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Comparing three different modes of electroretinography in experimental glaucoma: diagnostic performance and correlation to structure

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Abstract

Purpose—To compare diagnostic performance and structure-function correlations of multifocal electroretinogram (mfERG), full-field flash ERG (ff-ERG) photopic negative response (PhNR) and transient pattern-reversal ERG (PERG) in a non-human primate (NHP) model of experimental glaucoma (EG).

Methods—At baseline and after induction of chronic unilateral IOP elevation, 43 NHP had alternating weekly recordings of retinal nerve fiber layer thickness (RNFLT) by spectral domain OCT (Spectralis) and retinal function by mfERG (7F slow-sequence stimulus, VERIS), ff-ERG (red 0.42 log cd-sec/m² flashes on blue 30 scotopic cd/m² background, LKC UTAS-E3000), and PERG (0.8° checks, 99% contrast, 100 cd/m² mean, 5 reversals/sec, VERIS). All NHP were followed at least until HRT-confirmed optic nerve head posterior deformation, most to later stages. mfERG responses were filtered into low- and high-frequency components (LFC, HFC, >75 Hz). Peak-to-trough amplitudes of LFC features (N1, P1, N2) and HFC RMS amplitudes were measured and ratios calculated for HFC:P1 and N2:P1. ff-ERG parameters included A-wave (at 10 ms), B-wave (trough-to-peak) and PhNR (baseline-to-trough) amplitudes as well as PhNR:B-wave ratio. PERG parameters included P50 and N95 amplitudes as well as N95:P50 ratio and N95 slope. Diagnostic performance of retinal function parameters were compared using the area under the receiver operating characteristic curve (A-ROC) to discriminate between EG and control eyes. Correlations to RNFLT were compared using Steiger's test.

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Results—Study duration was 15 ± 8 months. At final follow-up, structural damage in EG eyes measured by RNFLT ranged from 9% above baseline (BL) to 58% below BL; 29/43 EG eyes (67%) and 0/43 of the fellow control eyes exhibited significant ($>7\%$) loss of RNFLT from BL. Using raw parameter values, the largest A-ROC findings for mfERG were: HFC (0.82) and HFC:P1 (0.90); for ff-ERG: PhNR (0.90) and PhNR:B-wave (0.88) and for PERG: P50 (0.64) and N95 (0.61). A-ROC increased when data were expressed as % change from BL but the pattern of results persisted. At 95% specificity, the diagnostic sensitivity of mfERG HFC:P1 ratio was best, followed by PhNR and PERG. The correlation to RNFLT was stronger for mfERG HFC ($R=0.65$) than for PhNR ($R=0.59$) or PERG N95 ($R=0.36$), ($p=0.20$ $p=0.0006$, respectively). The PhNR flagged a few EG eyes at the final time point that had not been flagged by mfERG HFC or PERG.

Conclusions—Diagnostic performance and structure-function correlation were strongest for mfERG HFC as compared with ff-ERG PhNR or PERG in NHP EG.

Keywords

glaucoma; retinal ganglion cells; electroretinography; optical coherence tomography; retinal nerve fiber layer

Introduction

Glaucoma is a progressive optic neuropathy that ranks as one of the leading causes of irreversible blindness worldwide [1,2]. Although loss of retinal ganglion cells (RGCs) and their axons is common to all optic neuropathies, the pattern of damage in glaucoma tends to be pathognomonic, including unique structural alterations of the optic nerve head (ONH) that reflect connective tissue deformation and remodeling [3–6]. Modern imaging techniques such as confocal scanning laser tomography and optical coherence tomography (OCT) provide means for reliable quantification of ONH anatomical parameters and macular or peripapillary retinal nerve fiber layer (RNFL) thickness, which may enable early detection of glaucomatous structural changes[7–9]. When such changes are accompanied by spatially corresponding defects of the visual field, the accurate diagnosis of glaucoma and/or its progression are more likely [4,10,11].

Although visual field testing (perimetry) is the mainstay for clinical assessment of vision function in glaucoma, other ancillary tests are occasionally employed to assist with diagnosis in equivocal cases or with patients that do not perform visual field tests reliably or in a research setting [12]. For these reasons, electrophysiological tests of vision function such as electroretinography (ERG) are thought to have a role in glaucoma management [13–15]. ERG testing also provides a non-invasive, objective measure of function that is easier to obtain in laboratory animals than those based on behavioral testing such as perimetry [16,17].

Several different ERG modes are known to be capable of monitoring RGC function in human glaucoma as well as in experimental glaucoma (EG) models [15,18]. For example, pattern ERG (PERG), full-field flash ERG (ff-ERG) and multifocal ERG (mfERG) techniques have all been studied extensively to determine their respective abilities to measure loss of RGC function [19–21,13,14,22–24,15,18]. Each technique has its

advantages and drawbacks[24,15], the balance of which may depend on the setting (e.g., clinical versus research) or the subjects under study (e.g., human patients versus laboratory animals). However, to our knowledge, no single study has yet evaluated all three modes simultaneously to compare their diagnostic performance and correlation to measures of glaucomatous structural damage. Therefore, in this study, we sought to compare mfERG, PERG and the photopic negative response (PhNR) of the ff-ERG in a large cohort of non-human primates (NHP) with unilateral EG.

Methods

Subjects

The subjects of this study were 43 rhesus macaque monkeys (*Macaca mulatta*), 35 female and 8 male, ranging in age from 1.4 to 22.6 years (mean $\pm$ standard deviation: 10.1 ± 6.7 years). All experimental methods and care procedures were approved and monitored by the Institutional Animal Care and Use Committee (IACUC) at Legacy Health (USDA license 92-R-0002 and OLAW assurance A3234-01) and carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the Association for Research in Vision and Ophthalmology's Statement for the Use of Animals in Ophthalmic and Vision Research.

Experimental Protocol

Prior to the initiation of unilateral EG, each animal was evaluated at baseline (BL) by spectral domain OCT (SDOCT) and by three modes of ERG (details below) a minimum of three times each; the average number of BL sessions was 5.2 ± 1.4 . One eye of each animal was then randomly assigned to EG, which was initiated by application of laser photocoagulation to the trabecular meshwork to induce chronic, mild-to-moderate elevation of intraocular pressure (IOP). Initially, 180° of the trabecular meshwork was treated in one session, then the remaining 180° was treated in a second session approximately two weeks later. If necessary, laser treatments were repeated in subsequent weeks (limited to a 90° sector) until an IOP elevation was first noted or if the initial post-laser IOP had returned to normal levels. The average number of laser treatments ($\pm$ SD) was 5.2 ± 2.6 . For each animal, the fellow eye served as control. IOP was measured in both eyes at the start of every session using a Tonopen XL (Reichert Technologies, Inc., Depew, NY). The value recorded for each eye was the average of three successive measurements.

Testing by SDOCT and ERG continued on alternating weeks for each animal until its pre-defined endpoint target had been reached. Those targets were based on the protocols for each of the four primary studies from which this cohort was assembled. Because those studies varied by design in their degree of severity at endpoint, the combined cohort provided a relatively wide range of damage available for analysis in this study.

Anesthesia

All experimental procedures began with induction of general anesthesia using ketamine (10–25 mg/kg IM) in combination with either xylazine (0.8–1.5 mg/kg IM) or midazolam (0.2 mg/kg IM), along with a single injection of atropine sulphate (0.05 mg/kg IM). Animals

were then intubated with an endotracheal tube to breathe a mixture of 100% oxygen and air for maintaining oxyhemoglobin saturation 95%, as close to 100% as possible. During ERG testing, anesthesia was maintained using a combination of ketamine (5 mg/kg/hr IV) and xylazine (0.8 mg/kg/hr IM). In some cases, a constant rate infusion (1.2–3.0 mg/kg/hr) was used for IV ketamine delivery after a loading dose of 3.0–8.0 mg/kg. For all SDOCT imaging sessions, anesthesia after initial induction was maintained using isoflurane gas (1–2%; typically 1.25%) mixed with 100% oxygen and delivered via endotracheal tube. For SDOCT imaging, a clear, rigid gas permeable contact lens filled with 0.5% carboxymethylcellulose solution was placed over the apex of each cornea. In all sessions, IV fluids (lactated Ringer's solution, 10–20 mL/kg/h) were administered via the saphenous vein, vital signs were monitored throughout and recorded every 10–15 mins, including, heart rate, blood pressure, arterial oxyhemoglobin saturation, endtidal CO₂ and body temperature; body temperature was maintained at 37°C, heart rate above 75 beats per min and oxygen saturation above 95%.

