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ORIGINAL RESEARCH ARTICLE

# Test–retest repeatability of the pattern electroretinogram and flicker electroretinogram

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**Abstract**

*Purpose* To evaluate the repeatability of the steady-state pattern electroretinogram (PERG) and full-field flicker electroretinogram (Flicker ERG) protocols, delivered by the office-based Neuro Optic Vision Assessment (NOVA)$^{TM}$ testing platform, in healthy subjects.

*Methods* Healthy individuals underwent PERG (16° and 24°) and Flicker ERG [fixed luminance (FL) and multi-luminance (ML)] testing protocols. Test–retest repeatability of protocols was calculated using intra-class correlation coefficients (ICC). Reference values of the parameters of the aforementioned tests were also calculated.

*Results* The ICCs for the PERG parameters ranged from 0.793 to 0.911 ($p < 0.001$). The ICCs for the Flicker ERG parameters ranged from 0.968 to 0.994 ($p < 0.001$). A linear regression analysis was applied to assess the impact of age on ERG responses. Age had a significant impact on all PERG parameters (16° or 24°). The phase response of the FL Flicker ERG significantly decreased with age ($\beta = -0.837$, $p \leq 0.001$). The FL Flicker ERG Magnitude was also impacted with a significant quadratic effect of age ($\beta = -0.0047$, $p = 0.0004$). Similarly, the Phase Area Under the Curve (Phase AUC) of the ML Flicker ERG significantly declined with age ($\beta = -0.007$, $p = 0.009$), and the impact on the Magnitude AUC was significant as well, with a negative quadratic age effect.

*Conclusions* The PERG and Flicker ERG protocols, delivered by an office-based testing platform, were shown to have good-to-excellent test–retest repeatability when tests were performed in the same order and in immediate succession.

**Keywords** Steady-state pattern electroretinogram · Flicker electroretinogram · Repeatability · Electrophysiology · Reference database

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## Introduction

Diagnosis and follow-up of ocular diseases have long relied on evaluation of the structural and functional condition of the visual pathway. However, functional analysis of the visual pathway has continued to rely on subjective measures that include visual acuity (VA) and visual field (VF) techniques. Reports from the National Institute of Health and the Food and Drug Administration (FDA) have recommended the employment of visual function tests as a primary endpoint in clinical studies [1–5]. This underscores the need for tests of visual function that are not influenced by subjective patient-to-patient inconsistencies [6]. One such possibility is the use of electrophysiological testing.

Since 1989, the International Society for Clinical Electrophysiology of Vision (ISCEV) has provided the scientific community with frequently revised and updated standardized protocols for electrophysiological testing in an effort to yield comparable results. ISCEV encourages developers to extend test protocols, as required, in order to maximize the diagnostic value of electrophysiology for patient care and research [7, 8].

Pattern electroretinography (PERG) is a modality of electrophysiological testing that uses a pattern with temporal modulation, alternating between brighter and darker elements [9]. From the PERG stimulus, a constant mean luminance is created, canceling the responses from cone receptors and bipolar cells. Therefore, PERG results mainly reflect the responses elicited from retinal ganglion cells (RGC) [10]. The steady-state variation of the PERG is a recently described testing sequence that arises from the superposition of overlapping PERG responses, and has an increased sensitivity to inner retinal disorders. Steady-state PERG has particular value in discerning between glaucomatous and healthy eyes, as an altered RGC response can precede significant visual field defects [7, 11–13].

Full-field ERG records the global response of the retina to flashes of light and provides an assessment of the general retinal function. Flicker electroretinography (Flicker ERG) presents a light stimulus at a rate of approximately 30 Hz per second to selectively reflect cone- and bipolar-driven response, as rods are unable to respond at this rate [8]. Flicker ERG is known to be a valuable marker of neurovascular coupling integrity and a useful tool for the evaluation of ischemic retinal states [14, 15].

This study aimed to evaluate the repeatability of the steady-state PERG (16° and 24°) and Flicker ERG (fixed luminance and multi-luminance) protocols in healthy subjects. The data from this study were used to develop a reference database for these tests.

## Methods

This was a cross-sectional study of healthy subjects with normal retinal function. Individuals were prospectively enrolled, and the study visits were carried out at the Glaucoma Research Center at Wills Eye Hospital in Philadelphia, PA. This study was conducted following the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the Wills Eye Hospital. Informed consent was obtained from all individual participants included in the study.

