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ORIGINAL RESEARCH

RE-PERG, a new procedure for electrophysiologic diagnosis of glaucoma that may improve PERG specificity

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Purpose: A significant variability of the second harmonic (2ndH) phase of steady-state pattern electroretinogram (SS-PERG) in intrasession retest has been recently described in glaucoma patients (GP), which has not been found in healthy subjects. To evaluate the reliability of phase variability in retest (a procedure called RE-PERG or REPERG) in the presence of cataract, which is known to affect standard PERG, we tested this procedure in GP, normal controls (NC), and cataract patients (CP).

Methods: The procedure was performed on 50 GP, 35 NC, and 27 CP. All subjects were examined with RE-PERG and SS-PERG and also with spectral domain optical coherence tomography and standard automated perimetry. Standard deviation of phase and amplitude value of 2ndH were correlated by means of one-way analysis of variance and Pearson correlation, with the mean deviation and pattern standard deviation assessed by standard automated perimetry and retinal nerve fiber layer and the ganglion cell complex thickness assessed by spectral domain optical coherence tomography. Receiver operating characteristics were calculated in cohort populations with and without cataract.

Results: Standard deviation of phase of 2ndH was significantly higher in GP with respect to NC ($P<0.001$) and CP ($P<0.001$), and it correlated with retinal nerve fiber layer ($r=-0.5$, $P<0.001$) and ganglion cell complex ($r=-0.6$, $P<0.001$) defects in GP. Receiver operating characteristic evaluation showed higher specificity of RE-PERG (86.4%; area under the curve 0.93) with respect to SS-PERG (54.5%; area under the curve 0.68) in CP.

Conclusion: RE-PERG may improve the specificity of SS-PERG in clinical practice in the discrimination of GP.

Keywords: glaucoma, pattern electroretinogram, optical coherence tomography, ganglion cells, visual field

Introduction

Glaucoma is a progressive optic neuropathy characterized by the apoptosis of retinal ganglion cells (RGCs), which becomes clinically evident anatomically as typical alterations of the optic nerve head (ONH) and retinal nerve fiber layer (RNFL) and functionally as visual field defects.

Standard automated perimetry (SAP) is the main tool for the detection of functional impairment of the visual field. However, it has been reported that at least 25%–40% of RGCs must to be lost before any visual field damage occurs.[1,2]

In addition, it has been reported that 60% of ocular hypertensive patients who become glaucomatous show ONH and RNFL damage before the occurrence of visual field damage.[3]

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For the anatomical analysis, spectral domain optical coherence tomography (OCT) can provide the objective measures of ONH and RNFL.[4–6]

Because most of the RGCs are located in the macula, the evaluation of this area and, in particular, of the ganglion cell complex (GCC) has been proposed as a diagnostic tool for the early diagnosis of glaucoma, in addition to the evaluation of ONH and RNFL.[7–11]

It is well known that in the diagnosis of glaucoma, a variable percentage of RGCs must die before any evidence of damage can be found by using the tools actually available (ie, among the most used, OCT for anatomical impairment and standard achromatic perimetry for functional impairment), regardless of the specific tool that, case by case, is able to detect the damage before the other one.[3] On the other hand, it is reasonable to think that, in the natural history of glaucoma, there should be an early stage in which the RGCs are damaged, but still alive. The aim of all the electrophysiologic studies performed on glaucoma patients (GP) was to find an examination able to recognize this early stage of the disease.

Pattern electroretinogram (PERG) has been shown to be able to analyze the electric activity of RGCs[12,13] and, for this reason, to be suitable for the diagnosis of early glaucoma.[14,15]

PERG has been shown to be abnormal even before the occurrence of visual field defects, as measured by SAP, and of RNFL loss, as assessed by OCT.[14]

Cross-sectional studies have shown that the PERG is frequently altered in glaucoma suspects and in patients with early glaucoma more than in normal controls (NC).[16–20]

A specific model of PERG for glaucoma screening (steady-state pattern electroretinogram [SS-PERG]), which is fast and user-friendly, has been developed for the evaluation RGCs dysfunction.[21]

The PERG is recorded in response to a noninvasive stimulus based on contrast variation of low (transient response) or high (steady-state response) temporal frequency.[21] A steady-state stimulus (fast) shows better glaucomatous dysfunction than a transient stimulus (slow), since RGCs are submitted to a greater metabolic stress.[22] The steady-state stimulus determines a sinusoidal response that is analyzed by the Fourier transform.[23,24] In this way, the second harmonic, that is, the harmonic that has a frequency twice that of the stimulus, can be isolated. Amplitude and phase of this harmonic show typical alterations in glaucoma. In particular, the amplitude is reduced in patients with glaucoma and ocular hypertension (OHT) compared to healthy subjects, while the phase remains constant or at the most tends to delay with age and with the disease.[25] SS-PERG has been reported to have high test–retest repeatability, providing, also, a good signal-to-noise ratio.[26] In particular, the phase variability was very limited in the retest within and between trials.[17,27]