SDOCT Measurements of Peripapillary RNFL Thickness

SDOCT (Spectralis, Heidelberg Engineering GmbH) was used to measure the average peripapillary RNFL thickness (RNFLT) from a single circular, 12° diameter B-scan consisting of 1536 A-scans (Fig. 1) [25–27]. Nine to sixteen individual sweeps were averaged in real time to comprise the final stored B-scan at each session. The position of the scan was centered on the ONH at the first imaging session and all follow-up scans were acquired at this same location using the instrument's automatic active eye tracking software. A trained technician masked to the specific purpose of this study manually corrected the accuracy of the instrument's native automated layer segmentations when the algorithm had obviously erred from the inner and outer borders of the RNFL to an adjacent layer. All SDOCT scans were performed 30 min after IOP was manometrically stabilized to 10 mmHg.

ERG Recordings

Custom-designed Burian-Allen contact lens electrodes (10 mm diameter, +3.0 diopter; Hansen Ophthalmics, Iowa City, IA) were used for all ERG testing; the corneal ring on the stimulated eye served as the active electrode, while the corneal ring of the unstimulated (patched) contralateral eye served as the reference electrode. A subcutaneous ground electrode was placed on a rear limb. Electrode impedance was accepted if <5 kΩ. Prior to insertion of ERG contact lens electrodes, one drop of topical anesthetic (0.5% proparacaine) and an ocular lubricating agent (1% carboxymethylcellulose sodium, Celluvisc, Allergan, Irvine, CA) were applied to each eye. Head position was stabilized using a bite bar capable of rotation in three axes. The ERG protocol required 2–2.5 hours to complete.

Multifocal ERG

Multifocal ERG (mfERG) recordings were obtained using VERIS™ (Electro-Diagnostic Imaging, Inc., Redwood City, CA) as previously described [28–31]. The stimulus consisted of 103 un-scaled hexagonal elements subtending a total field size of ~55° at the test distance of 30 cm (Fig. 2). The luminance of each hexagon was independently modulated between dark (1 cd/m²) and light (200 cd/m²) according to a pseudorandom, binary m-sequence. The

temporal stimulation rate was slowed by insertion of 7 dark frames into each m-sequence step (“7F”). The m-sequence exponent was set to 12, thus the total duration of each recording was 7 min 17 sec. Signals were amplified (gain = 100,000), band-pass filtered (10–300 Hz; with an additional 60 Hz line filter), sampled at 1.2 kHz (i.e. sampling interval = 0.83 ms), and digitally stored for subsequent off-line analyses. Two such recordings were obtained for each eye at each time point and averaged. Alignment of the stimulus on the visual axis was achieved via adjustment during a series of brief, standard m-sequence (“0F”) multifocal ERG recordings prior to the pair of 7F recordings.

From the average of the two 7F recordings at each time point, a subset of local responses from the full array, limited to the central element and the three rings surrounding it (37 local responses in total, Fig. 2B–2C), was processed to derive summary outcome parameters. A high-pass filter (5-pole, >75 Hz) was applied to each local mfERG response to extract the high frequency components (HFC, Fig. 2C). The low frequency component (LFC) of each response was represented as the raw response minus the HFC. The amplitude of the HFC was calculated as the root mean square (RMS) for the epoch between 0–80 ms of each filtered record. Peak amplitudes for LFC features were quantified as follows: the first negative feature (N1) was calculated as the maximum negative excursion from baseline in the epoch up to 30 ms; the amplitude of the first positivity (P1) was calculated as the voltage difference between the maximum peak and the N1 trough; and the second negativity (N2) was calculated as the difference between baseline and the minima from 30–80 ms. The global average (of 37 response locations) for each parameter represented the measurement for each eye at each ERG session.

Pattern-Reversal ERG (PERG)

Transient PERG recordings were obtained using the VERIS™ (Electro-Diagnostic Imaging, Inc.) immediately following the mfERG recordings in each eye. The checkerboard stimulus consisted of 0.8° checks subtending a total field size of 48° × 40° with a mean luminance of 100 cd/m² and contrast of ~99% modulated at 5 reversals/sec. PERG signals were bandpass filtered between 1–100 Hz and sampled at 2025 Hz. Two records were obtained for each eye with each record consisting of an average of 200 sweeps. Peak amplitudes for the first positive feature (P₅₀) were calculated as the voltage difference between the first prominent negative feature and the first prominent positive feature (Fig. 3); the amplitude of the first negative feature (N₉₅) was calculated as the voltage difference between the maximum peak and the subsequent trough. The N95 slope was calculated as the amplitude of the N₉₅ feature divided by the difference between the implicit time of the N₉₅ feature and implicit time of the P₅₀ feature. Data from two records per eye were averaged at each session.

Full-field Flash ERG (ff-ERG) Photopic Negative Response (PhNR)

Full-field flash ERG (ff-ERG) recordings were obtained using a UTAS-E3000 system (LKC Technologies, Inc., Gaithersburg, MD). Stimuli were red flashes (Wrattan #29; 0.42 log cd-sec/m²) presented via a Ganzfeld integrating sphere after a 5 min light adaptation to a blue background (Wrattan #78; 30 scotopic cd/m²). Each recording was an average of 10 sweeps with an interstimulus interval of 2 sec. Signals were band-pass filtered between 0.3–500 Hz and sampled at 2000 Hz. A-wave amplitude was measured at a criterion time of 10 ms (Fig.

4). B-wave amplitude was measured as the voltage difference between the maximum peak and the A-wave trough. PhNR amplitude was measured as the voltage difference between the ERG baseline and the trough (minima) following the B-wave peak (see Fig. 4 and Appendix). Data from two recordings per eye were averaged at each session.

Analysis and Statistics

The diagnostic performance of ERG parameters was compared using receiver operator characteristic (ROC) curve analysis and the chi-square test as well as by comparing diagnostic sensitivity at 95% specificity using the z-test for proportions. The strength of the Pearson correlation between ERG parameters and structure (peripapillary RNFLT) were compared using Steiger's test. Overlap of diagnostic capability across ERG parameters was evaluated using Euler plots. Statistical analyses were performed using commercial software packages (Prism 5, GraphPad Software, Inc., La Jolla, CA and SigmaPlot 11, Systat Software, Inc., San Jose, CA).

Results

Range of EG Severity at Final Follow-up

Across the 43 NHP of this study, the average study duration was 15.1 ± 8.0 months and the average duration of post-laser follow-up was 9.3 ± 7.8 months. Mean post-laser IOP ranged from 10.4 to 31.0 mmHg in the group of 43 EG eyes, with a group average of 19.0 ± 5.4 mmHg. Over the same period, mean IOP in the group of 43 fellow control eyes was 11.2 ± 2.0 mmHg. The peak IOP observed during post-laser follow-up period was 42.2 ± 11.5 in the EG eyes (range: 15.3 to 63.0 mmHg) and 18.2 ± 6.2 mmHg in control eyes.

At final follow-up, structural damage in EG eyes measured by RNFLT ranged from 9% above BL to 58% below BL, with an average loss of $16.2 \pm 15.9\%$. 29/43 EG eyes (67%) and 0/43 of the fellow control eyes exhibited statistically significant ($>7\%$) loss of RNFLT from baseline[32,33,27]; thus 33% of the EG eyes were still early-stage EG at final follow-up[25–27].