Subjects were volunteers aged 21 and older. To ensure data were obtained from all adult age groups (21–30, 31–40, 41–50, 51–60, 61–70, $> 70$), we conducted age-specific recruitment.

Subjects underwent a complete ophthalmologic examination, including best corrected visual acuity (BCVA) (Snellen), intraocular pressure (IOP) measurement (Goldmann Applanation Tonometer, Haag-Streit, Harlow, Essex), slit-lamp biomicroscopy, fundus examination, and visual field testing (24-2 Swedish Interactive Threshold Algorithm Standard Strategy, Carl Zeiss Meditec, Inc., Dublin, CA). Retinal function was tested using the commercially available Diopsys® NOVA™ PERG and Flicker ERG (Diopsys Inc., Pine Brook, NJ).

Subjects were excluded if they had a spherical refraction outside $\pm$ 5.0 D or a cylindrical correction outside of $\pm$ 3.0 D, an IOP $\geq$ 21 mmHg, history of glaucoma in either eye, history of intraocular surgery in the study eye (except non-complicated cataract surgery more than 1 year before enrollment), BCVA worse than 20/40, evidence of any systemic or ophthalmic condition known to affect the visual function, current or recent use of a medication known to affect visual function, or an inability to record a reliable PERG or Flicker ERG result.

## Electroretinography

The NOVA$^{TM}$ testing platform is an office-based method of administering electrophysiological tests. This platform is able to administer PERG and Flicker ERG testing in a short time and with easy placement of electrodes.

Prior to PERG and Flicker ERG testing, subjects were seated comfortably in front of the Diopsys device. The skin of the lower eyelid was cleansed using OCuSOFT® Lid Scrub (OCuSOFT, Inc., Rosenberg, TX), and the central forehead area was cleansed using NuPrep® Skin Prep Gel (Weaver & Co., Aurora, CO). Electrodes were placed in three locations, as seen in Fig. 1. Two adhesive Diopsys® ERG Lid Electrodes (Diopsys, Inc., Pine Brook, NJ) were placed on the lower eyelid of each eye to function as active and reference recording electrodes. A disposable electroencephalogram (EEG) electrode was secured to the forehead using Ten20 Conductive Paste (Weaver & Co., Aurora, CO) to function as a ground electrode. Pupils remained undilated and artificial tears were applied to each eye.

## PERG

Subjects were refracted and seated 24 inches from the stimulus monitor. Subjects were instructed to focus on a target in the center of the stimulus screen while continuing to blink freely. An occluding lens was fit into the trial lens to cover the eye not being tested.

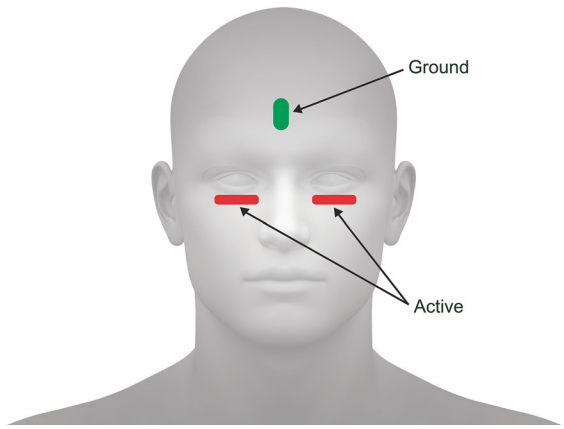
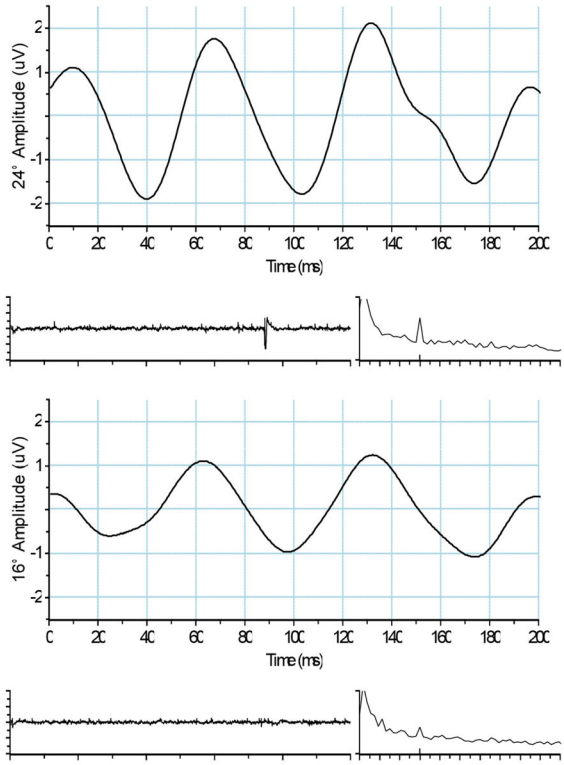



