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Article

Measuring the Photopic Negative Response: Viability of Skin Electrodes and Variability Across Disease Severities in Glaucoma

Zhichao Wu[1,2], Xavier Hadoux[1,2], Jennifer C. Fan Gaskin[1,2], Marc G. Sarossy[1,2], and Jonathan G. Crowston[1,2]

[1] Centre for Eye Research Australia, Royal Victorian Eye and Ear Hospital, East Melbourne, Australia
[2] Ophthalmology, Department of Surgery, The University of Melbourne, Melbourne, Australia

Correspondence: Zhichao Wu, Glaucoma Research Unit, Centre for Eye Research Australia, Level 1, 32 Gisborne Street, East Melbourne VIC 3002, Australia. e-mail: wu.z@unimelb.edu.au

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Purpose: The purpose of this study was to determine the feasibility of measuring the photopic negative response (PhNR) of the full-field electroretinogram (ERG) using skin electrodes compared to conjunctival electrodes and its test–retest variability over a range of disease severities in open-angle glaucoma.

Methods: Recordings were performed twice (100 sweeps each) within the same session in 43 eyes of 23 participants with glaucoma to determine its intrinsic variability. The ratio between the PhNR and B-wave amplitude (PhNR/B ratio) was determined for each trace and computed across 5 to 100 sweeps of each recording. Spectral-domain optical coherence tomography was used to measure the average peripapillary retinal nerve fiber layer (RNFL) thickness.

Results: The PhNR/B ratio and its magnitude of variability were not significantly different between skin and conjunctival electrodes ($P \leq 0.197$), and the degree of variability decreased substantially with increasing number of sweeps. For skin electrodes, the intraclass correlation coefficient was 0.89 and 0.91 for right and left eyes, respectively. The variability of the PhNR/B ratio decreased with lower RNFL thickness values and larger B-wave amplitudes ($P \leq 0.002$).

Conclusions: Skin electrodes are a viable alternative to conjunctival electrodes when measuring the PhNR in open angle glaucoma, and increasing the number of sweeps substantially reduced its intrinsic variability; the extent of variability was also lower with worsening disease severity.

Translational Relevance: The feasibility of performing ERG recordings widely across a range of disease severities in glaucoma can be achieved through using skin electrodes and increasing the number of sweeps performed to improve measurement repeatability.

Introduction

The lack of sufficiently sensitive and specific clinical markers of glaucoma is a major challenge in its clinical management and development of novel neuroprotective therapies to prevent irreversible vision loss. Intraocular pressure remains the only modifiable risk factor currently, although it is not specific for this disease and is an imperfect correlate for clinical outcomes when evaluating treatment efficacy.[1] More sensitive, specific, and clinically applicable markers are therefore clearly required to improve the management of glaucoma.

Given that glaucoma is characterized by the progressive loss of retinal ganglion cells (RGCs), an electrophysiological evaluation of the functional state of the RGCs could potentially provide such a specific marker. This can be achieved through using the full-field electroretinogram (ERG) or the pattern ERG. For the full-field ERG, a slow negative potential that follows the a- and b-waves of the photopic ERG—termed the photopic negative response (PhNR)—has been reported from animal studies to originate from the spiking activity of the RGCs and their axons, with contributions from amacrine and surrounding glial cells.[2–5]

Clinical studies have shown that the PhNR is reduced in eyes with glaucoma,[6–14] with the degree of functional loss often reported to be moderately associated with the extent of neural loss.[6–11] The loss of RGC function measured using the pattern ERG (PERG) has also been found to precede neural loss[15] and visual function loss.[16,17] More pertinently, partial reversal of such functional loss measured with both techniques has been demonstrated in interventional studies for glaucoma,[18–21] which collectively highlight the potential for electrophysiological measures of RGC function such as the PhNR to be used as a specific marker of disease in glaucoma. Although RGC function can also be measured using the PERG, measuring the PhNR with the full-field ERG is advantageous as it does not require clear optics, refractive correction, or exact foveal placement of the stimulus, while having a similar ability to detect glaucoma.[13]