Diagnostic Performance of ERG Parameters at Final Follow-up

Figure 5A shows receiver operator characteristic (ROC) curves derived by plotting the proportion of 43 EG eyes flagged at a given ERG parameter criterion value ("sensitivity") against the proportion of 43 fellow control eyes flagged at the same criterion (i.e., false alarm rate). Only the best two or three parameters from each ERG mode are plotted. The accompanying Table 1 lists the area under the ROC curve (A-ROC) for all ERG parameters evaluated. The data in Fig. 5A and Table 1 show that the mfERG HFC and the ff-ERG PhNR had better diagnostic performance (larger A-ROC) than any PERG parameter ($P = 0.001$ each). Figure 5B and Table 2 present the ROC curves and A-ROC values, respectively, for the same parameters expressed in each case as a change from the baseline average. These data demonstrate that diagnostic performance of all parameters improved after accounting for inter-individual variance of ERG amplitudes by normalization to baseline amplitude (e.g., compare Fig. 5B to 5A and Table 2 to Table 1). Further, normalization of the mfERG HFC and N2 parameter values to the mfERG P1 (another feature of the same mfERG

response) also improved diagnostic performance (e.g., mfERG HFC:P1 ratio or mfERG N2:P1 ratio). However, this enhancement did not occur for the PhNR amplitude when normalizing to the B-wave amplitude, nor for the PERG N95 amplitude when normalizing to the PERG P50 amplitude.

Table 3 lists the observed sensitivity for each parameter at a fixed specificity of 95% and Table 4 lists the same for each parameter expressed as a change from baseline. The mfERG HFC:P1 ratio had the highest diagnostic sensitivity for this high degree of specificity, and its sensitivity was increased further by normalization to pre-glaucoma baseline values. Sensitivity at 95% specificity was nearly as good for the PhNR amplitude and PhNR:B-wave amplitude ratio, but their point estimates fell below the lower bound of the confidence interval for the mfERG HFC:P1 ratio change from baseline. PERG parameters all had significantly lower sensitivity than the mfERG HFC or ff-ERG PhNR amplitudes. These results comparing sensitivity at high specificity are also apparent in the upper left corner of the ROC plots in Fig. 5.

In order to determine if the relative sensitivity of these ERG parameters differed at earlier stages, the same analysis was repeated while marching backward through the three previous recording sessions prior to the final available time point for each eye (see Figure 6). The results of that analysis revealed that: 1) sensitivity of all ERG parameters increased as functional loss progressed in EG; 2) sensitivity of the mfERG HFC:P1 ratio was highest at the earlier time points; 3) the PhNR amplitude and PhNR:B-wave ratio increased most rapidly over the final three time points.

Correlation to Structural Measure of Retinal Ganglion Cell Axon Loss

Figure 7 presents scatter plots to compare relationships between each ERG parameter and the measure of structural damage used in this study (peripapillary RNFL thickness). While only the best performing pair of parameters for each ERG mode are included as plots, Table 3 lists the corresponding Pearson correlation coefficients for these and all other ERG parameters evaluated. The mfERG HFC amplitude had the strongest correlation to structure of any ERG parameter studied, significantly stronger than PERG N95 amplitude ($P=0.0006$) but only marginally stronger than the ff-ERG PhNR amplitude ($P=0.20$). Figure 7 shows the results for the same parameters expressed as a change from baseline and plotted against the change of RNFL thickness from its baseline average value in each eye; Table 4 lists the corresponding Pearson correlation coefficients. Normalization to baseline values improved the strength of correlation to structure for PERG more than it did for the mfERG HFC or ff-ERG PhNR, yet the mfERG HFC correlation to structure remained significantly stronger than that for the PERG ($P=0.03$). Another salient finding is highlighted by the plots in Figs. 6 and 7: although the dynamic range for PhNR and PERG appear to be larger than mfERG HFC, their population variation and test-retest repeatability are worse, especially for PERG (see spread of control eyes along Y-axis), thus undermining diagnostic performance.

Overlap of Diagnostic Information

There is a possibility that different ERG parameters might offer complementary diagnostic information even if some exhibit lower overall diagnostic performance. To address this

question, the EG eyes flagged by each of the best performing ERG parameters at their 95% specificity criterion were plotted in area-proportional Euler diagrams in Figure 9. The results show that all but one of the EG eyes flagged by PERG were also flagged by mfERG and 25/30 (83%) eyes flagged by the PhNR were also flagged by mfERG. However, since 5 EG eyes were flagged only by PhNR, these results do suggest that complementary information is available through testing by multiple ERG modalities.

Discussion

The purpose of this study was to compare three modes of ERG testing in terms of their diagnostic performance and correlation to structure in a non-human primate (NHP) model of experimental glaucoma (EG). The three modes selected were mfERG, ff-ERG and PERG because they have each been evaluated extensively and proven effective in clinical studies of human glaucoma [19,21,13,14,22,23,20,24,15] as well as in previous laboratory studies in NHP eyes after retinal ganglion cell or optic nerve injury [34,28], including EG [35–39,17,40,41,29–31]. To our knowledge, however, no prior study has compared all three modes in the same cohort of glaucomatous eyes.

Specifically, based on previous evidence, we anticipated that the HFC amplitude of the slow-sequence mfERG [17,28–31], the PhNR amplitude of the red-on-blue ff-ERG [36,38,42,43,28] and the N95 of the transient PERG [19,38,34,28] would be the parameters from each mode that would have the strongest diagnostic capacity and correlation to structural damage. These expectations were confirmed by the results, yet the findings also demonstrate that the mfERG HFC amplitude normalized to the mfERG LFC P1 amplitude had the highest sensitivity and strongest correlation to structural loss. The PhNR amplitude and PhNR:B-wave ratio were nearly as sensitive to detect functional damage, nearly as well correlated to structural loss and had the best overall diagnostic accuracy of any ERG parameter according to the full A-ROC analysis. The latter finding reflects that the PhNR gains relatively more than the mfERG HFC at lower levels of specificity. The results also show that the transient PERG is hindered by wider population variance and poorer test-retest reliability, which undermines diagnosis by cross-sectional and longitudinal analysis, respectively, as well as its correlation to structure. This has been reported previously in a study of human glaucoma by Hood and colleagues [44]. Of the three ERG modes evaluated in this study, the PERG is most dependent on clear optics, which may explain its poorer performance, at least in part, since contact lens electrodes were used to maximize signal-to-noise ratio.

It is possible that use of steady-state stimulation and corresponding analysis, perhaps also including a normalization step by check size would have resulted in better PERG performance [45–47], though it is unlikely that these approaches would have led to PERG performance surpassing that of the ff-ERG PhNR [48,24]. We did find that normalization to other features of the same ERG response improved both diagnostic performance and correlation to structure for the mfERG HFC amplitude (e.g., HFC:P1 ratio) but not for the ff-ERG PhNR or PERG N95 amplitudes. This suggests that there is more of an effect of EG on the ff-ERG B-wave and PERG P50 than there is on the mfERG LFC P1 amplitude, consistent with results presented in Tables 1–4, which show that some diagnostic signal is

present for both B-wave and P50 amplitudes. Although the results in Fig. 9 suggest that the ff-ERG PhNR offers complementary diagnostic information to the mfERG HFC, the results analyzing multiple longitudinal time points (Fig. 6) indicate that the conclusion of this study is robust and that the mfERG HFC provides the single best ERG measure of retinal ganglion cell function, at least in NHP EG. The latter is an important caveat because the mfERG appears to provide only modest diagnostic benefit in human glaucoma [49–54]. It remains unknown why the mfERG of the NHP seems to be relatively enriched with retinal ganglion cell contributions as compared to the human eye [49,55]. Indeed, this phenomenon might be even more specific to rhesus monkeys among primates [40].