**Fig. 1** Electrode placement for pattern electroretinogram and flicker electroretinogram testing. Active electrodes are placed under the eyelids, and ground electrode is placed on the forehead

Testing was performed in a dark room. The PERG stimulus was presented on a gamma corrected Acer V173BM 27-inch LCD monitor with a refresh rate of 75 frames/second. Successive stimuli consisting of a pattern of horizontal gratings either within 16° or 24° concentric circular fields at a time reversing at 15 reversals/second were presented. A contrast level of 100% Michelson was used, with a mean luminance of 102.14 cd $m^{-2}$. The total display viewing angle was 24.97°, with each bar subtending 0.39°, and the fixation target subtending 0.78°. The stimulus duration was 25 s per eye, for a total duration of 50 s per eye. The PERG test was repeated three times, with a 2–3-min break between tests.

Signals were amplified 20,000 times and band-pass filtered with cutoff frequencies of 0.5 and 100 Hz. The voltage range of the analog to digital (A/D) converter was ± 5.0 V. Sweeps contaminated by eye blinks or gross motor saccades were rejected over a threshold voltage of 50 μV. Synchronized single-channel ERGs were recorded, generating a time series of 384 data points per analysis frame (200 ms). A fast Fourier transformation (FFT) was applied to the PERG waveforms to isolate the desired component at 15 rps. Other frequencies, such as those originating from eye muscles, were rejected.

Recorded PERG parameters included Magnitude, Magnitude D, Magnitude D/Magnitude Ratio, and signal-to-noise ratio (SNR) at 15 Hz. The Magnitude represents the amplitude, or strength, of the subject's response. The Magnitude is calculated by dividing the signal into one-second segments, performing FFTs on each one-second segment, and then averaging the FFTs. The Magnitude D also represents the amplitude of the signal, but is highly influenced by the phase variability during the signal acquisition period, unlike the Magnitude parameter which is minimally influenced by intra-test variability. Magnitude D is calculated by averaging the one-second segments of the signal and then performing an FFT on the averaged time-domain signal. Using the Magnitude and Magnitude D parameters, the Magnitude D/Magnitude Ratio can be calculated [16]. The Magnitude D/Magnitude Ratio aims to provide a parameter that is less influenced by inter-subject variability than either Magnitude or Magnitude D absolute values. The Magnitude D/Magnitude Ratio values fall between 0 and 1, where values




| Parameter | OD 24° | OD 16° |
|---|---|---|
| Magnitude (uV) | 2.01 | 1.28 |
| MagnitudeD | 1.82 | 0.99 |
| MagD/Mag Ratio | 0.91 | 0.77 |
| SNR (dB) | 7.3 | 3.8 |
| Artifacts | 0 | 0 |

**Fig. 2** PERG sample of a subject's right eye for representation of waveforms and parameters yielded

closer to 1 indicate greater consistency of the signal during the acquisition time (Fig. 2). The application of Magnitude, Magnitude D, and Magnitude D/Magnitude Ratio parameters has been found to be a very sensitive approach for identifying early retinal dysfunction [17, 18], given the fact that some diseases like glaucoma tend to affect the consistency of the RGC response [19], even before and structural damage is established [20].