The use of electrophysiological measures is currently hindered by the limitations associated with its ease of widespread clinical implementation and large magnitude of measurement variability.[22,23] For example, placement of corneal or conjunctival electrodes that are typically used for recordings requires highly skilled technicians and compliant patients. However, a recent study has shown that skin electrodes exhibited measurement repeatability comparable to that of conjunctival electrodes when recording the PhNR.[23] Another recent study also demonstrated that the PhNR amplitude exhibited a high level of measurement repeatability when using skin electrodes in a pediatric population.[24] These findings collectively suggested that skin electrodes may be a viable alternative that is more clinically applicable for measuring the PhNR, in a manner similar to what has been shown for PERG recordings.[25]

Nonetheless, to our knowledge no study to date has examined whether skin electrodes are comparable to conjunctival electrodes for measuring the PhNR in eyes with glaucoma. Furthermore, no study has examined whether the test–retest variability of the PhNR is related to disease severity in glaucoma, which is important when seeking to determine whether short-term and long-term functional changes are truly occurring. Therefore, we sought to investigate the test-retest variability of the PhNR in eyes with glaucoma across a range of disease severities using both skin and conjunctival electrodes in this study. Given that measurement error is also dependent on the number of sweeps made, we also investigated the test–retest variability of the PhNR over a large number of sweeps.

Methods

This study was approved by the Human Research Ethics Committee of the Royal Victorian Eye and Ear Hospital and was conducted in adherence with the Declaration of Helsinki. Informed consent was obtained from all participants.

Participants

The inclusion criteria for participants in this study included having an eye with the diagnosis of glaucoma with open angles on gonioscopy examination, a best-corrected visual acuity (BCVA) of 6/12 or better, a spherical refractive error within ±5.00 diopter (D), and cylindrical refractive error within ±3.00 D. All participants were also required to have performed standard automated perimetry testing with a 24-2 Swedish interactive threshold algorithm (Carl Zeiss Meditec, Inc., Dublin, CA) in the study eye within 6 months from the time of the visit, with ≤33% fixation losses and false-negative errors and ≤15% false-positive errors. The exclusion criteria for study eyes included a history of intraocular surgery (except uncomplicated cataract or glaucoma surgery, unless this was performed within the past 3 months from the time of the visit) or glaucoma due to secondary causes (e.g., trauma). Participants with any systemic or ocular disease (e.g., diabetes, age-related macular degeneration) or condition that affected cognition (e.g., dementia, stroke) or who were taking any medication known to affect retinal function (e.g., hydroxychloroquine) were excluded from this study. Participants were also excluded if they had any physical or mental impairment preventing them from participating in this study and/or providing informed consent.

Procedures

All participants first performed standard measurements of BCVA on a 4-m Early Treatment for Diabetic Retinopathy chart before pupillary dilation and retinal imaging, followed by electroretinography and a standard ophthalmic examination by a glaucoma specialist.

Full-Field Electroretinography

Pupils were dilated to ≥7 mm using 1% tropicamide prior to electroretinography recordings, and all