In addition to HFC amplitude, the mfERG LFC N2 amplitude exhibited diagnostic capability that was significantly better than chance and was as strongly correlated to structure as the PERG N95. Similar to HFC amplitude, both aspects improved when the N2 was normalized to the LFC P1 component and further still when normalized to baseline values. It has been suggested that the mfERG N2 component is analogous to the PhNR of the ff-ERG [Viswanathan S, et al. *IOVS* 2009;50: ARVO E-Abstract 4758], which in turn may share retinal generators with the PERG N95 [38,56]. Thus it is not surprising that the mfERG N2 appears to reflect glaucomatous damage [23,57,58]. Significant reduction of LFC amplitudes in mfERG responses to a more complicated stimulus have also been reported in NHP EG [41], which might reflect a similar underlying functional abnormality.

The utility of the PhNR for assessment of retinal ganglion cell function in glaucoma was first described by Viswanathan and colleagues [36,42] and has since been confirmed by numerous other investigators in clinical studies, particularly when normalized to B-wave amplitude [59,39,60,22,24,61–64] as well as in laboratory studies of both rodent [65,66] and NHP optic nerve injury models [28]. It is possible that limiting the stimulus to localized areas of the macula [67–69,62,23] and/or to a narrower spectral range (with a goal of stronger chromatic contrast) [43] might offer greater improvement of diagnostic performance of the PhNR as compared to the conditions used in this study. Meanwhile, there were substantial effects related to the derivation of PhNR amplitude alone (see Appendix), which are worth considering with/or without other methodological changes.

In summary, among the ERG modes evaluated in this study, the mfERG HFC had the highest diagnostic sensitivity and strongest correlation to structure in this cohort of non-human primates with experimental glaucoma. Consistent with numerous other reports, accounting for inter-eye differences by normalization to baseline amplitudes and/or by normalization to other features of the same ERG response less sensitive to glaucomatous damage improved both diagnostic performance and correlation to a structural measure of damage severity for the best parameters of all three ERG modes. After normalization, the mfERG HFC had the highest diagnostic sensitivity and strongest correlation to RNFL thickness and missed only a few EG eyes flagged by PhNR or PERG. The mfERG also offers an opportunity to evaluate focal loss, unlike the ff-ERG; future studies are planned to evaluate whether any benefit can be realized from this potential advantage, even in this EG model, which tends to manifest as more diffuse progressive damage rather than as sequential focal loss. Nevertheless, further research is required to determine whether these observations will successfully translate to clinical management of human glaucoma.

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References

1. Quigley HA, Broman AT. The number of people with glaucoma worldwide in 2010 and 2020. *Br J Ophthalmol.* 2006; 90(3):262–267. DOI: 10.1136/bjo.2005.081224 [PubMed: 16488940]
2. Tham YC, Li X, Wong TY, Quigley HA, Aung T, Cheng CY. Global prevalence of glaucoma and projections of glaucoma burden through 2040: a systematic review and meta-analysis. *Ophthalmology.* 2014; 121(11):2081–2090. DOI: 10.1016/j.ophtha.2014.05.013 [PubMed: 24974815]
3. Weinreb RN, Khaw PT. Primary open-angle glaucoma. *Lancet.* 2004; 363(9422):1711–1720. DOI: 10.1016/S0140-6736(04)16257-0 [PubMed: 15158634]
4. Quigley HA. Glaucoma. *Lancet.* 2011; 377(9774):1367–1377. DOI: 10.1016/S0140-6736(10)61423-7 [PubMed: 21453963]
5. Burgoyne C. The morphological difference between glaucoma and other optic neuropathies. *J Neuroophthalmol.* 2015; 35(Suppl 1):S8–S21. DOI: 10.1097/WNO.0000000000000289 [PubMed: 26274837]
6. Fortune B, Reynaud J, Hardin C, Wang L, Sigal IA, Burgoyne CF. Experimental Glaucoma Causes Optic Nerve Head Neural Rim Tissue Compression: A Potentially Important Mechanism of Axon Injury. *Invest Ophthalmol Vis Sci.* 2016; 57(10):4403–4411. DOI: 10.1167/iovs.16-20000 [PubMed: 27564522]
7. Chauhan BC, O'Leary N, Almobarak FA, Reis AS, Yang H, Sharpe GP, Hutchison DM, Nicoletta MT, Burgoyne CF. Enhanced detection of open-angle glaucoma with an anatomically accurate optical coherence tomography-derived neuroretinal rim parameter. *Ophthalmology.* 2013; 120(3): 535–543. DOI: 10.1016/j.ophtha.2012.09.055 [PubMed: 23265804]
8. Gardiner SK, Boey PY, Yang H, Fortune B, Burgoyne CF, Demirel S. Structural Measurements for Monitoring Change in Glaucoma: Comparing Retinal Nerve Fiber Layer Thickness With Minimum Rim Width and Area. *Invest Ophthalmol Vis Sci.* 2015; 56(11):6886–6891. DOI: 10.1167/iovs.15-16701 [PubMed: 26501416]
9. Blumberg DM, De Moraes CG, Liebmann JM, Garg R, Chen C, Thevenithiran A, Hood DC. Technology and the Glaucoma Suspect. *Invest Ophthalmol Vis Sci.* 2016; 57(9):OCT80–85. DOI: 10.1167/iovs.15-18931 [PubMed: 27409509]
10. De Moraes CG, Liebmann JM, Ritch R, Hood DC. Understanding disparities among diagnostic technologies in glaucoma. *Arch Ophthalmol.* 2012; 130(7):833–840. DOI: 10.1001/archophthalmol.2012.786 [PubMed: 22776920]
11. Raza AS, Zhang X, De Moraes CG, Reisman CA, Liebmann JM, Ritch R, Hood DC. Improving glaucoma detection using spatially correspondent clusters of damage and by combining standard automated perimetry and optical coherence tomography. *Invest Ophthalmol Vis Sci.* 2014; 55(1): 612–624. DOI: 10.1167/iovs.13-12351 [PubMed: 24408977]
12. De Moraes CG, Liebmann JM, Ritch R, Hood DC. Clinical use of multifocal visual-evoked potentials in a glaucoma practice: a prospective study. *Doc Ophthalmol.* 2012; 125(1):1–9. DOI: 10.1007/s10633-012-9324-3 [PubMed: 22476612]

