Photopic Negative Response versus Pattern Electroretinogram in Early Glaucoma

Dunja Preiser,1 Wolf A. Lagrèze,2 Michael Bach,1,3 and Charlotte M. Poloschek1–3

PURPOSE. Photopic negative response (PhNR) and pattern electroretinogram (PERG) are electrophysiological markers of retinal ganglion cell function; both are reduced in glaucoma. We compared PhNR and PERG in different stages of the disease.

METHODS. Eleven eyes with preperimetric glaucoma (glaucomatous optic disc with normal field); 18 with manifest glaucoma; and 26 normals were included. We obtained PhNR (flash strength from 0.1–4 cd/s/m²) and steady-state PERG and analyzed PhNR amplitude (baseline to 72 ms trough); PhNR/b-wave ratio; PERG amplitude; and PERG ratio (0.8/16").

RESULTS. Identification of PhNR structure was only reliable ≥1 cd/s/m² flash strength; amplitude and receiver operating characteristics (ROC) area under curve (AUC) changed little from 1 to 4 cd/s/m². Both PhNR and PERG (amplitude and ratio) were reduced in preperimetric and more so in manifest glaucoma. AUCs based on PhNR/PERG amplitudes were not significantly different from chance in preperimetric glaucoma (AUCs 0.61/0.59), but were significant in manifest glaucoma (0.78/0.76); ratios were significant in both glaucoma groups (0.80/0.73 and 0.80/0.79). In spite of that, PhNR ratio and PERG ratio were not significantly correlated (r = 0.22 across all groups); an ROC based on a combination of both reached AUCs of 0.85/0.90 for preperimetric/manifest glaucoma.

CONCLUSIONS. Both PhNR and PERG performed similarly to detect glaucoma; for both, ratios performed better than amplitudes. The PhNR has the advantage of not requiring clear optics and refractive correction; the PERG has the advantage of being recorded with natural pupils. (Invest Ophthalmol Vis Sci. 2013;54:1182–1191) DOI:10.1167/iovs.12-11201

Glaucoma is characterized by apoptotic retinal ganglion cell (RGC) death, whereas bipolar cells and photoreceptors remain nearly normal. In the course of the disease, this may lead to vision loss and ultimately blindness. The exact reasons for RGC loss in glaucoma are still unclear. Several mechanisms have been suggested so far, such as impaired axonal transport of neurotrophic factors, intrinsic and extrinsic apoptosis activation, neurotoxicity via oxidative stress, altered biomechanics around the lamina cribrosa, and an altered immune response promoting neuroinflammatory processes.1–4

Early detection of glaucoma and patients at risk for visual dysfunction is essential. One means of detecting glaucoma prior to visual field loss lies in electrophysiology. An established method to assess RGC function is the pattern electroretinogram (PERG).5–8 A checkerboard stimulus reverses its local luminances while keeping space-averaged luminance constant. Thus, the ERG signals are largely cancelled out and nonlinearities remain that are known to originate mainly in the RGCs.8–11 Bach et al.12 described that early glaucoma reduces the PERG amplitude to smaller checks prior to a reduction in amplitudes to large stimulus checks, and this can be quantified by taking the PERG ratio (amplitude to smaller checks/amplitude to larger checks).13 Longitudinal studies revealed that the PERG ratio can distinguish glaucoma patients from ocular hypertension (OHT) patients up to four years before measurable visual field defects occur.14–16

Another method to assess RGC function is the photopic negative response (PhNR),17–19 a component of the full-field ERG. The PhNR is a relatively slow negative potential that occurs after the positive b-wave of the ERG; it reaches a trough around 70 ms. The PhNR most likely arises from the activity of retinal ganglion cells and their axons, because it is markedly reduced in monkeys with experimental glaucoma and can be eliminated pharmacologically by tetrodotoxin (TTX).17,18,20 Normalizing (“PhNR/b-wave ratio”) has also been suggested.21

In the present study, we compared PhNR and PERG regarding their sensitivity and specificity to detect preperimetric and manifest glaucoma. The ultimate aim is to develop a methodology that identifies those patients with an elevated IOP who will convert to glaucoma. Thus, therapy could be applied before irreversible retinal damage and visual field loss have occurred, while sparing patients with pure ocular hypertension.

