PhNR is around 70 msec [18, 22]. Recently, the International Society of Clinical Electrophysiology and Vision published an extended protocol for the PhNR recordings and measurements. They recommended that the measurement at the fixed time point especially in diseased eyes with small PhNR amplitudes [46].

In conclusion, significant function-function and function-structure correlations were found in glaucomatous eyes. Because a stronger correlation was seen between the GCC thickness and linear visual sensitivity than the focal PhNR amplitude, the GCC thickness may be a better biomarker than the focal PhNR amplitude to track the visual sensitivities in glaucomatous eyes.

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Figure legends

Figure 1: How the focal macular electroretinograms (fmERGs), retinal sensitivities by microperimetry (MP), and thickness of the ganglion cell complex (GCC) were measured. (a) Location of the stimulus spot for the focal macular ERGs (fmERGs). A circular, superior semicircular, or inferior semicircular spot with a diameter of 15 degrees was used. (b) The location of 32 measuring points of the visual sensitivity for MP-3. The measurment points were uniformly distributed in the circular area (white line) where the fmERGs were recorded. (c) Retinal thickness map and chart of the GCC. Inner and middle circles with 1.5 and 4.5 mm in diameter were placed on the center of the macular area. Fifteen degrees approximately correspond to 4.5 mm. Averaged thicknesses for values which are marked by squares in the GCC chart were used for the analyses.

Figure 2: The SD-OCT, MP-3 (a), and focal macular ERG (fmERG) (b) findings obtained from a patient with early glaucoma. The fmERGs were elicited by a 15-degree circular, superior semicircular or inferior semicircular stimuli. The focal PhNR amplitude elicited by the circular and inferior semicircular stimulus spots were smaller than that of the normal subject, and the GCC thickness and visual sensitivity were also reduced. SD-OCT: spectral-domain optical coherence tomography, MP:

558 microperimetry, ERG: electroretinogram, PhNR: photopic negative response, GCC:
559 ganglion cell complex.
560
561 **Figure 3:** The amplitudes of the focal PhNR are plotted against the means of the
562 visual sensitivity (dB) measured for the circular (a), superior semicircular (b) and
563 inferior semicircular retinal regions (c). The visual sensitivity (dB) was converted to a
564 linear scale (1/Lambert) and plotted against the focal PhNR amplitude for the circular
565 (d), superior semicircular (e) and inferior semicircular retinal regions (f). The visual
566 sensitivity (dB) was measured by MP-3, a microperimeter. The focal PhNR amplitude
567 was linearly and significantly correlated with the linear visual sensitivity (1/Lambert).
568 PhNR: photopic negative response.
569
570 **Figure 4:** The GCC thicknesses are plotted against the means of the visual
571 sensitivities (dB) measured for the circular (a), superior semicircular (b) and inferior
572 semicircular retinal regions (c). The visual sensitivity (dB) was converted to a linear
573 scale (1/Lambert) and plotted against the GCC thicknesses for the circular (d),
574 superior semicircular (e) and inferior semicircular retinal regions (f). The visual
575 sensitivity (dB) was measured by MP-3, a microperimeter. The GCC thickness is

576 significantly and linearly correlated with the linear visual sensitivity (1/Lambert).

577 GCC: ganglion cell complex

578

579 **Figure 5:** The focal PhNR amplitudes are plotted against the means of the GCC

580 thicknesses for the circular (a), superior semicircular (b), and inferior semicircular

581 retinal regions (c). The focal PhNR amplitude is significantly correlated with the GCC

582 thickness. PhNR: photopic negative response, GCC: ganglion cell complex

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