Pattern Electroretinogram in Glaucoma Suspects: New Findings from a Longitudinal Study

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PURPOSE. Early detection of glaucoma remains a challenging problem and needs long-term, prospective studies. The pattern electroretinogram (PERG) directly reflects retinal ganglion cell function. The PERG was evaluated by extending a prospective study of patients with ocular hypertension and evaluated amplitude, PERG ratio, peak time, and trends thereof.

METHODS. One hundred twenty eyes of 64 patients with intraocular pressure greater than 25 mm Hg (or \( \geq 23 \) mm Hg with additional risk factors), normal visual fields, normal optic disc appearance, and visual acuity \( \geq 0.8 \) were included in the study. Mean follow-up time was 10.3 years. The per-visit measures of amplitude at 15 reversals/s to 0.8° check size, PERG ratio (0.8°/16°), peak time, visual field, and their trends were analyzed.

RESULTS. Over the course of the study 13 eyes converted to glaucoma according to a visual field definition. Amplitude to 0.8° check size, PERG ratio, and peak time were significantly lower in converters. Amplitude and PERG ratio predicted conversion 4 years ahead with a sensitivity/specificity of 75%/76%. Slope analysis revealed significant differences in the PERG ratio; however, trends did not predict conversion as successfully as single-visit measures.

CONCLUSIONS. The PERG, especially the PERG ratio, detected glaucoma patients 4 years before visual field changes occurred, with a sensitivity/specificity of 75%/76%. Slope analysis required multiple visits, but provided little additional information in detecting converters. (Invest Ophthalmol Vis Sci. 2011;52: 4300 – 4306) DOI:10.1167/iovs.10-6381

Glaucoma is characterized by chronic retinal ganglion cell (RGC) damage that does not become apparent in standard automated perimetry as visual field defects until 25% to 35% of the RGCs have been lost.1 An additional tool would be helpful in identifying glaucoma among patients with high intraocular pressure (IOP) or suspicious optic nerve morphology.

The retinal response to pattern stimulation, the pattern electroretinogram (PERG), predominantly reflects RGC activity.2–6 In patients with glaucoma or high-risk ocular hypertension (OHT), both the P50- and N95 amplitude in transient and steady state stimulations are reduced.7–9 In glaucoma, the loss of PERG amplitude can appear before visual field changes occur.7,10–17 So far, one long-term study has found pathologic changes up to 1 year before visual field conversion.18 Sensitivity to detect glaucomatous RGC damage is optimal for a check size of 0.8° and markedly lower for very large check sizes (e.g., 16°).7–19,20 Sensitivity to glaucoma damage increases with higher temporal presentation rate (steady state stimulation, e.g., 15 reversals/s [rps]) compared with transient stimulation (<4 rps).12,21–23 Intra-individual and inter-individual variability can be reduced by using the PERG ratio, the ratio of the amplitudes at 0.8° and 16° checks, which also obviates the need for age correction.19,21–23 The PERG may also detect potentially reversible RGC malfunction, making it a potent tool in selecting a therapeutic regimen.24,25

We will refer to patients who developed criteria-compliant visual field defects during the study as “converters” and to those who did not as “nonconverters.”

METHODS

Population Data

This study adhered to the tenets of the Declaration of Helsinki26 and was approved by the local review board. PERGs were recorded within the Freiburg OHT study from 1993 to 2007. Mean follow-up time of the present study was 10.3 years (range, 3.1–14.4 years), thus adding 35 months to the published Freiburg OHT study.18 That study comprised 120 eyes of 64 patients with high-risk OHT, defined as \( \geq 25 \) mm Hg or \( \geq 25 \) mm Hg if additional risk factors (glaucoma in the fellow eye or positive family history) were present. Examinations were conducted every 6 months and included visual acuity (had to be \( \geq 0.8 \), measured at the stimulation distance of 57 cm with appropriate correction7). initial normal visual field perimetry (Octopus 123; Haag Streit, König, Switzerland) and initial normal optical disc cupping on clinical examination and photographs. Patients with diabetic retinopathy, secondary glaucoma, and any other disease affecting the visual field or the optic nerve were excluded. The use of IOP-lowering medication or ocular surgery was not an exclusion criterion.18,28

We extended and re-examined data from a prospective, long-term study, to determine the ability of steady state PERG to detect glaucoma. We evaluated different PERG recordings of high-risk patients to identify those who would develop visual field defects later on. We analyzed the PERG measures amplitude, PERG ratio, and peak time. Two modes of analysis were compared: single-visit values and trends and the slopes thereof across time.