13. Bach M. Electrophysiological approaches for early detection of glaucoma. *Eur J Ophthalmol*. 2001; 11(Suppl 2):S41–49. [PubMed: 11592530]
14. Bach M, Poloschek CM. Electrophysiology and glaucoma: current status and future challenges. *Cell Tissue Res*. 2013; 353(2):287–296. DOI: 10.1007/s00441-013-1598-6 [PubMed: 23525754]
15. Wilsey LJ, Fortune B. Electroretinography in glaucoma diagnosis. *Curr Opin Ophthalmol*. 2016; 27(2):118–124. DOI: 10.1097/ICU.0000000000000241 [PubMed: 26720775]
16. Harwerth RS, Crawford ML, Frishman LJ, Viswanathan S, Smith EL 3rd, Carter-Dawson L. Visual field defects and neural losses from experimental glaucoma. *Prog Retin Eye Res*. 2002; 21(1):91–125. [PubMed: 11906813]
17. Rangaswamy NV, Zhou W, Harwerth RS, Frishman LJ. Effect of experimental glaucoma in primates on oscillatory potentials of the slow-sequence mfERG. *Invest Ophthalmol Vis Sci*. 2006; 47(2):753–767. [PubMed: 16431977]
18. Porciatti V. Electrophysiological assessment of retinal ganglion cell function. *Exp Eye Res*. 2015; 141:164–170. DOI: 10.1016/j.exer.2015.05.008 [PubMed: 25998495]
19. Holder GE. Pattern electroretinography (PERG) and an integrated approach to visual pathway diagnosis. *Prog Retin Eye Res*. 2001; 20(4):531–561. [PubMed: 11390258]
20. Hood DC. Assessing retinal function with the multifocal technique. *Prog Retin Eye Res*. 2000; 19(5):607–646. [PubMed: 10925245]
21. Ventura LM, Porciatti V. Pattern electroretinogram in glaucoma. *Curr Opin Ophthalmol*. 2006; 17(2):196–202. DOI: 10.1097/01.icu.0000193082.44938.3c [PubMed: 16552256]
22. Machida S. Clinical applications of the photopic negative response to optic nerve and retinal diseases. *Journal of ophthalmology*. 2012; 2012:397178.doi: 10.1155/2012/397178 [PubMed: 23133741]
23. Kaneko M, Machida S, Hoshi Y, Kurosaka D. Alterations of photopic negative response of multifocal electroretinogram in patients with glaucoma. *Curr Eye Res*. 2015; 40(1):77–86. DOI: 10.3109/02713683.2014.915575 [PubMed: 24832792]
24. Preiser D, Lagreze WA, Bach M, Poloschek CM. Photopic negative response versus pattern electroretinogram in early glaucoma. *Invest Ophthalmol Vis Sci*. 2013; 54(2):1182–1191. DOI: 10.1167/iovs.12-11201 [PubMed: 23307968]
25. Cull GA, Reynaud J, Wang L, Cioffi GA, Burgoyne CF, Fortune B. Relationship between orbital optic nerve axon counts and retinal nerve fiber layer thickness measured by spectral domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2012; 53(12):7766–7773. DOI: 10.1167/iovs.12-10752 [PubMed: 23125332]
26. Cull GA, Reynaud J, Wang L, Cioffi GA, Burgoyne CF, Fortune B. Erratum in: “Relationship Between Orbital Optic Nerve Axon Counts and Retinal Nerve Fiber Layer Thickness Measured by Spectral Domain Optical Coherence Tomography”. *Invest Ophthalmol Vis Sci*. 2014; 55(4):2619–2620. DOI: 10.1167/iovs.12-10752a
27. Fortune B, Hardin C, Reynaud J, Cull G, Yang H, Wang L, Burgoyne CF. Comparing Optic Nerve Head Rim Width, Rim Area, and Peripapillary Retinal Nerve Fiber Layer Thickness to Axon Count in Experimental Glaucoma. *Invest Ophthalmol Vis Sci*. 2016; 57(9):OCT404–412. DOI: 10.1167/iovs.15-18667 [PubMed: 27409499]
28. Fortune B, Cull GA, Burgoyne CF. Relative course of retinal nerve fiber layer birefringence and thickness and retinal function changes after optic nerve transection. *Invest Ophthalmol Vis Sci*. 2008; 49(10):4444–4452. DOI: 10.1167/iovs.08-2255 [PubMed: 18566463]
29. Fortune B, Burgoyne CF, Cull GA, Reynaud J, Wang L. Structural and functional abnormalities of retinal ganglion cells measured in vivo at the onset of optic nerve head surface change in experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2012; 53(7):3939–3950. DOI: 10.1167/iovs.12-9979 [PubMed: 22589428]
30. Fortune B, Cull G, Reynaud J, Wang L, Burgoyne CF. Relating Retinal Ganglion Cell Function and Retinal Nerve Fiber Layer (RNFL) Retardance to Progressive Loss of RNFL Thickness and Optic Nerve Axons in Experimental Glaucoma. *Invest Ophthalmol Vis Sci*. 2015; 56(6):3936–3944. DOI: 10.1167/iovs.15-16548 [PubMed: 26087359]

31. Wilsey LJ, Reynaud J, Cull G, Burgoyne CF, Fortune B. Macular Structure and Function in Nonhuman Primate Experimental Glaucoma. *Invest Ophthalmol Vis Sci*. 2016; 57(4):1892–1900. DOI: 10.1167/iovs.15-18119 [PubMed: 27082305]
32. Fortune B, Burgoyne CF, Cull G, Reynaud J, Wang L. Onset and progression of peripapillary retinal nerve fiber layer (RNFL) retardance changes occur earlier than RNFL thickness changes in experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2013; 54(8):5653–5661. DOI: 10.1167/iovs.13-12219 [PubMed: 23847322]
33. Fortune B, Reynaud J, Wang L, Burgoyne CF. Does optic nerve head surface topography change prior to loss of retinal nerve fiber layer thickness: a test of the site of injury hypothesis in experimental glaucoma. *PLoS One*. 2013; 8(10):e77831. [PubMed: 24204989]
34. Fortune B, Wang L, Bui BV, Burgoyne CF, Cioffi GA. Idiopathic bilateral optic atrophy in the rhesus macaque. *Invest Ophthalmol Vis Sci*. 2005; 46(11):3943–3956. DOI: 10.1167/iovs.04-1160 [PubMed: 16249467]
35. Johnson MA, Drum BA, Quigley HA, Sanchez RM, Dunkelberger GR. Pattern-evoked potentials and optic nerve fiber loss in monocular laser-induced glaucoma. *Invest Ophthalmol Vis Sci*. 1989; 30(5):897–907. [PubMed: 2722446]
36. Viswanathan S, Frishman LJ, Robson JG, Harwerth RS, Smith EL 3rd. The photopic negative response of the macaque electroretinogram: reduction by experimental glaucoma. *Invest Ophthalmol Vis Sci*. 1999; 40(6):1124–1136. [PubMed: 10235545]
37. Frishman LJ, Saszik S, Harwerth RS, Viswanathan S, Li Y, Smith EL 3rd, Robson JG, Barnes G. Effects of experimental glaucoma in macaques on the multifocal ERG. Multifocal ERG in laser-induced glaucoma. *Doc Ophthalmol*. 2000; 100(2–3):231–251. [PubMed: 11142748]
38. Viswanathan S, Frishman LJ, Robson JG. The uniform field and pattern ERG in macaques with experimental glaucoma: removal of spiking activity. *Invest Ophthalmol Vis Sci*. 2000; 41(9):2797–2810. [PubMed: 10937600]
39. Rangaswamy NV, Frishman LJ, Dorotheo EU, Schiffman JS, Bahrani HM, Tang RA. Photopic ERGs in patients with optic neuropathies: comparison with primate ERGs after pharmacologic blockade of inner retina. *Invest Ophthalmol Vis Sci*. 2004; 45(10):3827–3837. [PubMed: 15452095]
40. Nork TM, Kim CB, Heatley GA, Kaufman PL, Lucarelli MJ, Levin LA, Ver Hoeve JN. Serial multifocal electroretinograms during long-term elevation and reduction of intraocular pressure in non-human primates. *Doc Ophthalmol*. 2010; 120(3):273–289. DOI: 10.1007/s10633-010-9231-4 [PubMed: 20422254]
41. Luo X, Patel NB, Harwerth RS, Frishman LJ. Loss of the low-frequency component of the global-flash multifocal electroretinogram in primate eyes with experimental glaucoma. *Invest Ophthalmol Vis Sci*. 2011; 52(6):3792–3804. DOI: 10.1167/iovs.10-6667 [PubMed: 21421870]
42. Viswanathan S, Frishman LJ, Robson JG, Walters JW. The photopic negative response of the flash electroretinogram in primary open angle glaucoma. *Invest Ophthalmol Vis Sci*. 2001; 42(2):514–522. [PubMed: 11157891]
43. Rangaswamy NV, Shirato S, Kaneko M, Digby BI, Robson JG, Frishman LJ. Effects of Spectral Characteristics of Ganzfeld Stimuli on the Photopic Negative Response (PhNR) of the ERG. *Invest Ophthalmol Vis Sci*. 2007; 48(10):4818–4828. DOI: 10.1167/iovs.07-0218 [PubMed: 17898309]
44. Hood DC, Xu L, Thienprasiddhi P, Greenstein VC, Odel JG, Grippio TM, Liebmann JM, Ritch R. The pattern electroretinogram in glaucoma patients with confirmed visual field deficits. *Invest Ophthalmol Vis Sci*. 2005; 46(7):2411–2418. DOI: 10.1167/iovs.05-0238 [PubMed: 15980229]
45. Bach M, Holder GE. Check size tuning of the pattern electroretinogram: a reappraisal. *Doc Ophthalmol*. 1996; 92(3):193–202.
46. Bode SF, Jehle T, Bach M. Pattern electroretinogram in glaucoma suspects: new findings from a longitudinal study. *Invest Ophthalmol Vis Sci*. 2011; 52(7):4300–4306. DOI: 10.1167/iovs.10-6381 [PubMed: 21372021]
47. Banitt MR, Ventura LM, Feuer WJ, Savatovsky E, Luna G, Shif O, Bosse B, Porciatti V. Progressive loss of retinal ganglion cell function precedes structural loss by several years in glaucoma suspects. *Invest Ophthalmol Vis Sci*. 2013; 54(3):2346–2352. DOI: 10.1167/iovs.12-11026 [PubMed: 23412088]