METHODS

Participants

The study was performed according to the tenets of the Declaration of Helsinki22 and was approved by the local ethics committee. Informed consent was obtained prior to examination from all participants.

We included either one or two eyes per patient/healthy subject. When both eyes were included, their results were averaged, except when they were classified with different stages of glaucoma (the latter occurred for two participants; see Data Analysis).

Exclusion criteria were: visual acuity < 20/25, insufficient quality of either PhNR or PERG recordings (e.g., trial glass rim artifacts recognizable in subharmonics of the Fourier analysis, excessive blink artifacts causing marked baseline drift) and any other eye disease (apart from cataracta incipiens, that did not reduce visual acuity to less than

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Table 1. Overview of Participant Groups

<table>
<thead>
<tr>
<th>Preperimetric Glaucoma</th>
<th>Manifest Glaucoma</th>
<th>Normal Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of “eyes”/number of patients</td>
<td>11/11</td>
<td>18/16</td>
</tr>
<tr>
<td>Age, 49–71 y</td>
<td>61.5 ± 6.1</td>
<td>61.5 ± 5.4</td>
</tr>
<tr>
<td>MD, dB</td>
<td>0.35 ± 2.00</td>
<td>−4.48 ± 5.73</td>
</tr>
<tr>
<td>ONH assessment via HRT</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Borderline</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>Outside normal</td>
<td>9</td>
</tr>
<tr>
<td>ONH fundus photography</td>
<td>Normal</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Glaucomatous</td>
<td>11</td>
</tr>
<tr>
<td>Visual acuity</td>
<td>≥ 0.8decimal or ≥ 20/25Snellen</td>
<td></td>
</tr>
</tbody>
</table>

ONH assessment via HRT used the Moorfields regression analysis. All were classified as at least “satisfactory quality.”

20/25). The number of eyes given below and in Table 1 reflect the situation after exclusion.

Manifest Glaucoma Group. We included 16 glaucoma patients; 11 patients with primary open angle glaucoma (POAG), four with normal tension glaucoma (NTG), and one with pigment dispersion glaucoma. All glaucoma patients’ eyes met the following criteria:

1. Glaucomatous appearance of the optic disc: general enlargement of the cupping defined as vertical cup-to-disc ratio ≥ 0.7 or a local notching of the rim, as assessed by glaucoma experts. Agreement between these raters and objective ONH assessment via HRT (using Moorfields regression analysis) can be judged in Table 1, row entitled “ONH assessment....”
2. Visual field defect in automated perimetry (Octopus 1-2-3 perimeter [Haag-Streit AG, Koeniz, Switzerland], program dG1X, or Octopus 301 perimeter with program G1 [Haag-Streit AG]). Visual field defect was defined as three or more adjacent points of ≥5 dB loss or two or more adjacent points of ≥10 dB loss in the absence of any other abnormalities that could explain the defect.

We averaged the data of fellow eyes when they were in a similar glaucoma stage (Glaucoma Staging System 2 [GSS2] stage difference ≤ 1; stage between 1 and 5)22-24. This resulted in 18 “eyes” of 16 subjects for this group’s final analysis.

Preperimetric Group. We included 11 eyes of 11 patients with preperimetric glaucoma, 8 patients with POAG, two with NTG, and one with pigment dispersion glaucoma. These patients met the same criteria as the glaucoma group, apart from the absence of a visual field defect; their mean MD was −0.35 ± 2.00. Thus, all but the two NTG patients had at least two risk factors: glaucomatous appearance of the optic disk and elevated IOP or pigment dispersion syndrome.

Normal Control Group. We recruited an age-matched group of 26 healthy subjects without any known eye disease. Data from fellow eyes were averaged when both eyes met the inclusion criteria, resulting in 26 normal “eyes.”