## Flicker ERG

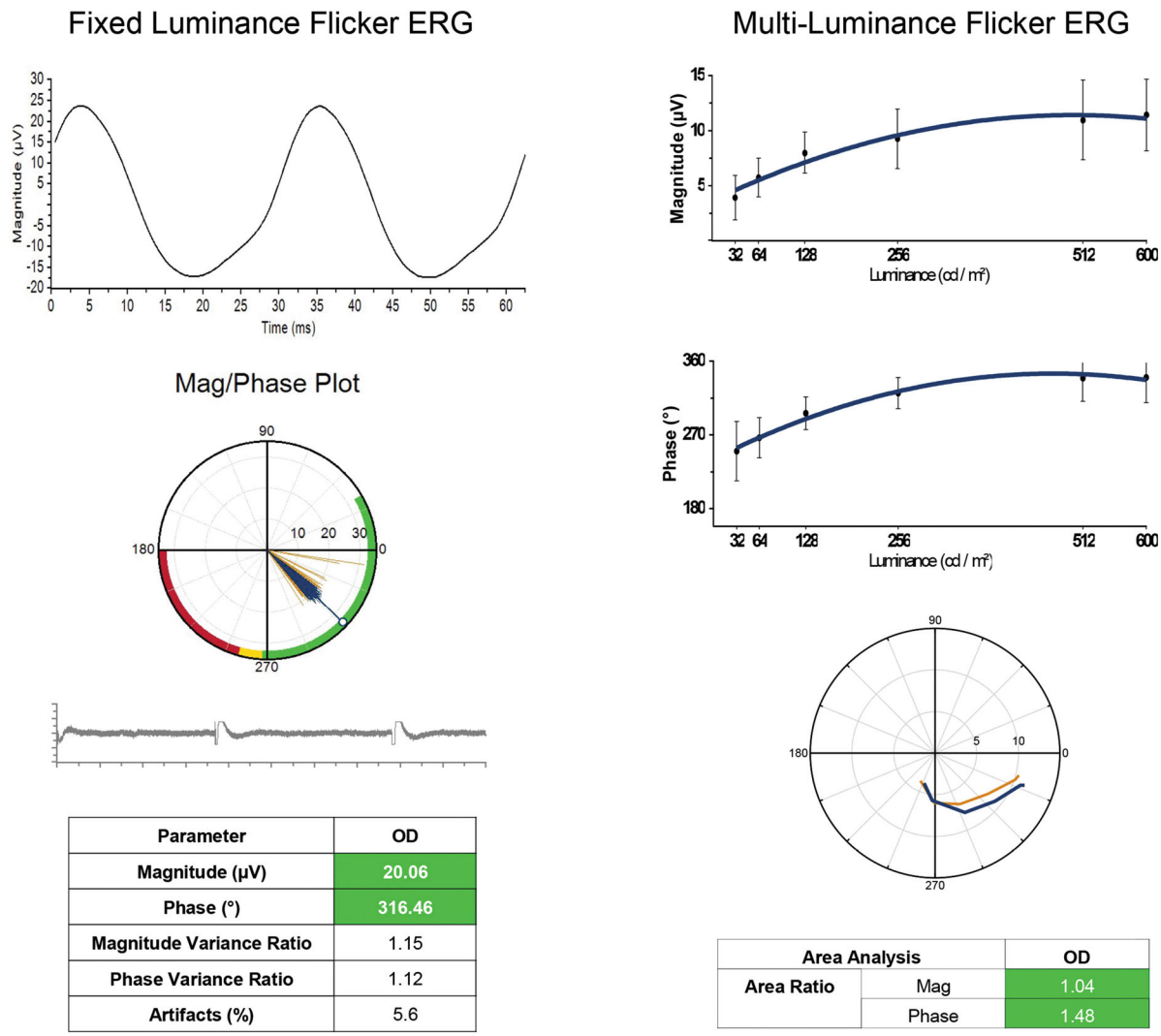
Subjects were asked to place the handheld mini-Ganzfeld dome over the testing eye, resting it on the cheek and upper brow, while the non-testing eye was left uncovered. With the non-testing eye, subjects were asked to focus on a stationary object in the room. Subjects were able to blink freely throughout the testing period. Subject preparation for both variations of the Flicker ERG test remained constant.

The Flicker ERG tests were conducted in an illuminated room. The fixed luminance (FL) Flicker ERG stimulus, presented within the mini-Ganzfeld dome, consisted of white flashes at 3 cd s $m^{-2}$ on a yellow background of 30 cd $m^{-2}$. The stimulus was presented at a rate of 32 flashes/second, with each flash lasting 5 ms. The stimulus pattern was repeated for 20 s for each eye. The FL Flicker ERG test was repeated three times, with a 1-min break between tests.