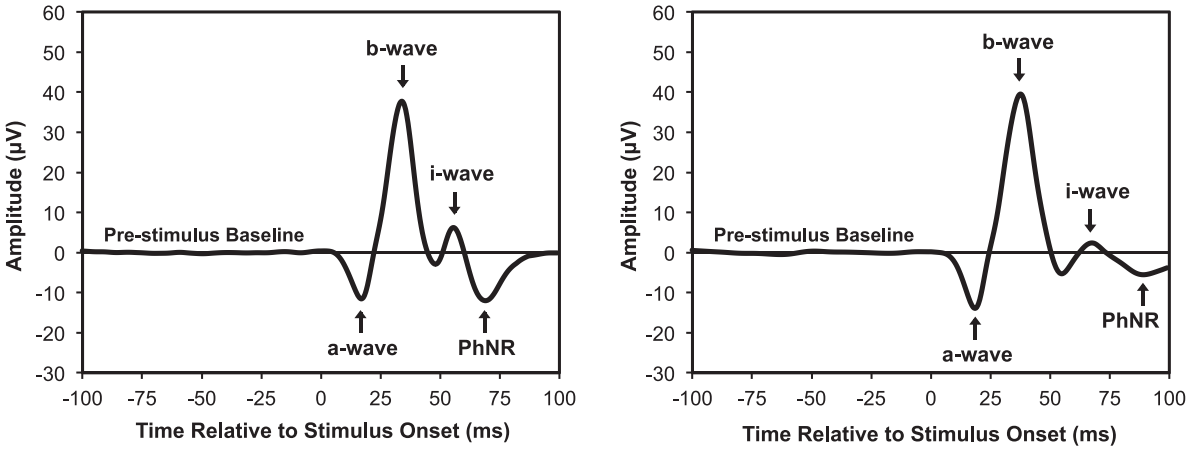

Figure 1. Illustration of the parameters of the ERG waveform measured in this study with two examples using skin electrodes (using 200 sweeps). The a- and b-waves were considered to be the first negative trough and positive peak, respectively. The i-wave was often present as a second positive peak, and the PhNR was considered to be the negative trough between 60 and 100 ms after stimulus onset. The second example (*right*) illustrates the rationale for choosing this window for locating the PhNR, since the second negative trough between the b-wave and i-wave peaks can exhibit amplitude similar to the PhNR when the PhNR is reduced.

stimulus generation and signal recordings were performed with an Espion System (E2/ColorDome; Diagnosys LLC, Lowell, MA). Responses were recorded with Dawson-Trick-Litzkow (DTL) fiber electrodes placed inside the inferior conjunctival fornix, and gold cup electrodes (Grass Technologies; Astro-Med, Inc., West Warwick, RI) were placed on skin surface at the inferior orbital rim below the pupil in primary gaze[23,25]; these two electrodes are henceforth referred to as the DTL and skin electrodes, respectively. Both the DTL and skin electrodes were referred to gold cup electrodes positioned at the ipsilateral lateral canthus and grounded with a gold cup electrode placed on the forehead in the midfrontal position. An impedance level of <5 kΩ at 25 Hz for all channels was achieved through skin preparation before recordings began.

Preadaptation to a blue background (of 10 cd/m^2, peak wavelength 465 nm) was performed for 3 minutes. Electroretinography recordings were then performed using brief red flashes (of 1.50 cd-s/m^2; peak wavelength, 635 nm) of 4 ms in duration, presented at a frequency of 2 Hz against the blue background, in a manner similar to previous studies.[7,22] All signals were filtered with a band-pass filter of 0.1 to 100 Hz, before being digitized to a resolution of 12 bits at a sampling rate of 1 kHz. Signals were recorded 100 ms before and after the stimulus onset and were considered a single sweep, and a sweep was automatically rejected if it exceeded ±125 μV (typically due to blink or eye movement artifacts). Two measurements consisting of 100 sweeps were recorded from each eye within the same session without removing the electrodes.

Signal Analysis

A linear trend correction was first applied to each individual sweep using the slope and offset computed from the prestimulus baseline (100 ms preceding stimulus onset). In order to analyze the variability obtained with increasing number of sweeps, the final trace was computed using 5 to 100 randomly selected sweeps, in steps of five sweeps. The median value at each time point of all sweeps included was used to determine the final trace.

The parameters of the ERG waveform analyzed in this study are shown in Figure 1. The a- and b-waves were considered to be the first negative trough (between 0 and 25 ms poststimulus onset) and positive peak (between 26 and 50 ms poststimulus onset), respectively. The a-wave trough to b-wave peak was considered to be the B-wave amplitude and was considered as a covariate for an analysis in this study. The PhNR was considered to be the negative trough between 60 and 100 ms poststimulus onset to avoid including the trough between the b-wave and i-wave peaks when the PhNR is reduced in eyes with glaucoma.