Table 2. Flash Strengths in Previous Studies

<table>
<thead>
<tr>
<th>Reference</th>
<th>Flash Strength, cd/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Viswanathan et al. 200128</td>
<td>0.78</td>
</tr>
<tr>
<td>Machida et al. 200821</td>
<td>4.8</td>
</tr>
<tr>
<td>Sustar et al. 200928</td>
<td>2.5</td>
</tr>
<tr>
<td>North et al. 201029</td>
<td>0.3</td>
</tr>
<tr>
<td>Machida et al. 201130</td>
<td>4.8</td>
</tr>
<tr>
<td>Kremers et al. 201231</td>
<td>0.03 to 3.0</td>
</tr>
</tbody>
</table>

Confined to recordings in humans and short (3–5 ms) red stimulation on a blue background.

Recording

Prior to electrophysiologic testing, all subjects were refracted and visual acuities evaluated with the Freiburg Visual Acuity Test (FrACT)25 at the stimulation distance for the PERG. We obtained photopic full-field ERG (ERG, optimized for the PhNR) and steady-state PERG; PERG was recorded according to the ISCEV standard,26 using steady-state and an additional checksize.

PhNR

The stimulus, a red flash (655 nm) on a blue background (450 nm) of 10 cd/m², was presented with a full-field LED-stimulator (Q450 SN 66-99-705; Roland Consult, Brandenburg/Havel, Germany). After a review of the existing literature (Table 2), we chose to stimulate with seven different flash strengths between 0.1 and 4 cd·s/m² with a flash duration of 5 ms.27 Flash strength was calibrated with an integrating photometer (P-9710; Gigahertz-Optik, Turkenfeld, Germany) using a photopic Vₛ probe. For each flash strength, we presented 26 flashes at a frequency of 1 Hz.

Retinal potentials were recorded with corneal DTL-like electrodes32 placed near the lower limbus. Gold cup electrodes at the outer ipsilateral canthus served as reference. Signals were amplified and filtered with an analog band-pass filter of 0.5 to 300 Hz and digitized to a resolution of 12 bits at a sampling rate of 1 kHz. Traces exceeding 100 μV or −100 μV in a time window of 100 ms after stimulus onset were rejected as artifacts. The mean of the flash responses that were not rejected as artifacts was calculated for each flash strength.

PhNR amplitude can be measured in different ways: by identifying the PhNR trough around 70 ms, then obtaining the baseline-trough amplitude; or by identifying the amplitude at a fixed time after stimulus onset, again with respect to baseline. Baseline was the mean value across 50 ms before the stimulus flash. When we screened our recordings, we noted that in some cases, the exact location of the PhNR trough is ill-defined. So we turned to the fixed-time approach: Viswanathan et al.28 averaged responses within age bins of 10 years and measured the mean implicit time of the PhNR in each age bin. The implicit time of the PhNR in the group-averaged traces was used for fixed-time point measurements of individual traces, even if this did not coincide with the exact trough of the PhNR on visual inspection. The mean implicit time of all their PhNR data for our age range (50–72 years) was 72 ms. Mortlock et al.35 who also averaged their PhNR data, obtained a mean implicit time of 72 ms as well. Further analysis was based on amplitude at 72 ms as PhNR and also the PhNR/b-wave ratio.

PERG

The stimulus subtended 40° × 30° of visual angle at a distance of 57 cm and was presented on a 21-inch monochrome monitor (GD402 FIMI; Philips, Saronno, Italy) driven at a frame rate of 75 Hz. The
checkboards had a mean luminance of 45 cd/m², a contrast of 98%, and a checksize of 0.8° or 16°, and were counterphased at 15 reversals per second. Luminance variation across the monitor was less than 10% as measured with a spot meter (Minolta Luminance Meter 1°, Minolta Camera Co., Ltd., Osaka, Japan).

Electrodes and their montage were the same as for PhNR recordings. Signals were amplified and filtered with an analog bandpass of 0 to 40 Hz and digitized to a resolution of 16 bits at a sampling rate of 1 kHz by a computer that simultaneously generated the stimuli (System EP2000).34 Traces exceeding ≥ 100 μV were rejected as artifacts; we averaged 2 × 80 sweeps of 1.066 seconds duration. Further analysis was based on the amplitude for 0.8° checks and the PERG ratio.13

Data Analysis
Analysis was automated as much as possible. For the PhNR, peaks/troughs need to be defined, which proved particularly difficult to automate for all records. After comparing various automatic peak finders and reviewing the literature, we proceeded as follows:

- All single flash responses (traces) underwent a phase-neutral Fourier-based low-pass filter with a 45 Hz cutoff frequency. While this reduces the a-wave amplitude, it rejects main interference (50 Hz) and noise, rendering peak/trough detection markedly more robust. Since all groups were treated likewise, no confounds are expected.
- Baseline was estimated as the average in the time window —45 ms to 0 ms, and subtracted from the trace.
- Traces exceeding ≥ 100 μV in a time window from 0 to 100 ms were rejected as artifacts (but remain visible in Figs. 3, 4 as light gray traces).
- All remaining traces were averaged; definition of a-, b-wave, and PhNR was as follows:
  - a-wave: trough in the time window 10 to 30 ms,
  - b-wave: the maximum in a time window of 50 ms after the a-wave trough,
  - PhNR: amplitude at 72 ms.
- Calculation of the “PhNR/b-wave ratio” (abbreviated to PhNR ratio), namely the ratio of the PhNR divided by the preceding b-wave as defined above.21

For the PERG, the following steps took place:

- Detrending of the 1066-ms traces by subtracting a linear-regression “saw-tooth.”
- Discrete Fourier transform,35 scaled to provide magnitudes in microvolts at the reversal rate.
- Calculation of “true amplitude” and signal-to-noise ratio35,36; all response P values were below 0.01.
- Calculation of the “PERG ratio,” namely the ratio of the “true amplitude” evoked by 0.8° checks and the one by 15° checks.

All the above steps were programmed into a technical graphing and data analysis program (IGOR Pro; WaveMetrics, Portland, OR) as an extension to the EP2000 analysis package.34 Peak times and amplitudes were semi automatically transferred to a master spreadsheet, which was arranged in an eye-per-row fashion and also comprised all other relevant data (visual acuity, age, group, visual field measures, PERG, etc.). From this spreadsheet, we performed all further analysis and figure production with the R statistical system.57 For quantitative receiver operating characteristics (ROC) analysis,58 the new “pROC” package59 was employed; this runs faster than the version we had programmed previously.16

In ophthalmological studies, one needs to deal with the “eyes or patients” problem40,41 because the data from the two eyes often correlate, reducing the effective number of cases. We took the following approach here: when the two eyes of a manifest glaucoma patient did not differ in their GSS2 index23,24 by more than 1, the values of the two eyes were averaged. The resulting numbers of “eyes” and patients were given in Table 1. For both the control and the preperimetric groups, all fellow eyes were averaged (as follows from their definition). Only in the manifest glaucoma group, there were two patients where the two eyes differed to such degrees that they were included individually.

RESULTS
PhNR
Figure 1 shows sample ERGs of a normal control (left) and a glaucoma patient (right). The a-wave (first trough) and b-wave (first peak) are followed by two negative troughs. The second, deeper one is the PhNR. It is reduced in the glaucomatous eye compared with the control eye. The peak between the two troughs is the i-wave. In the examples in Figure 1, the positioning of PhNR at 72 ms seems suboptimal; this is not typical, but highlights the problems of objective automatic analysis.

The most important stimulus parameter for the PhNR is arguably the flash strength. In Figure 2, we depict the ERG across all seven flash strengths in one control subject. While here the peaks and troughs are well defined for all flash strengths, an overview of all recordings revealed that only from

![Figure 1](http://tvst.arvojournals.org/)
Among the three groups (normal subjects, preperimetric glaucoma, and manifest glaucoma) we compared PhNR amplitudes and ratios. PhNR mean amplitude is reduced in both glaucoma groups, not significantly in preperimetric glaucoma ($P = 0.69$) and significantly in the manifest group ($P = 0.0018$; Fig. 3, left). The PhNR reduction in the preperimetric glaucoma group is not statistically significant ($P = 0.69$), whereas it is highly significant ($P = 0.0018$) in the manifest glaucoma group. However, for the PhNR ratio (Fig. 3, right), in both preperimetric and manifest groups, the reduction is highly significant ($P < 0.01$) and rather similar in the median.