Stimulation, Recording, and Analysis of the PERG

Retinal potentials were recorded simultaneously from both eyes with corneal DTL electrodes placed near the lower limbus.29–31 Gold-cup electrodes at the ipsilateral outer canthus were used as the reference. After amplification of the signals by 50,000, they were filtered with an analog band-pass filter of 1 to 100 Hz and then digitized to a resolution of 12 bits at a sample rate of 1 kHz. The stimuli were generated by the same computer that acquired the data, thus ensuring close time
correspondence between stimulus and averager.\textsuperscript{35} We averaged 2 × 80 sweeps of 1066-ms duration. Traces exceeding 130 µV were automatically rejected as artifacts and repeated.

For stimulation, checkerboard patterns appeared on a video monitor covering 35° × 30° of visual angle at a distance of 57 cm. Check size was either 0.8° or 16°, the latter being shorthand for 17.5° × 15°, where four checks filled the entire screen. Check borders always met at the screen’s edge, ensuring that no net luminance occurred on contrast reversal. The patterns had a contrast of 98% and a mean luminance of 45 cd/m², undergoing phase reversal at 15 rps (equivalent to 7.5 Hz), thus evoking a steady state PERG response. Analysis was based on the magnitude (called amplitude) at 15 Hz after discrete Fourier transformation.\textsuperscript{33,34}

We report herein on the data collected from January 1993 to December 2007. Only eyes with a minimum follow-up time of 3 years were included. Our results from 1993 to January 2005 have been reported previously.\textsuperscript{18} The peak times of recordings from 1999 to 2007 have not been analyzed before and are reported here for the first time; peak time data before 1999 were not accessible. Peak times of the steady state recordings were derived after Fourier analysis from the phase of the recordings\textsuperscript{36} and represent the timing of the response at steady state recordings were derived after Fourier analysis from the peak time data before 1999 were not accessible. Peak times of the recordings from 1999 to 2007 were included. Our results from 1993 to January 2005 have been reported previously.\textsuperscript{18} The peak times of recordings from 1999 to 2007 have not been analyzed before and are reported here for the first time; peak time data before 1999 were not accessible. Peak times of the steady state recordings were derived after Fourier analysis from the phase of the recordings\textsuperscript{36} and represent the timing of the response at 15 Hz.

The response phase was defined according to

\[
 f(t) = \cos(\omega \cdot t - \varphi)
\]

where \( t \) is time (e.g., in seconds), \( f(t) \) is the amplitude time course of the spectral component considered (here, double the fundamental frequency), \( \omega \) is the angular frequency, and \( \varphi \) is the phase.

Thus, a delayed response corresponds to higher phase values. Furthermore, use of cosine rather than sine ensures that a peak at 0 ms corresponds to a phase of 0° and a peak time of 0 ms. Although the relation between peak time and phase is not unique, the stimulation period (1/15 Hz ≈ 67 ms) was longer than the normal peak time of the PERG (~ 50 ms), so that wraparound is not expected. This longer peak time is also apparent from Figure 2C, showing an actual phase range of 220° to 350° in our recordings. The peak time thus derived refers to the peak of the sinusoid at the reversal frequency and consequently can deviate slightly from actual peak time as higher harmonics affect the shape.

\[ f(t) = \cos(\omega \cdot t - \varphi) \]

Statistics were calculated with the R statistical system.\textsuperscript{38} We used the \textit{t}-test for group comparison, subsequently applying the Bonferroni-Holm correction\textsuperscript{39} for multiple testing. In the figures (Figs. 2, 6) we report the raw \( P \) in the text where the correction result is given. We computed ROCs (receiver operating characteristics of converting versus the nonconverting eyes) based on single-visit amplitudes, ratios, and peak times and their slopes. These ROCs were computed for different times before conversion. Sensitivity, specificity, and the area under the curve (AUC) of the ROCs were used to quantify the PERG’s potential to diagnose glaucoma before conversion. Confidence intervals for the AUCs were obtained by bootstrapping (or resampling),\textsuperscript{40–42} as follows: To keep the proportion of converters constant, the patient group was divided into converters and nonconverters. For each of these subgroups a random sample was assembled (with replacement, using the Sample procedure of R, Ref. 41, pages 46 – 49); then, the groups were recombined to calculate the ROC and the AUC. These steps were repeated typically 10,000 times for each condition, and various percentiles (2.5%, 25%, lower quartile; 50%, median; 75%, upper quartile, and 97.5%) of the resulting AUC distributions were calculated.