The primary outcome measure used in this study was considered to be the ratio between the b-wave peak to PhNR trough amplitude and the B-wave (b-wave peak to a-wave trough) amplitude (PhNR/B ratio). We chose to use the PhNR/B ratio to normalize the values from different types of electrodes

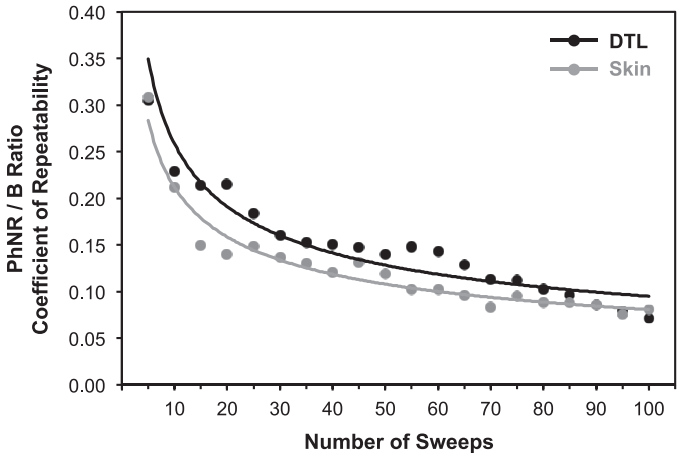

Figure 2. The CoR for the PhNR/B ratio for the DTL (*black*) and skin (*gray*) electrodes within the same session are shown over different number of sweeps. A power function was fitted over the points for each electrode to illustrate the trend of decreasing variability with the increasing number of sweeps.

(which produce a different overall signal amplitude) and because previous studies have demonstrated that this parameter may be a more robust measure.[13,26]

Spectral-Domain Optical Coherence Tomography (SD-OCT)

Spectral-domain optical coherence tomography scans were performed to obtain the average peripapillary retinal nerve fiber layer (RNFL) thickness measurement in all participants, using a SD-OCT imaging system (Spectralis HRA+OCT device; Heidelberg Engineering, Heidelberg, Germany). A peripapillary scan consisting of 1536 A-scans on a 3.5-mm-diameter circle centered on the optic nerve head was performed on the high-speed setting, with 100 image frames averaged.

Statistical Analysis

Generalized estimating equation (GEE) models were used for the analyses in this study due to the hierarchical and repeated measures nature of the data. These models were used to examine the intrasession change and coefficient of repeatability (CoR; a value representing where 95% of the test–retest difference is expected to lie) of the PhNR/B ratio, as well as comparing the raw PhNR/B ratio between the two electrodes. Intraclass correlation coefficients (ICC) of the PhNR/B ratio were also calculated as a measure of relative reliability, and the full details of the ICC model used, calculation of the CoR, and details of the GEE models are described in Supplementary Material 1.

Results

A total of 40 eyes from 23 participants with open-angle glaucoma were included in this study, and they were on average 70.7 ± 10.2 years old (range 48–91 years). The median visual field mean deviation was −3.05 dB (interquartile range [IQR], −1.14 to −5.52 dB), and median RNFL thickness was 80 μm (IQR, 72–85 μm).

Intrinsic Variability over Different Number of Sweeps

The PhNR/B ratio did not exhibit a significant change between the first and second measurement when considering the final traces computed from different numbers of sweeps individually, with both the DTL ($P \geq 0.107$) and skin electrode ($P \geq 0.067$). Therefore, the intrasession CoR of the PhNR/B ratio (representing intrinsic variability) was determined over the different number of sweeps and compared between the two electrode types, as illustrated in Figure 2. No significant difference in the CoR of the PhNR/B ratio between the two electrodes was observed over all numbers of sweeps ($P \geq 0.209$), including no significant difference at 100 sweeps ($P = 0.689$). The average PhNR/B ratios were 0.93 ± 0.03 and 0.95 ± 0.02 at 100 sweeps for the DTL and skin electrodes, respectively, and were not significantly different between the two types of electrode ($P = 0.197$).

The CoR of the PhNR/B ratio and ICC values at 10 and 100 sweeps for both types of electrodes are summarized in Table 1, illustrating the reduced level of intrinsic variability with a larger number of sweeps. At 100 sweeps, the ICC for the skin electrodes was 0.89 and 0.91 for the right and left eyes analyzed, respectively. Given that both electrodes obtained the similar PhNR/B ratio values and exhibited similar intrinsic variability, subsequent analyses are shown only for the skin electrodes.