The Figure 3 results were obtained with a flash strength of 1 cd·s/m². Across the four flash strengths from 1 to 4 cd·s/m², PhNR amplitude has a shallow peak at 1.7 cd·s/m² (Fig. 4, left). In an ANOVA, the factor flash strength had no significant effect for AUCs ($P = 0.82$), whereas the factor group was highly significant for PhNR amplitude ($P < 0.0001$). Interestingly, when performing the ROC analysis, for the PhNR at these four flash strengths, the AUC was highest at 1 cd·s/m² (Fig. 4, right, a very shallow effect). Thus, we chose the PhNR responses at 1 cd·s/m² for comparison with the PERG (Kremers et al. 31 also found that this flash strength performed best).

**PERG**

Figure 5 depicts sample PERG recordings of a normal and a glaucoma eye. At a check size of 16⁰, the amplitudes of the glaucomatous eye and the normal eye are essentially the same, whereas at a check size of 0.8⁰, the amplitude in glaucoma is considerably smaller compared with the normal eye.

Similar to the PhNR, a group comparison of PERG findings in glaucoma patients and normal controls reveals the following differences (Fig. 6): in preperimetric glaucoma, the amplitude at 0.8⁰ and the PERG ratio are reduced compared with normal controls, but only the PERG ratio is significantly reduced ($P = 0.021$; Fig. 6, right). In the manifest glaucoma group, both the amplitude at 0.8⁰ and the PERG ratio are significantly reduced (both $P < 0.01$).

**Comparing PhNR and PERG**

To avoid arbitrary choices of normal/pathologic thresholds in the comparison between PhNR and PERG, we calculated ROCs. Figure 7 and Table 3 present the results for all combinations of glaucoma disease stage, electrophysiological measure (PhNR versus PERG), and analysis procedure (amplitude versus ratio). The findings in Figure 7 and Table 3 follow relatively simple rules: AUCs increase from preperimetric to manifest glaucoma, and they increase from ROCs being based on raw amplitudes to those based on ratios. PhNR and PERG perform roughly equally, the PhNR a little better.

**Are PhNR and PERG Correlated on an Individual Basis?**

Since both PhNR and PERG are affected in glaucoma—though somewhat differing per stage—the question arises whether the two measures are correlated in individual eyes. Compar-
ing raw PhNR and PERG 0.8° amplitudes finds a significant correlation ($r = 0.34$, $P = 0.010$). In contrast, the normalized measures PhNR ratio and PERG ratio—which are more successful in reflecting glaucoma damage—were not significantly correlated (Fig. 8): While the regression lines (not depicted) have a similar slope, the correlations all fail to reach significance ($r = 0.22$, $P = 0.11$ across groups, and $P \geq 0.36$ for each individual group).

The lack of correlation between PhNR and PERG ratios could be explained in two ways: either the measures are too noisy or the two measures tap different disease mechanisms. If the latter were true to some degree, then a linear combination of the two measures should be a better discriminator. To test this hypothesis, the PERG- and PhNR ratios were $z$-transformed, and then an ROC was calculated based on $z$(PERG ratio) $+ z$(PhNR ratio). ROC-AUCs based on this combination rose markedly (Table 3, rightmost column).

**DISCUSSION**

We recorded PhNR and PERG in glaucoma patients, preperimetric glaucoma patients and normal controls to compare their potential for detecting glaucoma in different stages of the disease. Both PhNR and PERG amplitude were reduced in glaucoma and more so manifest compared with preperimetric glaucoma; both measures profited from normalizing. Assessing diagnostic efficacy on a single-subject basis via a ROC (Fig. 7), the PhNR ratio and PERG ratio achieved similar AUCs in the manifest glaucoma group (0.80 and 0.79, respectively) with a mean MD of $-4.5$. In the preperimetric glaucoma group, AUCs were lower but the 95% confidence bands for the ratios did not include the 0.5-chance level. Thus, PhNR and PERG are nearly equal in detecting manifest glaucoma, and normalizing improves efficacy of both measures similarly.