48. Bach M, Ramharter-Sereinig A. Pattern electroretinogram to detect glaucoma: comparing the PERGLA and the PERG Ratio protocols. *Doc Ophthalmol*. 2013; 127(3):227–238. DOI: 10.1007/s10633-013-9412-z [PubMed: 24126828]
49. Hood DC, Greenstein VC, Holopigian K, Bauer R, Firoz B, Liebmann JM, Odel JG, Ritch R. An attempt to detect glaucomatous damage to the inner retina with the multifocal ERG. *Invest Ophthalmol Vis Sci*. 2000; 41(6):1570–1579. [PubMed: 10798678]
50. Fortune B, Johnson CA, Cioffi GA. The topographic relationship between multifocal electroretinographic and behavioral perimetric measures of function in glaucoma. *Optom Vis Sci*. 2001; 78(4):206–214. [PubMed: 11349928]
51. Fortune B, Bearse MA Jr, Cioffi GA, Johnson CA. Selective loss of an oscillatory component from temporal retinal multifocal ERG responses in glaucoma. *Invest Ophthalmol Vis Sci*. 2002; 43(8):2638–2647. [PubMed: 12147597]
52. Stiefelmeyer S, Neubauer AS, Berninger T, Arden GB, Rudolph G. The multifocal pattern electroretinogram in glaucoma. *Vision Res*. 2004; 44(1):103–112. [PubMed: 14599576]
53. Harrison WW, Viswanathan S, Malinovsky VE. Multifocal pattern electroretinogram: cellular origins and clinical implications. *Optom Vis Sci*. 2006; 83(7):473–485. DOI: 10.1097/01.opx.0000218319.61580.a5 [PubMed: 16840872]
54. Ledolter AA, Monhart M, Schoetzau A, Todorova MG, Palmowski-Wolfe AM. Structural and functional changes in glaucoma: comparing the two-flash multifocal electroretinogram to optical coherence tomography and visual fields. *Doc Ophthalmol*. 2015; 130(3):197–209. DOI: 10.1007/s10633-015-9482-1 [PubMed: 25616700]
55. Hood DC, Frishman LJ, Saszik S, Viswanathan S. Retinal origins of the primate multifocal ERG: implications for the human response. *Invest Ophthalmol Vis Sci*. 2002; 43(5):1673–1685. [PubMed: 11980890]
56. Luo X, Frishman LJ. Retinal pathway origins of the pattern electroretinogram (PERG). *Invest Ophthalmol Vis Sci*. 2011; 52(12):8571–8584. DOI: 10.1167/iops.11-8376 [PubMed: 21948546]
57. Kato F, Miura G, Shirato S, Sato E, Yamamoto S. Correlation between N2 amplitude of multifocal ERGs and retinal sensitivity and retinal nerve fiber layer thickness in glaucomatous eyes. *Doc Ophthalmol*. 2015; 131(3):197–206. DOI: 10.1007/s10633-015-9519-5 [PubMed: 26521929]
58. Golemez H, Yildirim N, Ozer A. Is multifocal electroretinography an early predictor of glaucoma? *Doc Ophthalmol*. 2016; 132(1):27–37. DOI: 10.1007/s10633-016-9524-3 [PubMed: 26792427]
59. Colotto A, Falsini B, Salgarello T, Iarossi G, Galan ME, Scullica L. Photopic negative response of the human ERG: losses associated with glaucomatous damage. *Invest Ophthalmol Vis Sci*. 2000; 41(8):2205–2211. [PubMed: 10892864]
60. North RV, Jones AL, Drasdo N, Wild JM, Morgan JE. Electrophysiological evidence of early functional damage in glaucoma and ocular hypertension. *Invest Ophthalmol Vis Sci*. 2010; 51(2):1216–1222. DOI: 10.1167/iops.09-3409 [PubMed: 19850843]
61. Niyadurupola N, Luu CD, Nguyen DQ, Geddes K, Tan GX, Wong CC, Tran T, Coote MA, Crowston JG. Intraocular pressure lowering is associated with an increase in the photopic negative response (PhNR) amplitude in glaucoma and ocular hypertensive eyes. *Invest Ophthalmol Vis Sci*. 2013; 54(3):1913–1919. DOI: 10.1167/iops.12-10869 [PubMed: 23385794]
62. Machida S, Kaneko M, Kurosaka D. Regional variations in correlation between photopic negative response of focal electroretinograms and ganglion cell complex in glaucoma. *Curr Eye Res*. 2015; 40(4):439–449. DOI: 10.3109/02713683.2014.922196 [PubMed: 24871085]
63. Wu Z, Hadoux X, Fan Gaskin JC, Sarossy MG, Crowston JG. Measuring the Photopic Negative Response: Viability of Skin Electrodes and Variability Across Disease Severities in Glaucoma. *Translational vision science & technology*. 2016; 5(2):13.doi: 10.1167/tvst.5.2.13
64. Kirkiewicz M, Lubinski W, Penkala K. Photopic negative response of full-field electroretinography in patients with different stages of glaucomatous optic neuropathy. *Doc Ophthalmol*. 2016; 132(1):57–65. DOI: 10.1007/s10633-016-9528-z [PubMed: 26831670]
65. Chrysostomou V, Crowston JG. The photopic negative response of the mouse electroretinogram: reduction by acute elevation of intraocular pressure. *Invest Ophthalmol Vis Sci*. 2013; 54(7):4691–4697. DOI: 10.1167/iops.13-12415 [PubMed: 23766473]

66. Liu Y, McDowell CM, Zhang Z, Tebow HE, Wordinger RJ, Clark AF. Monitoring retinal morphologic and functional changes in mice following optic nerve crush. *Invest Ophthalmol Vis Sci.* 2014; 55(6):3766–3774. DOI: 10.1167/iov.14-13895 [PubMed: 24854856]
67. Machida S, Toba Y, Ohtaki A, Gotoh Y, Kaneko M, Kurosaka D. Photopic negative response of focal electroretinograms in glaucomatous eyes. *Invest Ophthalmol Vis Sci.* 2008; 49(12):5636–5644. DOI: 10.1167/iov.08-1946 [PubMed: 18641287]
68. Machida S, Gotoh Y, Toba Y, Ohtaki A, Kaneko M, Kurosaka D. Correlation between photopic negative response and retinal nerve fiber layer thickness and optic disc topography in glaucomatous eyes. *Invest Ophthalmol Vis Sci.* 2008; 49(5):2201–2207. DOI: 10.1167/iov.07-0887 [PubMed: 18436853]
69. Machida S, Tamada K, Oikawa T, Gotoh Y, Nishimura T, Kaneko M, Kurosaka D. Comparison of photopic negative response of full-field and focal electroretinograms in detecting glaucomatous eyes. *Journal of ophthalmology.* 2011; 2011doi: 10.1155/2011/564131
70. Fortune B, Bui BV, Cull G, Wang L, Cioffi GA. Inter-ocular and inter-session reliability of the electroretinogram photopic negative response (PhNR) in non-human primates. *Exp Eye Res.* 2004; 78(1):83–93. [PubMed: 14667830]
71. Mortlock KE, Binns AM, Aldebasi YH, North RV. Inter-subject, inter-ocular and inter-session repeatability of the photopic negative response of the electroretinogram recorded using DTL and skin electrodes. *Doc Ophthalmol.* 2010; 121(2):123–134. DOI: 10.1007/s10633-010-9239-9 [PubMed: 20607349]
72. Wu Z, Hadoux X, Hui F, Sarossy MG, Crowston JG. Photopic Negative Response Obtained Using a Handheld Electroretinogram Device: Determining the Optimal Measure and Repeatability. *Translational vision science & technology.* 2016; 5(4):8.doi: 10.1167/tvst.5.4.8