Factors Associated with Intrinsic Variability

The association between the absolute test–retest difference of the PhNR/B ratio (representing the intrinsic variability) at 100 sweeps and several factors including age, the average PhNR/B ratio (representing the magnitude of the RGC response), the average B-wave amplitude (representing the magnitude of the

Table 1. Indices of Intrinsic Variability of the PhNR/B Ratio (Units) for DTL and Skin Electrodes within the Same Session

	DTL		Skin	
	10 Sweeps	100 Sweeps	10 Sweeps	100 Sweeps
CoR				
Both eyes	0.23 (0.14–0.32)	0.07 (0.04–0.11)	0.21 (0.17–0.26)	0.08 (0.05–0.10)
ICC				
Right eyes	0.51 (0.12–0.76)	0.91 (0.79–0.96)	0.58 (0.21–0.80)	0.89 (0.75–0.95)
Left eyes	0.76 (0.51–0.89)	0.98 (0.94–0.99)	0.62 (0.27–0.82)	0.91 (0.81–0.96)

All values are reported with its 95% CI in parentheses. Analyzed separately when including only the right and left eye of participants where both eyes were included in this study.

overall signal), and RNFL thickness (representing disease severity) were examined. Only the B-wave amplitude and RNFL thickness were found to be associated with the extent of the intrinsic variability ($P < 0.001$), but not age and the PhNR/B ratio ($P \geq 0.127$); these two factors remained independently associated with the extent of the intrinsic variability in a multivariate analysis ($P \leq 0.002$; Table 2).

The absolute test–retest difference of the PhNR/B ratio over a range of B-wave amplitude and RNFL thickness is plotted in Figure 3, illustrating how the magnitude of intrinsic variability increases with a decrease in the B-wave amplitude and an increase in the estimated number of RGCs. Two examples from this study are also shown in Figure 4 to illustrate the latter association.

Association between Disease Severity and PhNR/B Ratio

The PhNR/B ratio exhibited a significant association with the RNFL thickness, decreasing by 0.02 units on average for every decrease in 10 μm in RNFL thickness (95% confidence interval [CI] = −0.00 to −0.04 units per 10 μm; $P = 0.010$). However, there was no significant association between the PhNR/B ratio and age (−0.03 units per decade, 95% CI = −0.6 to 0.01 units per decade; $P = 0.101$). A plot of the PhNR/B ratio against the RNFL thickness is shown in Figure 4.

Discussion

This study found that the PhNR (represented by the PhNR/B ratio) and its intrinsic variability were comparable between the conjunctival and skin electrodes and highlighted the substantial reduction in intrinsic variability that can be achieved by increasing the number of sweeps performed. Greater intrinsic variability was also found to be independently associated with lower overall signal amplitude and larger RNFL thickness. These findings are important considerations when seeking to determine whether short-term and long-term functional changes are truly occurring in eyes with glaucoma.

The findings that the PhNR and its intrasession test–retest repeatability were comparable between conjunctival and skin electrodes in eyes with

Table 2. Analysis of Factors Associated with the Intrinsic Variability of the PhNR/B Ratio Using Univariate and Multivariate GEE Models

	Univariate			Multivariate		
Parameter	β	95% CI	*P* Value	β	95% CI	*P* Value
Age, year	−0.03	−0.12 to 0.06	0.553	-	-	-
PhNR/B ratio, units	6.30	−1.80 to 14.39	0.127	-	-	-
B-wave amplitude, μV	−0.13	−0.19 to −0.06	<0.001	−0.09	−0.15 to −0.04	0.001
RNFL thickness, μm	0.06	0.03 to 0.10	<0.001	0.05	0.02 to 0.08	0.002

PhNR/B ratio scaled by a factor of 100. Values for the PhNR/B ratio and B-wave amplitude are obtained from the average of the two trials with 100 sweeps.