The multi-luminance (ML) Flicker ERG stimuli were presented within the mini-Ganzfeld dome and consisted of white flashes with no background illumination. The stimulus consisted of six luminance levels (0.16 cd s $m^{-2}$, 0.32 cd s $m^{-2}$, 0.64 cd s $m^{-2}$, 1.28 cd s $m^{-2}$, 2.56 cd s $m^{-2}$, and 3 cd s $m^{-2}$), which were presented at a rate of 32 flashes/second. Each luminance level was presented for 4 s with a 600-ms break between stimuli. The ML Flicker ERG test was recorded once per subject.

Analog signals were amplified 20,000 times, band-pass filtered with cutoff frequencies of 0.5 and 100 Hz, and digitized at 2048 samples/second. The A to D converter (A/D) had a resolution of 12 bits and the voltage range was ± 5.0 V. Synchronized single-channel ERGs were recorded, generating a time series of 128 data points per analysis.

Recorded FL Flicker parameters included Magnitude, Phase, Magnitude Variance, and Phase Variance. ML Flicker ERG parameters included Magnitude and Phase for each luminance level, but were aggregated into Magnitude Area Under the Curve (Magnitude AUC) and Phase Area Under the Curve (Phase AUC). The Magnitude AUC unit of measurement is (μV cd $m^{-2}$) × $10^{-3}$ and was derived from the polynomial regression line of the magnitude of the subject's response across the 6 increasing luminance levels. Similarly, the Phase AUC unit of measurement is (° cd $m^{-2}$) × $10^{-3}$ and was derived from the polynomial regression line of the phase of the




| Parameter | OD |
|---|---|
| Magnitude (µV) | 20.06 |
| Phase (°) | 316.46 |
| Magnitude Variance Ratio | 1.15 |
| Phase Variance Ratio | 1.12 |
| Artifacts (%) | 5.6 |

| Area Analysis | | OD |
|---|---|---|
| Area Ratio | Mag | 1.04 |
| | Phase | 1.48 |

**Fig. 3** Fixed luminance and multi-luminance flicker of a subject's right eye for representation of waveforms and parameters yielded

subject's responses across the 6 increasing luminance levels (Fig. 3).

## Statistical analysis

Demographic data were summarized using means and standard deviations (SD), or as frequency and percentages when appropriate. Good-quality ERG tests were determined by a good sensor connection and absence of excessive artifacts. Poor sensor connection was determined when the noise level was above a set threshold of 130 dBµV. An artifact was defined as an eye blink or gross saccade $> 50$ µV. PERG tests were excluded from the analysis if $\geq 4$ artifacts were recorded, and FL Flicker ERG was excluded if the artifact percent exceeded 30%. ML Flicker ERG tests were excluded if any one luminance level or the overall test exhibited artifact recording greater than 25%.

Repeatability of PERG and FL Flicker ERG parameters was assessed using intra-class correlation coefficients (ICC). Subjects included in the ICC repeatability measurements were required to have three quality tests of a given ERG. Classification of repeatability used the following definitions: ICC values of 0.80 or greater indicated excellent repeatability, values between 0.60 and 0.79 indicated good repeatability, and values between 0.40 and 0.59 or less

than 0.40 were considered to have fair and poor repeatability, respectively [21]. Reference values were obtained by calculating the mean, ± SD, and 95% confidence intervals (95% CI) for PERG and Flicker ERG parameters, these are unadjusted ground means for each of the four tests. One eye per subject was used in the aforementioned analyses. If a subject recorded quality ERGs from both eyes, the study eye was chosen randomly [22]. To assess the impact of age on the PERG and Flicker ERG parameters both eyes were included and a linear regression that accounted for inter-eye correlation was employed.

An alpha level of 0.05 was considered statistically significant. Statistical analyses were performed using SPSS 20.0 (IBM, Chicago, IL).

## Results

### Demographics

Fifty subjects were enrolled in this study. The mean age (± SD) of tested subjects was 53.3 (± 16.6) years. Subjects' ages ranged from 21 to 81. Age-specific recruitment ensured individuals from all decades within that range were included. Fifty-six percent ($n$ = 28) of subjects were female. Demographic data are shown in Table 1.