\section*{RESULTS}

There is some inconsistency in the literature on the sign of phase: Ventura and Porciatti,\textsuperscript{7} for example, use the convention opposite to ours; peak time, in contrast, is unambiguous. In accordance with the recent ISCEV standards,\textsuperscript{36,37} we use the term peak time rather than latency or implicit time.

The amplitude at 0.8° checks, the PERG ratio (the ratio of the amplitude to 0.8° divided by the amplitude at 16°), and the peak time at 0.8° checks were analyzed both as single-visit measures and slopes over time.

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**RESULTS**

Thirteen of the 120 eyes included in the study converted to glaucoma, according to a strict visual field definition, over a median follow-up time of 10.3 years (range, 3.1–14.4 years). Converters and nonconverters did not differ significantly in age or IOP. Figure 1 shows examples of PERG recordings in a

![Non-converter eye](image1.png)

![Converter eye](image2.png)

**Figure 1.** Example PERGs from one eye of a nonconverter (last visit, left) and a converter (right, 25 months after conversion). Time-domain (left) and Fourier spectrum (right) are shown. Top: 0.8° stimuli; bottom: 16° stimuli. Above the spectra the amplitude in µV, signal-to-noise ratio (snr), significance of the response, and phase-derived peak time are given. In this converter eye, the amplitudes are altogether lower (note differing scales), and the amplitude for the 0.8° stimulus is smaller than the one to 16° stimulus; furthermore, the peak time is shorter.
nonconverted (left) and a converted (right) eye. For each recording, the time series is displayed on the left and the frequency magnitude spectrum—after Fourier transform—on the right. In the top row are responses to 0.8° check size; in the bottom row, to 16° check size.

**Single-Visit PERGs**

We first report on PERG measures obtained at the last visit. The median age was 60.6 years, with upper and lower quartile from 51.0 to 70.3 years. The converters presented reduced PERG amplitudes, ratios, and peak times. We observed a significant difference from the nonconverters in all PERG measures (Fig. 2), remaining significant after correction for multiple testing.

We calculated ROCs (Fig. 3) for the three PERG measures to determine the predictive power ahead of conversion to glaucoma. Four years before conversion, the AUC of the amplitude to 0.8° stimuli showed an AUC of 0.64 (sensitivity 67%, specificity 64%). The ratio 0.8°/16° reached an AUC of 0.75 (sensitivity 75%, specificity 76%) and peak time an AUC of 0.49 (sensitivity 50%, specificity 88%). Going back in time, the number of steps in the ROC decreased, corresponding to the lower number of converters in the respective time range.

The area under the ROC curve is a handy indicator of prediction accuracy; the AUC time courses in Figure 4 are depicted starting at 5 years before conversion. As we would expect, the AUCs decrease going back in time. The 95%-confidence intervals for the PERG ratio do not include the chance AUC of 0.5 until 5 years before conversion, indicating significant prediction above chance; the raw amplitude does not consistently reach this criterion, nor does peak time. Ear-

**Figure 2.** Group comparison of nonconverters to converters at the last PERG recording. (A) Amplitude 0.8°; (B) ratio 0.8°/16°; (C) peak time (left axis) and phase (right axis) using 0.8° checks. *P* values are shown in the graphs. Box plot details: thick horizontal bars: median; notches: 67% confidence interval of the median; box: interquartile range (25%–75%); whiskers: range; circles: outliers (C, data >1.5 times the interquartile range away from the box). There are marked group differences, most for the PERG ratio, then the amplitude, then the peak time.

**Figure 3.** Single visit ROCs at 4, 2, 1, 0.5 and 0 years before conversion. All recordings at 15 rps. Below the *dashed diagonal* the respective AUC, sensitivity and specificity are denoted. Below them, the ROC threshold of minimum error score (amplitude [μV], PERG ratio and peak time [ms]) is given, its position is indicated by a *star* along the ROC curve. Note that the ratio 0.8°/16° shows the highest sensitivity, specificity, and AUC over the entire period. See Figure 4 for the large confidence intervals of the AUC.
lier than 5 years, the AUCs declined further (not shown), but the interpretation becomes less reliable as it is based on a decreasing number of converters (12 converters at \( \text{H11002} \) 4 years, 9 at \( \text{H11002} \) 5 years, 4 at \( \text{H11002} \) 6 years, and so forth).

Slopes over Time: Determining the Trends

We computed slopes over time for the amplitude 0.8°, the ratio 0.8°/16°, and the peak time. The number of visits on which the slope was based varied from 3 to 11, depending on the follow-up time point for which the preceding slope had been calculated. The steepness of the slopes represents the rate of functional RGC loss, and was used to analyze the predictive potential of the PERG for conversion to glaucoma. Figure 5 shows examples of one nonconverter and one converter eye (each of different patients).