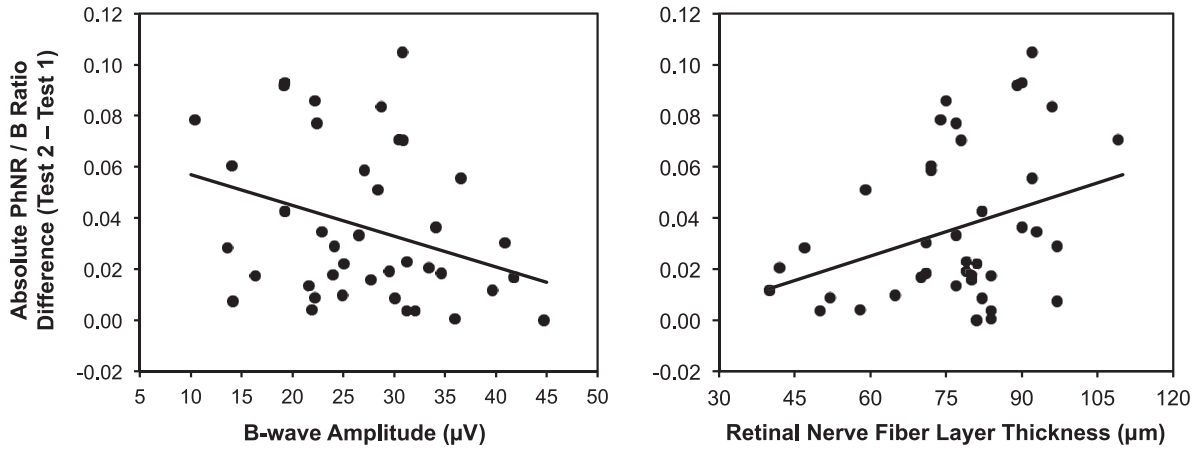

Figure 3. Plots of the absolute test–retest difference of the PhNR/B ratio against the B-wave amplitude (*left*) and RNFL thickness (*right*) using data from the skin electrodes. These plots illustrate the increasing magnitude of intrinsic variability with the decreasing magnitude of the B-wave amplitude and larger RNFL thickness. A *black line* representing the modeled fit from the univariate GEE models is also shown on each graph.

glaucoma are consistent with a previous study that examined normal eyes from younger participants.[23] It is also consistent with a previous study that examined eyes with glaucoma using both of these electrode types with the PERG.[25] The similar extent of intrinsic variability for the PhNR/B ratio between these two electrode types is due to the similar ratio between the intrinsic variability of the PhNR compared to the overall signal amplitude (where the PhNR trough to b-wave peak amplitude for the skin electrodes was on average 36% of the amplitude for conjunctival electrodes; data not shown).[23,25] Although traditionally frowned upon by many clinical electrophysiologists, skin electrodes are easier to use (requiring less skill and training), are less costly, and present a lower risk of corneal