### Repeatability

PERG repeatability measurements were calculated from 44 eyes for the 16° stimulus and 39 eyes for the 24° stimulus. Forty-seven eyes were included in the FL Flicker ERG measurements. The PERG ICCs ranged from 0.793 to 0.911, all of which were statistically significant with $p$ values $< 0.001$. The Flicker ERG ICCs ranged from 0.968 to 0.994, all of which were also statistically significant with $p$ values $< 0.001$. The repeatability results of PERG and Flicker ERG tests are detailed in Table 2.

**Table 1** Demographic characteristics from 50 subjects

| Variables | Total ($N$ = 50 subjects) |
|---|---|
| Age (years), mean [SD, range] | 53.3 [16.6, 21–81] |
| Gender, $N$ (%) | |
| Female | 28 (56%) |
| Race, $N$ (%) | |
| Asian | 19 (38.0%) |
| Caucasian | 16 (32.0%) |
| African–American | 9 (18.0%) |
| Unknown | 6 (12%) |

*SD* standard deviation

### Reference values

To avoid bias, only data from subjects' first PERG and Flicker ERG tests were used to calculate the reference values. The PERG 16° and 24° reference values were derived from 46 eyes with good-quality tests, while FL Flicker reference values were derived from good-quality tests of 47 eyes. ML Flicker ERG reference ranges were derived from good-quality tests of 32 eyes. Reference values for PERG and Flicker ERG tests are detailed in Table 3. These results are mean values of the above tests without adjusting for the effect of age; to obtain patient level parameter values of a test with a given age, we conducted further analysis as described in the following section.

### Impact of subject age

Data from subjects' first ERG tests were used to assess the impact of age on ERG parameters. Age had a significant impact on all PERG parameters (16° or 24°). The Phase response of the FL Flicker ERG significantly decreased with age ($\beta$ = -0.837, $p \leq 0.001$). FL Flicker ERG Magnitude was also impacted with a significant quadratic effect of age ($\beta = -0.0047$, $p = 0.0004$). Similarly, the Phase AUC ML Flicker ERG significantly declined with age ($\beta = -0.007$, $p = 0.009$), while the impact on the Magnitude AUC was significant as well, with a negative quadratic age effect. Linear regression results are detailed in Table 4. All significant negative quadratic age effects shown in Table 4 indicate the overall age effect decreases after a certain age (*e.g.* 50 years) even if the parameter estimate for the main age effect is positive.

**Table 2** Repeatability of PERG and FL Flicker ERG tests over three sessions

| Test | Parameter | ICC (95% CI) | *p* value |
|---|---|---|---|
| PERG 16° | Magnitude | 0.852 (0.756–0.914) | < 0.001 |
| *N* = 44 eyes | | | |
| | Magnitude D | 0.871 (0.787–0.925) | < 0.001 |
| | Magnitude D/Magnitude Ratio | 0.793 (0.659–0.880) | < 0.001 |
| PERG 24° | Magnitude | 0.911 (0.849–0.950) | < 0.001 |
| *N* = 39 eyes | | | |
| | Magnitude D | 0.906 (0.841–0.948) | < 0.001 |
| | Magnitude D/Magnitude Ratio | 0.829 (0.710–0.905) | < 0.001 |
| FL Flicker ERG | Magnitude | 0.968 (0.949–0.981) | < 0.001 |
| *N* = 47 eyes | | | |
| | Phase | 0.994 (0.990–0.996) | < 0.001 |

*PERG* pattern electroretinography, *FL* fixed luminance, *ICC* intra-class correlation coefficient, two-way random, absolute agreement

**Table 3** Reference data for PERG and flicker ERG tests

| Test | Parameter | Mean, ± SD (95% CI) |
|---|---|---|
| PERG 16° | Magnitude | 1.24, ± 0.34 (1.13–1.34) |
| *N* = 46 eyes | | |
| | Magnitude D | 0.88, ± 0.41 (0.76–1.01) |
| | Magnitude D/Magnitude Ratio | 0.70, ± 0.17 (0.65–0.75) |
| PERG 24° | Magnitude | 1.76, ± 0.54 (1.60–1.924) |
| *N* = 46 eyes | | |
| | Magnitude D | 1.51, ± 0.59 (1.33–1.68) |
| | Magnitude D/Magnitude Ratio | 0.83, ± 0.11 (0.80–0.87) |
| FL Flicker ERG | Magnitude | 10.14, ± 3.52 (9.11–11.17) |
| *N* = 47 eyes | | |
| | Phase | 305.24, ± 25.55 (297.74–312.74) |
| ML Flicker ERG | Magnitude AUC | 5.16, ± 1.83 (4.50–5.81) |
| *N* = 32 eyes | | |
| | Phase AUC | 156.03, ± 12.23 (151.62–160.44) |