All PERG measures had a mean negative slope over the course of the study, which was considerably steeper in converters. However, only the slope of the ratio 0.8°/16° differentiated significantly between converters and nonconverters (see Fig. 5, \( P < 0.001 \), remaining significant after correction for multiple testing).

We calculated the ROCs of the slopes at different time points before conversion. The AUCs of these ROCs have markedly lower values than do the AUCs of the measures based on the single-visit PERGs. The AUCs based on slope increased as the subject drew closer to conversion. Both the slope of the ratio 0.8°/16° and that of amplitude 0.8° revealed similar potential for identifying converters, the peak time’s slope demonstrated a lower AUC.

One year before conversion, the ROC of the slope of the amplitude to 0.8° stimuli had an AUC of 0.54 (sensitivity 61%, specificity 52%). The slope of the ratio 0.8°/16° showed an AUC of 0.61 (sensitivity 62%, specificity 70%), and the peak time’s slope an AUC of 0.61 (sensitivity 33%, specificity 87%).

DISCUSSION

In this longitudinal study, we compared PERG measures from eyes that converted to glaucoma with eyes that did not convert. Measures analyzed were amplitude to 0.8° check size, PERG-ratio (0.8°/16°) and peak time. We used two modes of analysis: single-visit values and their slopes across time.

Single-Visit PERGs

Amplitude. In agreement with previous studies, the PERG amplitude was significantly lower in patients who converted to glaucoma.\(^9,12,18,21,43\) The amplitude to 0.8° stimuli had an AUC

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**FIGURE 4.** AUCs for amplitude 0.8°, ratio 08°/16°, and peak time 5 years before conversion until conversion, based on all patients; the vertical bars cover the 95% CI (2.5%–97.5%). Horizontal dashed line at an AUC of 0.5 represents chance prediction. For the PERG ratio (blue) the confidence interval does not include 0.5 until 5 years before conversion, indicating significant prediction above chance; the amplitude does not consistently reach this criterion, nor does peak time.

**FIGURE 5.** Examples of PERG changes over time. Shown are changes over time in the eyes of one nonconverter (A) and one converter (B). (B, vertical line, time of conversion). The x-axis represents years in the study.
of 0.64 (sensitivity 67%, specificity 64%) 4 years before conversion. Because of the current paucity of longitudinal studies in this field, we can only compare our results with one earlier analysis of this database that demonstrated an AUC of 0.68 (sensitivity 70%, specificity 73%), 1 year before conversion. A comparison of our converters to a matched, fully normal control group would be likely to yield higher sensitivity/specificity, as the present comparison group consists of OHT patients who potentially also will develop visual field defects in the future. However, it is worth noting that the AUC remains roughly constant from conversion to 4 years before conversion (Fig. 4), fitting with the view that PERG changes occur early and then saturate, thus rendering the PERG a poor biomarker for monitoring advanced disease.

In further agreement with the literature (Philippin H, et al. IOVS 2005;46:ARVO EAbstract 3757), we found the PERG ratio to be superior to other PERG measures in identifying converters. Our data demonstrate an AUC of 0.75 (sensitivity 75%, specificity of 76%), 4 years before conversion. The previous study reported an AUC of 0.78 (sensitivity 80%, specificity 71%), 1 year before conversion. The PERG ratio thus remains the most potent PERG measure to identify converters correctly.

A likely confounder in PERG interpretation is age. We tested for this by applying the age correction that we obtained for a normal group in a previous study to the PERG amplitude and PERG ratio. Since the age correction is rather similar for the 0.8° and 16° responses, it was no surprise that the PERG ratio results did not change appreciably. For the 0.8°-amplitude-based group difference, the P-value (at last visit, Fig. 2) changed from P < 0.001 to P = 0.005. This result suggests that early identification of converters is not an age artifact.

Another possible confounder is optical degradation—namely, media opacities and misrefraction. This problem was avoided by the acuity requirement of 0.8 (decimal acuity) or higher. We have found earlier that in this acuity range the effect on the 0.8° amplitude is negligible, although appreciable when smaller check sizes would be used.