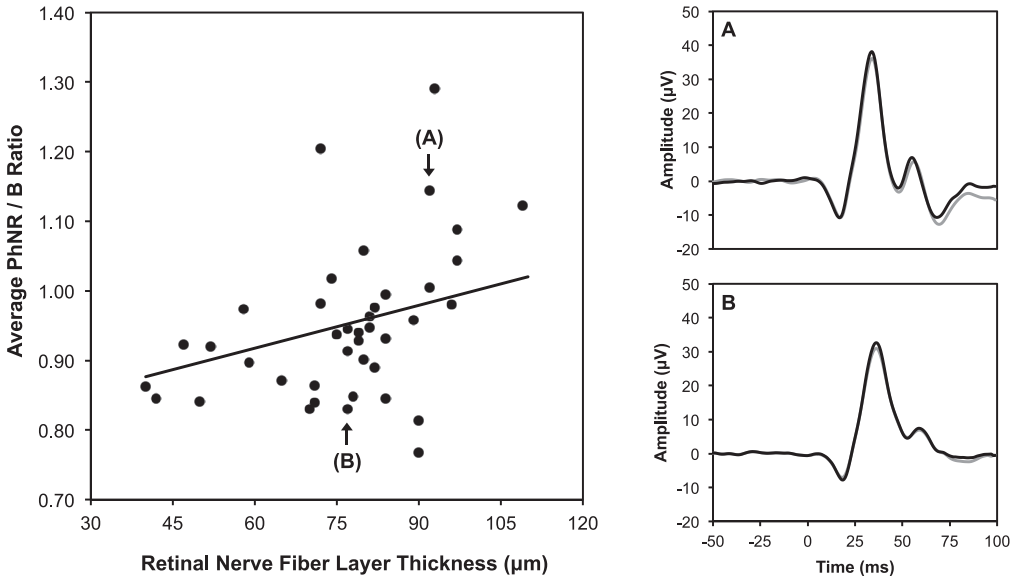

Figure 4. The PhNR/B ratio is plotted against the RNFL thickness using data from the skin electrodes (*left*); the association between these two parameters from the GEE model is shown (*solid line*). Two examples of the ERG traces from this study are also shown (*right*), with each example consisting of two measurements (*black* and *gray lines*) averaged from 100 sweeps. These examples are shown to illustrate how an eye with a larger RNFL thickness exhibits a greater degree of intrasession variability (A) than an eye with a smaller RNFL thickness (B).

abrasion or infection, overcoming some limitations in using the PhNR in a widely clinically applicable manner.

While increasing the number of sweeps substantially reduced the intrinsic variability of the PhNR, we sought to examine whether there were other factors associated with the intrinsic variability for a protocol that involved measuring the PhNR with 100 sweeps using skin electrodes. Larger intrinsic variability was independently associated with lower B-wave amplitude (representing the overall signal amplitude) and a greater RNFL thickness (or milder disease severity). For skin electrodes, the overall signal amplitude is markedly influenced by the distance of the electrode placement below the pupil.[27] It is possible that measurements with electrodes placed farther below the pupil have a smaller signal-to-noise ratio (perhaps because of a disproportionate decrease in signal relative to the noise level), therefore resulting in a greater magnitude of intrinsic variability. However, there are also other factors associated with larger overall signal amplitude, including the duration of adaptation[28–30] and extent of pupillary dilation,[31] but this study is unable to determine which of these factors underlie the association between the magnitude of intrinsic variability and overall signal amplitude. While some studies have reported a reduction in either the a- or b-wave amplitude (components of the B-wave amplitude in this study) in eyes with glaucoma[8,32] and others have not,[6,7,13] we did not find an association between the B-wave amplitude and disease severity in this study (data not shown). We therefore concluded that the independent association between the intrinsic variability and B-wave amplitude must be attributed to factors other than the disease severity.

The decrease in intrinsic variability with worsening disease severity in eyes with glaucoma is also consistent with previous findings using PERG,[33] although neither the previous study or the present study are able to determine the exact mechanism behind this finding. The absence of a significant association between the absolute test–retest difference and the average PhNR/B ratio indicates that the intrinsic variability is not due merely to the magnitude of the PhNR,[34] leaving the significant association between the intrinsic variability and disease severity suggestive of biological origins to this finding.

These findings have important implications for future studies that seek to perform measurements of the PhNR in eyes with glaucoma. First, the viability of skin electrodes as an alternative to conjunctival electrodes allows these measurements to be performed by less-experienced examiners with better patient compliance, improving its clinical applicability. Second, future studies seeking to measure the PhNR should consider obtaining more sweeps than historically used, especially when seeking to accurately detect short-term or long-term changes in glaucoma. Using a protocol that performs 100 sweeps at a frequency of 2 Hz requires only approximately 1 minute for both eyes. Finally, this study highlights how the intrasession test–retest repeatability should not be considered uniform across the range of disease severity in glaucoma, which is important, especially when considering whether longitudinal disease progression or short-term change with an intervention or a provocative test has taken place.

Limitations of this study include the sample size, short duration of adaptation, and the intrasession design for examining test–retest variability. However, the duration of adaptation was the same for all participants in this study, and we did not observe a systematic change in the PhNR/B ratio between the two measurements. Furthermore, investigating the measurement variability within the same session allows us to determine what factors (such as number of sweeps or disease severity) are associated with the variability. Future studies are required to examine the viability of both electrode types between sessions in addition to the within-session variability examined in this study, as well as to examine the influence of adaptation duration on the PhNR/B ratio measurements.

In conclusion, this study demonstrated that skin electrodes are a viable alternative to conjunctival electrodes for the measurement of the PhNR, with the intrinsic variability of this measure being substantially reduced with an increased number of sweeps. This study highlights the importance of considering disease severity when examining whether short-term or long-term changes in PhNR are likely to have occurred.

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