Data derived from each patient's first testing session and unadjusted for age

*PERG* pattern electroretinography, *AUC* area under curve. Phase AUC (° cd $m^{-2}$) × $10^{-3}$ is derived from the polynomial regression line of the phase of the patient's responses across the 6 increasing luminance levels, Magnitude AUC (μV cd $m^{-2}$) × $10^{-3}$ is derived from the polynomial regression line of the magnitude of the patient's responses across the 6 increasing luminance levels

## Discussion

In this study, we tested subjects using PERG and Flicker ERG protocols in order to determine the test–retest repeatability and develop a reference database for an office-based testing platform. Glaucoma and DR, as well and many other ocular diseases, may display an erratic or slow pattern of progression, which makes the test–retest repeatability one of the most important features when evaluating tests to include in clinical management strategies [23]. Overall, the steady-state PERG 16° and 24° stimulus protocols, as well as the fixed luminance Flicker ERG protocol, were shown to have good-to-excellent repeatability. Multi-luminance Flicker ERG was recorded only once, so test–retest repeatability could not be determined. Our repeatability results are better or comparable to findings from previous reports that have assessed the reliability of other testing platforms for PERG and Flicker ERG [24–26]. The excellent

**Table 4** Regression analysis assessing the impact of age on PERG and Flicker ERG parameters

| Test | Parameter | $\beta$ of Age/$Age^2$ ± SE | $Z$ | $p$ value |
|---|---|---|---|---|
| PERG 16° | Magnitude | − 0.029 ± 0.0139 | 2.09 | 0.0366 |
| $N$ = 92 eyes | | **− 0.0003 ± 0.0001 | − 2.20 | 0.0278 |
| | Magnitude D | 0.0473 ± 0.0127 | 3.72 | 0.0002 |
| | | **− 0.0005 ± 0.0001 | − 4.04 | < 0.0001 |
| | Magnitude D/Magnitude Ratio | 0.0172 ± 0.0053 | 3.26 | 0.0011 |
| | | **− 0.0002 ± 0.0001 | − 3.66 | 0.0002 |
| PERG 24° | Magnitude | − 0.0599 ± 0.0226 | 2.65 | 0.0081 |
| $N$ = 92 eyes | | **− 0.0007 ± 0.0002 | − 3.12 | 0.0018 |
| | Magnitude D | 0.0699 ± 0.0228 | 3.066 | 0.0022 |
| | | **− 0.0008 ± 0.0002 | − 3.58 | 0.0003 |
| | Magnitude D/Magnitude Ratio | 0.0118 ± 0.0052 | 2.29 | 0.0222 |
| | | **− 0.0001 ± 0.0001 | − 2.47 | 0.0137 |
| FL Flicker ERG | Magnitude | 0.450 ± 0.13 | 3.50 | 0.0005 |
| $N$ = 94 eyes | | **− 0.0047 ± 0.0013 | − 3.54 | 0.0004 |
| | Phase | − 0.837 ± 0.207 | − 4.05 | < 0.0001 |
| ML Flicker ERG | Magnitude AUC | 0.032 ± 0.013 | 2.54 | 0.011 |
| $N$ = 64 eyes | | **− 0.0003 ± 0.0001 | − 2.74 | 0.00061 |
| | Phase AUC | − 0.007 ± 0.003 | − 2.62 | 0.009 |

*PERG* pattern electroretinography, *FL* fixed luminance, ML multi-luminance, *AUC* area under curve. Phase AUC (° cd $m^{-2}$) × $10^{-3}$ is derived from the polynomial regression line of the phase of the patient's responses across the 6 increasing luminance levels. Magnitude AUC (μV cd $m^{-2}$) × $10^{-3}$ is derived from the polynomial regression line of the magnitude of the patient's responses across the 6 increasing luminance levels

**Displaying results from the nonlinear effect of age. The effects of $Age^2$ and SEs in several models are rounded to the nearest four decimal points

repeatability observed in this study could be related to the testing time, patients' comfort, and easy-to-learn protocols for operators. One limitation of the present report is that the repeated tests were done without replacement of electrodes, which may limit some of the clinical applications of the repeatability findings.