**Comparison of PhNR and PERG in Other Studies**

Prior studies comparing PhNR and PERG in glaucoma arrived at the following results: Sustar et al.,28 who examined patients with a mean MD of 10.3 ± 6.3, had markedly better results for PhNR than for PERG (AUC 0.97 for PhNR and 0.89 for P95; their MD of $+10.3$ corresponds to $-10.3$ in the nomenclature used here). Drasdo et al.,43 who examined glaucoma patients with a mean MD of $-3.2$, found almost equal AUCs for PhNR and for PERG (0.86 for PhNR and 0.81 for the transient P50-P95). North et al.29 examined patients with early glaucoma...
(mean MD: −1.89) and reported considerably better results for PERG (AUC 0.78 for P95–P50 and 0.60 for PhNR). Together with our findings, the common finding is, the less advanced the glaucoma, the better the PERG fares compared with the PhNR when the PhNR is not normalized.

To date, the PERG has been studied more extensively in glaucoma compared with the PhNR. PERG changes have been reported to occur prior to visual field losses.\textsuperscript{12,15,16,44–49} In their longitudinal study, Bode et al.\textsuperscript{16} found that PERG detects glaucoma patients four years before visual field defects appear in standard automated perimetry. The present work suggests that the PhNR is altered prior to visual field in structurally defined glaucoma. There are less (and somewhat inconsistent) studies so far examining PhNR in ocular hypertension: North et al.\textsuperscript{29} and Viswanathan et al.\textsuperscript{18} found reduced PhNR amplitudes in patients with OHT. Colotto et al.\textsuperscript{50} and Horn et al.\textsuperscript{51} in their longitudinal study, Bode et al.\textsuperscript{16} found that PERG detects glaucoma patients four years before visual field defects appear in standard automated perimetry. The present work suggests that the PhNR is altered prior to visual field in structurally defined glaucoma. There are less (and somewhat inconsistent) studies so far examining PhNR in ocular hypertension: North et al.\textsuperscript{29} and Viswanathan et al.\textsuperscript{18} found reduced PhNR amplitudes in patients with OHT. Colotto et al.\textsuperscript{50} and Horn et al.\textsuperscript{51} in

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Influence of flash strength on PhNR amplitude (left) and ROC-AUC (right) in manifest glaucoma. PhNR amplitude is highest at 1.7 cd·s/m². AUC based on both amplitude and ratio has a shallow maximum at 1 cd·s/m². The horizontal dashed line on the right depicts an AUC of 0.5, corresponding to the chance level. AUC differences between flash strengths are small compared with the 95% confidence intervals (antennas); accordingly, in an ANOVA, the factor flash strength is not significant ($P = 0.82$).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5}
\caption{PERGs. (A) From one eye of a normal control. (B) From one eye of a glaucoma patient. Time-domain (left) and Fourier spectra (right) are shown. Top: 0.8° check size. Bottom: 16°. Above the spectra, the response frequency in Hz with $P$ value and amplitude in $\mu$V are given. $P$ values were calculated based on the signal-to-noise-ratio.\textsuperscript{36} At 16° stimulus size, the amplitudes of the glaucoma and the normal eye are nearly the same (after Fourier transformation, 2.1 $\mu$V and 2.4 $\mu$V, respectively); whereas at 0.8° stimulus size, the amplitude in glaucoma is considerably smaller than in the normal eye (1.0 $\mu$V and 2.3 $\mu$V, respectively).}
\end{figure}
contrast found no amplitude reduction in OHT. Different
stimulus conditions might be one reason for these discrep-
cyencies: North and Viswanathan used short flashes (5 ms),
Horn and Colotto used long flashes (200 ms). A second
reason might be small sample sizes. Furthermore, one must
keep in mind that only 1% to 2% of OHT patients develop
glaucoma per year. Thus, the fact that PhNR is not reduced in
OHT does not necessarily argue against PhNR as an early
marker of glaucoma, especially in studies with small cohorts.
To clarify whether the PhNR is able to detect glaucoma before
visual field defects occur, further studies with larger cohorts or
longitudinal studies are needed.