**Peak Time.** Peak time was significantly reduced in the converted eyes at and before conversion by approximately 3 ms lower peak time (Fig. 2). As a conversion predictor this measure performed poorer than other PERG measures (Fig. 4). We were initially surprised that incipient RGC damage should be associated with lower peak times. A review of the literature encountered difficulties, as the latency or phase of the PERG in glaucoma is rarely assessed; moreover, the terms “latency” and “phase” needs to be disentangled (taking into account the different definitions of the sign in phase, which are often not provided in the article). Finally, again, there is an increase in peak time with age; however, this effect is not as potent in predicting converters as the single-visit PERGs. However, consecutive single-visit recordings may prove helpful in detecting converters even when the AUCs were considerably smaller than those of the single-visit PERGs.

In the present study the converter eyes had a shorter peak time than did the nonconverter eyes. Equating our converters with glaucoma eyes in the earlier studies and our nonconverters with “normal” eyes, we noted a discrepancy among three of the aforementioned studies. We thus first questioned our analysis algorithms, which convert phase to the time domain (see the Methods Section) by three different techniques:

1. Digital low-pass filtering at 25 Hz (leaving only the dominant response at 15 Hz) combined with “averaging down” our ~1-second time series so that it contained only one period (67 ms). In the resulting sinusoidal trace, the time of the peak after 40 ms was easy to identify. These peak times were compared to the ones derived from the phase, and we found that they coincided within a millisecond.

2. We shifted a recording by 20 ms to the left and then applied our standard processing: the 20 ms were correctly picked up when converting phase to peak time from the Fourier-transformed data.

3. We recorded steady state PERG in author MB at a luminance of 45 and 300 cd/m² and found a consistent reduction of peak time to the higher luminance, as expected.

Concluding that reduced peak time in the converters is a reliable finding, we turned to evidence from fields other than glaucoma and noted the following: Viswanathan et al. recorded the PERG in a nonhuman primate model in a normal condition and after application of tetrodotoxin (blocking the voltage-activated sodium channels, thus suppressing action potentials). Under the condition in which the ganglion cells were unable to produce action potentials, PSO peak time was reduced by approximately 7 ms (measured from the published traces). This corresponds closely to the reduced PSO peak times in optic nerve disease. Quite recently, the PERG was compared before and after marked IOP reduction in glaucoma patients by surgical intervention. The authors found a 24% increase in PERG amplitude, thus a functional improvement; phase changed from 1.81 to 1.72 π · rad. With their phase definition this corresponds to an increase in peak time by 2.8 ms (unfortunately incorrectly stated in their discussion).

Possibly the discrepancy in the literature is just due to different phase definitions; in most papers the definition of phase is not explicitly stated. Thus, we interpret our observation “the lower the peak time, the more pronounced the pathology” as reliable and look forward to further studies targeting peak time in glaucoma.

**Change in PERG Measures Over Time**

We calculated slopes to assess the loss of RGC function in OHT patients over time. All three measures showed a considerably steeper mean negative slope in converters (Fig. 6); however, the only significant difference from the nonconverters was in the 0.8°/16° ratio. We also calculated ROCs for the slopes, and their AUCs were considerably smaller than those of the single-visit PERGs.

The slopes calculated over the whole observation period were not as potent in predicting converters as the single-visit PERGs. However, consecutive single-visit recordings may prove helpful in detecting converters or even monitoring therapy—in particular to indicate stable glaucoma after glaucoma surgery, or a change in medical therapy. The potential of slopes to evaluate therapeutic success should be analyzed in further studies.

**Clinical Utility**

To what degree can these findings be applied to clinical situations? On the one hand, our approach underestimates the value of PERG to detect glaucoma, because we did not compare glaucoma to normal, but rather to an OHT group of patients of whom a fraction would be expected to develop glaucoma in the future. On the other hand, the PERG ratio, while performing best, and the amplitude at 0.8° have a major shortcoming in their dependence on optical factors. When acuity is below criterion (0.8 decimal, corresponding to 20/25 Snellen or 0.1 logMAR) optical imaging confounds check-size-specific PERG changes. Furthermore, obtaining a PERG re-
recording demands more skill from the examiner than for example automated visual field measurement, even when skin electrodes\(^5\) rather than semiinvasive DTL electrodes are used. We thus see a role for PERG recording in high-throughput clinics (where recording skills develop rapidly and are well maintained), for patients with unreliable field tests, for glaucoma suspects with normal visual fields, and as a rapid surrogate measure for interventions. When asked the obvious question, “when to treat,” among the factors to be taken into account, we use the PERG ratio to determine the PERG contribution and note that the threshold for minimum error (Fig. 3) is between 1.0 and 1.1, suggesting that a PERG ratio below 1.0 should tip the balance toward treating.

References

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