Standardization of the testing sequence also entails the establishment of a reference database for each testing platform [8]. In this study, we report the mean and 95% confidence interval in order to determine reference values for these tests. We were able to note a normal or near-normal distribution in both PERG and Flicker ERG parameters. Previous studies have noted that full-field ERG amplitudes and b-waves implicit times do not follow a Gaussian distribution [8, 26, 27]. However, there are limited data describing the distribution of Flicker ERG parameters [28, 29]. Data from a normative study using a similar PERG protocol as the present study have also reported a normal distribution [30]. In addition, we have recruited an ethnically diverse population, which increases the external validity of this platform and provides objective data for clinicians to compare their patients' results accurately when using the same technique and testing sequence [8, 31]. We would like to note that the test sequences presented here do not follow the ISCEV guidelines, since they do not provide a protocol for steady-state PERG tests, and the Flicker ERG tests sequence was optimized for an office-based testing platform.

When investigating the impact of age, we found that the Flicker ERG Phase parameters were significantly influenced by age, findings consistent to previously published studies [28, 29]. We also noted a significant impact of age in the PERG parameters, which is in line with the previous reports [30, 32]. We chose not to exclude pseudophakic subjects from our study in an effort to have our normative results reflect a

representative sample of the patients who will be tested using these sequences, even though a statistically significant effect of cataract surgery with intraocular lens (IOL) implantation has been demonstrated in multifocal ERG parameters [33]. Further studies should be conducted to evaluate the effect of the current and diverse technologies of IOLs on other electrophysiological tests.

The diagnostic ability of PERG for glaucoma has been studied and validated in many ERG platforms [9, 34, 35]. The high repeatability reported in this study emphasizes the role of PERG for monitoring glaucoma progression, as well as for diagnostic purposes.

Psychophysical and electrophysiological examinations have been reported to have an increasingly important role in the screening, diagnosis, and management of diabetic retinopathy (DR) [36]. ERGs have been proven to have the ability to predict the progression from non-proliferative diabetic retinopathy (NPDR) to pre-proliferative stages [37]. Being a sensitive marker of trophic disorders of the retina, changes in ERG parameters have even been reported in preclinical stages of DR [14, 35, 38, 39].

Flicker ERG, a cone and bipolar pathway-driven response, is found to be especially useful in DR since it provides an evaluation of the retinal tissue's neurovascular coupling response to light stimuli [36, 40]. Studies using Flicker ERG have shown a reduction in vasodilator capacity in retinal vessels of diabetic patients in response to the light stimuli. The impairment in retinal vessels was found to be directly proportional to the degree of retinopathy, providing objective qualitative and quantitative data [41–43]. Other studies have shown a correlation between delayed Flicker ERG responses and disease severity in diabetic patients [44, 45].

The ML Flicker ERG modality tested in this study, with responses at different luminance levels, may provide additional information regarding the integrity and functional status of different retinal pathways. Starting at a far lower luminance level that the standard fixed luminance Flicker ERG protocol, the multi-luminance protocol may allow for greater sensitivity to retinal dysfunction, which can help detect earlier dysfunction or monitor progression [46].

Recent research on new treatment modalities of DR has changed the paradigms regarding follow-up and management of the disease. After results from Protocol S from the Diabetic Retinopathy Clinical Research Network (DRCR.net) and the RIDE and RISE studies, ranibizumab, an anti-vascular endothelium growth factor (anti-VEGF) agent, was approved by the FDA for use in any stage of DR regardless of the presence of macular edema [47, 48]. Protocols for the administration of anti-VEGF, however, require frequent visits and testing to assess the need for repeat injections. Studies have reported that Flicker ERG testing successfully detected an improvement in retinal function after treatment with anti-VEGF agents for a variety of retinal diseases [49–51]. Electroretinography is a valuable tool in this setting, as it provides an objective, noninvasive assessment of global retinal function in these patients, and could help guide their further management.

In this study, we found that PERG and Flicker ERG testing delivered by the NOVA$^{TM}$ platform is a reliable source of objective parameters that can be incorporated into ophthalmic care.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**Statement of human rights** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards.

**Statement on the welfare of animals** This manuscript does not contain any studies with animals performed by any of the authors.

**Informed consent** Informed consent was obtained from all individual participants included in the study.

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