Table 3. AUCs for All Combinations

<table>
<thead>
<tr>
<th>Glaucoma Group</th>
<th>PhNR Amplitude</th>
<th>PhNR Ratio</th>
<th>PERG Amplitude</th>
<th>PERG Ratio</th>
<th>PhNR + PERG Ratios Combined</th>
</tr>
</thead>
<tbody>
<tr>
<td>Preperimetric</td>
<td>0.614</td>
<td>0.797*</td>
<td>0.586</td>
<td>0.731*</td>
<td>0.850*</td>
</tr>
<tr>
<td>Manifest</td>
<td>0.779*</td>
<td>0.801*</td>
<td>0.765*</td>
<td>0.786*</td>
<td>0.897*</td>
</tr>
</tbody>
</table>

Includes glaucoma disease stage, electrophysiological measure (PhNR versus PERG), and analysis procedure (amplitude versus ratio).

* 95% CI did not include the chance value of 0.5.

Ratios Improve Detection Efficacy for PhNR and PERG

The ROC analyses (Fig. 7) clearly demonstrate that for both
PhNR and PERG, it is beneficial to calculate ratios, a time-
proven electrophysiological technique. For the PhNR,
following Machida et al., we used the b-wave. For the PERG,
we divided by the PERG amplitude to very large checks. Both
normalizers have limitations. On the one hand, the b-wave
might be reduced in glaucoma. The literature is inconsistent on
this; of the reports looking at both PhNR and b-wave in
glaucoma, two report no b-wave reduction in glaucoma and
two do find b-wave reduction. In our own data, for
preperimetric glaucoma we see an insignificant (P = 0.11) rise

Figure 6. Comparison of PERG amplitude and PERG ratio across normal controls, preperimetric glaucoma and manifest glaucoma eyes. Left: amplitude at 0.8°. Right: PERG ratio. Plot details as in Figure 4; the P values indicate that group’s difference from the normal controls. In preperimetric glaucoma, the PERG ratio is significantly reduced (P = 0.021), but not the raw PERG amplitude; in manifest glaucoma, both measures are highly significantly reduced (P = 0.003/0.002).
of the b-wave, and no change in manifest glaucoma compared with normals (P = 0.99). Thus, PhNR normalization currently looks promising. On the other hand, the large-check PERG is reduced in advanced glaucoma.11 The interindividual variance reduction by normalization has two likely major causes: individual amplitude, which might be related to eye size, and electrode location. Both are multiplicative effects.

Relation between PhNR and PERG
We explain the significant correlation between PhNR amplitude and PERG 0.88 amplitude by “trivial” factors like eye size or electrode position, which vary between eyes but are common for the two measures. Calculation of the ratio factors this out, leaving no significant correlation between individual PhNR ratio and PERG ratio values. The highest correlation was for preperimetric glaucoma with r = 0.3, thus explaining less than 10% of the variance. Because of this low correlation, it seemed interesting to combine the PhNR and PERG results, and indeed the area under curve of the ROC increased (Table 3, rightmost column). For manifest glaucoma, it reached 0.9—a rather high value; when calculating the ROC based on MD (which is part of the group definition for the manifest group), AUC is 0.87. (As an aside: in the preperimetric group, AUC based on MD was at chance level [0.48], as would be expected by group definition.) The highest AUC value results when using the GSS2 as discriminator, yielding an AUC of 0.99 in the manifest group; this is, of course, expected and a circular analysis, but it serves to highlight the robustness of the glaucoma stage attribution.

The findings that combination of PhNR and PERG increases individual discrimination, and that the two measures are not
correlated (Fig. 8) fit well together. However, is this just due to better noise rejection or could it be that these two physiological measures pick up different disease mechanisms? This situation brings to mind the well-corroborated finding that the PERG is pathologic in glaucoma at loci where the visual field is still normal.54–57 The recent findings by Machida et al.58 however, suggest that the PhNR is more closely related to populations, possibly differently affected in (early) glaucoma. Whether the two measures lean toward different ganglion cell populations, possibly differently affected in (early) glaucoma.

CONCLUSIONS

The aim of present investigation was to assess two early markers for glaucoma, namely the PhNR and the PERG. Both were similarly effective, especially when normalized. Assuming they reflect the same disease mechanisms, the choice of one over the other can currently be guided by practical considerations, namely PhNR requiring mydriasis and the PERG requiring an optimal retinal image (i.e., clear optics and adequate refractive correction).

Acknowledgments

We thank Jan Kremers and Gobinda Pangeni for lively lab exchanges while tackling related research questions.

References