Appendix

Measurement of PhNR amplitude has been done using a variety of methods in different laboratories. These various approaches include measuring the voltage difference between the pre-stimulus baseline or the peak of the B-wave and either the trough (minima) following the B-wave or a fixed post-stimulus criterion time [36,70,67,68,71,72]. Previously, we had determined that PhNR amplitude measurements from the peak of the B-wave to a fixed criterion time of 70 msec provided the best reproducibility for the flash intensity used in the current study. However, the larger sample size and inclusion of glaucomatous eyes available for the current study presented an opportunity to re-evaluate this question. Therefore, we compared diagnostic performance of 8 different derivations of PhNR amplitude and selected the best performing method to use in the main part of the study for comparison to mfERG and PERG parameters. The eight PhNR amplitude derivations evaluated were measured as follows: 1) from B-wave peak to a criterion time of 60 msec; 2) 65 msec; 3) 70 msec; 4) from the ERG baseline to a criterion time of 60 msec; 5) 65 msec; 6) 70 msec; 7) from the B-wave peak to the PhNR trough; 8) from the ERG baseline to the PhNR trough. The results of this comparison demonstrated unequivocally that the best diagnostic performance was obtained using method 8: from the ERG baseline to the PhNR trough (see Tables 5 and 6). Therefore, PhNR amplitude measurements obtained using that method were included in the main study for comparison to other parameters of the photopic full-field flash ERG, the mfERG and PERG.

Although diagnostic performance was clearly better for the baseline-to-trough PhNR amplitude derivation, it was also useful to compare repeat reliability across these eight approaches. To this end, we calculated the coefficient of variation (CoV) as the standard deviation of pre-laser baseline measurements divided by the mean of the same

measurements for each eye. Alternatively, since the average value of a given parameter in healthy eyes might not be an adequate representation of its dynamic range, we also scaled the median standard deviation of baseline measurements by the median effect size (i.e., the difference between the amplitude in the glaucomatous eye and the amplitude in the fellow control eye at the final time point). The results revealed that the median value for the CoV was always approximately half as large for PhNR amplitude measurements made from the B-wave peak as compared with those made from the ERG baseline (17% vs. 30%, on average, respectively). However, the inverse was true when inter-session variation was scaled by the effect size instead of by the average magnitude of each parameter: by this metric, the PhNR amplitude measurements made from the B-wave peak were about twice as variable as those made from the ERG baseline (65% vs. 27%, on average, respectively). Ironically, this result for repeat reliability did not manifest as better diagnostic performance of PhNR measurements made from the ERG baseline compared to those made from the B-wave peak for longitudinal (baseline normalized) data as compared with strictly cross-sectional data (compare Tables 5 and 6). In any case, the method of PhNR amplitude derivation based on ERG baseline-to-trough had the best diagnostic performance and among the best repeat reliability by either metric (median CoV=21%; inter-session variation scaled by effect size=29%). Thus it was clearly the measurement of choice for the comparison to other ERG parameters in the main portion of this study.

Table 5

Diagnostic Sensitivity Across Various PhNR Amplitude Derivations, Raw Values.

PhNR Measurement		A-ROC	Standard Error	P Value	Sensitivity at 95% Specificity	95% Confidence Interval
<i>Peak-to-Criterion Time</i>	60 msec	0.70	0.06	0.0012	37.2%	24.4% – 52.1%
	65 msec	0.69	0.06	0.0024	23.3%	13.2% – 37.7%
	70 msec	0.72	0.06	0.0005	34.9%	22.4% – 49.8%
<i>Baseline-to-Criterion Time</i>	60 msec	0.87	0.04	< 0.0001	67.4%	52.5% – 79.5%
	65 msec	0.82	0.05	< 0.0001	23.3%	13.2% – 37.7%
	70 msec	0.85	0.04	< 0.0001	51.2%	36.8% – 65.4%
<i>To trough</i>	peak-to-trough	0.70	0.06	0.0015	25.6%	14.9% – 40.2%
	BL-to-trough	0.90	0.03	< 0.0001	65.1%	50.2% – 77.6%

Table 6

Diagnostic Sensitivity Across Various PhNR Amplitude Derivations, Change from Baseline.

PhNR Measurement		A-ROC	Standard Error	P Value	Sensitivity at 95% Specificity	95% Confidence Interval
<i>Peak-to-Criterion Time</i>	60 msec	0.84	0.04	< 0.0001	34.9%	22.4% – 49.8%
	65 msec	0.82	0.05	< 0.0001	30.2%	18.6% – 45.1%
	70 msec	0.84	0.04	< 0.0001	39.5%	26.4% – 54.4%
<i>Baseline-to-Criterion Time</i>	60 msec	0.79	0.05	< 0.0001	9.3%	3.7% – 21.6%

PhNR Measurement		A-ROC	Standard Error	P Value	Sensitivity at 95% Specificity	95% Confidence Interval
<i>To trough</i>	65 msec	0.78	0.06	< 0.0001	7.0%	2.4% – 18.6%
	70 msec	0.76	0.06	< 0.0001	9.3%	3.7% – 21.6%
	peak-to-trough	0.80	0.05	< 0.0001	51.2%	36.8% – 65.4%
	BL-to-trough	0.93	0.03	< 0.0001	67.4%	52.5% – 79.5%

Figure 1. SDOCT peripapillary circular B-scans to measure RNFLT

Individual example of control eye and EG eye at first baseline (A, B) and at final imaging session (C, D). The insets show the B-scan location indicated by the green line overlaid onto the fundus infrared reflectance image.

Figure 2. Multifocal ERG (mfERG) methods and example

(A) Schematic of slow-sequence stimulus, 7 dark frames between m-sequence steps; 103 hexagons over $\sim 55^\circ$; mean luminance of m-steps: 100 cd/m^2 ; contrast: $\sim 99\%$. We analyzed the central 37 responses enclosed by red circle in (B); which are shown for same eye as Fig. 1B/1D in (C), color-coded by rings around the central element and numbered by location; baseline set is shown at left and final session set at right. Each local response was filtered at 75 Hz into low- and high-frequency components (LFC, HFC). Amplitudes of the LFC features N1, P1 and N2 were measured 'peak-to-trough', HFC amplitude measured as RMS

from 0–80 ms. Note that mfERG HFC amplitudes were reduced this EG eye with moderate damage at the final session.

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Figure 3. Pattern-reversal ERG (PERG) methods and example

P50 and N95 amplitudes measured 'trough-to-peak'; see inset. Gray traces show the recording from each of the five baseline sessions, red trace shows the recording from the final session in the same animal (left eye, OS) as shown Figs. 1–2. Note that N95 amplitude is reduced more than P50 in this EG eye with moderate damage at the final session.

Figure 4. Full-Field flash ERG (ff-ERG) photopic negative response (PhNR) methods and example

A-wave amplitude measured at 10 ms, B-wave at peak from A-wave trough, PhNR at 70 ms from B-wave peak; see inset. Gray traces show the recording from each of the five baseline sessions; red trace shows the recording from the final session in the same eye as Figs. 1–3. Note that PhNR amplitude is reduced more than A-wave or B-wave amplitude in this EG eye with moderate damage at the final session.

Figure 5. Diagnostic performance of ERG parameters at the final session
(A) Receiver operator characteristic (ROC) curves based on the raw parameter values at the final ERG recording session. **(B)** ROC curves based on the percentage change of parameter value at the final ERG recording session in each eye relative to its baseline average value. See Tables 1 and 2, respectively, for quantitative results corresponding to panels A and B.

Figure 6. Diagnostic sensitivity at earlier time points for the best parameters of each ERG mode
Sensitivity at 95% specificity is plotted for three additional time points prior to the final time point.

Figure 7. Relationship between ERG parameters and peripapillary RNFL thickness

Scatter plots show ERG parameter values versus RNFL thickness at the final session. Only the best performing parameters of each ERG mode are shown. (A) mfERG HFC amplitude (B) mfERG HFC:P1 (C) ff-ERG PhNR amplitude (D) ff-ERG PhNR:B-wave (E) PERG P50 amplitude (F) PERG N95 amplitude. See Table 3 for comparison of Pearson correlation coefficients.

Figure 8. Relationship between ERG parameters and peripapillary RNFL thickness change from baseline

Scatter plots show ERG parameter values expressed relative to the baseline (BL) average values of each eye (i.e., ERG change from BL versus RNFLT change from BL). Only the best performing parameters of each ERG mode are shown. (A) mfERG HFC amplitude (B) mfERG HFC:P1 (C) ff-ERG PhNR amplitude (D) ff-ERG PhNR:B-wave (E) PERG P50 amplitude (F) PERG N95 amplitude. See Table 4 for comparison of Pearson correlation coefficients.

Figure 9.

Overlap of diagnostic information. Area-proportional Euler diagrams indicate overlap in the number of EG eyes flagged by the best performing parameters of each ERG mode at their 95% specificity criterion; the total number (and proportion) of EG eyes flagged is listed for each parameter in the legend. Panel A shows that three EG eyes were flagged by PhNR amplitude and two by PERG N95 that were not among the 23 eyes flagged by mfERG HFC amplitude. Panel B shows that normalization to other components of the same ERG response, HFC:P1 amplitude in the case of the mfERG and PhNR:B-wave in the case of the ff-ERG, increased sensitivity at 95% specificity and also increased overlap such that only one EG eye was flagged by PhNR and one other by PERG N95 that were not otherwise flagged by mfERG HFC.

Table 1
A-ROC* Results Based on Raw Parameter Values

Parameters performing significantly better than chance (after Bonferroni correction) have A-ROC values listed in bold font.

		<u>A-ROC</u>	<u>Standard Error</u>	<u>P Value</u>
<i>mfERG</i>	N1	0.57	0.06	0.23
	P1	0.55	0.06	0.45
	N2	0.70	0.06	0.001
	HFC	0.82	0.04	<0.0001
	HFC:P1	0.90	0.03	<0.0001
	N2:P1	0.79	0.05	<0.0001
<i>ff-ERG</i>	A-wave	0.49	0.06	0.85
	B-wave	0.60	0.06	0.13
	PhNR	0.90	0.03	<0.0001
	PhNR:B-wave	0.88	0.04	<0.0001
<i>PERG</i>	P50	0.64	0.06	0.03
	N95	0.61	0.06	0.08
	N95:P50	0.53	0.06	0.67
	N95 slope	0.59	0.06	0.15

*
A-ROC: Area under receiver operator characteristic curve.

Table 2
A-ROC* Results Based on Parameter % Change from Baseline

Parameters performing significantly better than chance (after Bonferroni correction) have A-ROC values listed in bold font.

		<u>A-ROC</u>	<u>Standard Error</u>	<u>P Value</u>
<i>mfERG</i>	N1	0.64	0.06	0.02
	P1	0.57	0.06	0.27
	N2	0.73	0.06	0.0003
	HFC	0.87	0.04	<0.0001
	HFC:P1	0.92	0.03	<0.0001
	N2:P1	0.84	0.04	<0.0001
<i>ff-ERG</i>	A-wave	0.56	0.06	0.38
	B-wave	0.67	0.06	0.005
	PhNR	0.93	0.03	<0.0001
	PhNR:B-wave	0.90	0.03	<0.0001
<i>PERG</i>	P50	0.70	0.06	0.001
	N95	0.69	0.06	0.002
	N95:P50	0.55	0.06	0.42
	N95 slope	0.68	0.06	0.005

*
A-ROC: Area under receiver operator characteristic curve.

Table 3

Diagnostic Sensitivity and Correlation to Structure (RNFLT) for ERG Parameter Raw Values.

		Sensitivity at 95% Specificity	95% Confidence Interval	Correlation to RNFLT	95% Confidence Interval	P Value
<i>mERG</i>	N1	20.9%	11.4% – 35.2%	0.34	0.136 – 0.514	0.0014
	P1	11.6%	5.1% – 24.5%	0.23	0.015 – 0.418	0.0365
	N2	39.5%	26.4% – 54.4%	0.43	0.235 – 0.585	< 0.0001
	HFC	53.5%	38.9% – 67.5%	0.65	0.510 – 0.759	< 0.0001
	HFC:P1	74.4%	59.8% – 85.1%	0.61	0.460 – 0.730	< 0.0001
	N2:P1	25.6%	14.9% – 40.2%	0.44	0.252 – 0.596	< 0.0001
<i>ff-ERG</i>	A-wave	9.3%	3.7% – 21.6%	0.07	–0.144 – 0.278	0.5218
	B-wave	16.3%	8.1% – 30%	0.22	0.012 – 0.415	0.0389
	PhNR	65.1%	50.2% – 77.6%	0.59	0.431 – 0.712	< 0.0001
	PhNR:B-wave	55.8%	41.1% – 69.6%	0.50	0.318 – 0.641	< 0.0001
<i>PERG</i>	P50	32.6%	20.5% – 47.5%	0.40	0.205 – 0.563	0.0001
	N95	25.6%	14.9% – 40.2%	0.36	0.159 – 0.530	0.0007
	N95:P50	9.3%	3.7% – 21.6%	–0.01	–0.220 – 0.204	0.9371
	N95 slope	34.9%	22.4% – 49.8%	0.35	0.151 – 0.524	0.0009

Table 4

Diagnostic Sensitivity and Correlation to Structure (RNFLT) for ERG Parameter Change from Baseline.

		Sensitivity at 95% Specificity	95% Confidence Interval	Correlation to RNFLT	95% Confidence Interval	P Value
<i>mERG</i>	N1	18.6%	9.7% – 32.6%	0.37	0.168 – 0.537	0.0005
	P1	18.6%	9.7% – 32.6%	0.22	0.005 – 0.410	0.045
	N2	30.2%	18.6% – 45.1%	0.48	0.294 – 0.625	< 0.0001
	HFC	60.5%	45.6% – 73.6%	0.64	0.494 – 0.750	< 0.0001
	HFC:P1	83.7%	70% – 91.9%	0.67	0.533 – 0.772	< 0.0001
<i>ff-ERG</i>	N2:P1	34.9%	22.4% – 49.8%	0.58	0.415 – 0.702	< 0.0001
	A-wave	11.6%	5.1% – 24.5%	0.04	-0.173 – 0.25	0.7131
	B-wave	37.2%	24.4% – 52.1%	0.19	-0.018 – 0.39	0.0729
	PhNR	67.4%	52.5% – 79.5%	0.60	0.449 – 0.723	< 0.0001
	PhNR:B-wave	69.8%	54.9% – 81.4%	0.62	0.473 – 0.737	< 0.0001
<i>PERG</i>	P50	34.9%	22.4% – 49.8%	0.48	0.303 – 0.631	< 0.0001
	N95	30.2%	18.6% – 45.1%	0.49	0.310 – 0.636	< 0.0001
	N95:P50	4.7%	1.3% – 15.5%	0.21	-0.003 – 0.403	0.0536
	N95 slope	30.2%	18.6% – 45.1%	0.51	0.332 – 0.650	< 0.0001