Porciatti focused his attention on the phase delay, which tends to increase as the disease progresses. In general, he assumed that the PERG amplitude should be related to the number of surviving RGCs, while the phase should express diminished activity of the existing neurons (synaptic dysfunction). Therefore, he hypothesized that a reduced input to RGCs may be due to a synaptic dysfunction.[28] Dendritic modifications precede neuronal apoptosis and can result in reduced responsiveness of RGCs with lower ability to follow stimuli of increased temporal frequency. A phase delay without amplitude reduction could arise from synaptic and transport delays.[27,29]

The biologic variability of a measurement is not only a physiologic behavior inherent in the instrumental bias, as in diagnostic imaging,[30] but also in the different adaptation of the bioelectrical response to an external visual stimulus.[22,31]

Nevertheless, the amplitude can also be reduced in the presence of nonspecific causes (optic media opacities and others), while phase is a more specific indicator of disease,[28] showing, in addition, low variability in the retest within and between trials.[17,27]

The main problem in the use of electrophysiologic diagnosis in glaucoma is its variability, due to the fact that often, the patients present associated conditions (ie, cataract, diabetic retinopathy, low myopia) that can influence the measurements.[32,33] In clinical trials, these kinds of patients are excluded, but in the ordinary practice, they are very common. Probably, this is the reason why PERG is not usually performed worldwide, apart from the specialized laboratories, and is not included in the standard diagnosis tools for glaucoma in any scientific society guidelines.[34,35]

In a previous study,[36] we showed that the individual-intrinsic within-trial variability of the PERG signal in test–retest of the same eye (ie, five consecutive stimulations without pause, a procedure that we called RE-PERG or REPERG) of early GP was greater than the physiologic one present in healthy individuals; in addition, it was also correlated with markers of disease severity such as retinal thickness and visual field indices. The aim of this study was to verify that such variations are not influenced by optic media opacities and, therefore, that the RE-PERG can be more reliable than the standard SS-PERG in the discrimination of GP.

Materials and methods

From January to July 2015, 112 consecutive patients were enrolled in the study. All patients were recruited at the Glaucoma Center of the Brindisi Social Health District, Mesagne, Italy, and at the Department of Ophthalmology and Otolaryngology of the University of Bari, Italy.

The patients were divided into three groups: 50 glaucoma patients (GP), 62 age-matched patients further divided into 35 normal controls (NC), and 27 control cases with no glaucoma, but with various degrees of cataract (cataract patients [CP]).

The criteria for classification in the GP group, in accordance with the European Glaucoma Society (EGS) guidelines (http://www.eugs.org/eng/EGS_guidelines4.asp), were: appearance of optic disc and peripapillary nerve fiber layer glaucoma damage (increased ratio cup/disc, asymmetry ratio of cup/disc, notch or narrowing of the neuroretinal rim, disc hemorrhage, thinning of the peripapillary nerve fiber layer) or visual field suspicious for glaucomatous damage in the absence of clinical signs of other optic neuropathies (default sort, nasal step, paracentral scotoma, altitudinal defect) with a constant elevated intraocular pressure before therapy. The severity of glaucoma was evaluated functionally by SAP and anatomically by the measurement of RNFL and GCC thickness with spectral domain OCT. The NC group included 35 age-matched healthy subjects with no evidence of having any other ocular or general disease or undergoing any ocular or general therapy able to determine the influence on the visual function.

The CP group included 27 age-matched subjects with no evidence of having any other ocular or general disease or undergoing ocular or general therapy able to determine the influence on the visual function, apart from cataract.

Each participant underwent a comprehensive ophthalmic evaluation, including review of medical history, best-corrected visual acuity testing, intraoccular pressure (IOP) measurement by Goldmann applanation tonometry, ultrasound pachymetry (Pachmate GH55; DGH Technology, Inc., Exton, PA, USA), slit-lamp biomicroscopy, gonioscopy, and dilated fundus examination with a 78 lens. All participants had best-corrected visual acuity ≥20/40 (Snellen acuity), spherical refraction within ±5.0 D, and cylinder correction within ±2.0 diopters, and NC patients had transparent ocular media (nuclear color/opalescence, cortical, or posterior subcapsular lens opacity <1) according to the system of lens opacity Classification System III and open iridocorneal angles on gonioscopy. CP had cataract up to nuclear color N2. Patients with coexisting retinal diseases, diabetes, Parkinson's disease, or nonglaucomatous optic neuropathies able to determine nonspecific PERG abnormality[37,38] were excluded.

Only one eye of each patient who met the criteria mentioned above was randomly included in the study.

Spectral domain optical coherence tomography

Peripapillary RNFL thickness was assessed by a Zeiss Cirrus HD OCT-500 (software version 7.0.1.290; Carl Zeiss Meditec, Dublin, CA, USA). The protocol Optic Disc Cube 200×200 was used to perform a circular scan 3.46 mm in diameter and was automatically targeted around the optic disc to provide the RNFL thickness of the four quadrants and each of the 12-hour clock positions. The protocol Macular Cube 512×128 was used to obtain measurements of macular thickness.

All images were obtained by the same experienced technician with a signal larger/resistance at 7/10. Three scans of the optic disc and the macular region were consecutively acquired and analyzed for each eye. The measurements of RNFL and GCC were averaged using the data of each of the three scans.

Standard automated perimetry

The visual field was assessed by Humphrey Field Analyzer, model 745i II (Carl Zeiss Meditec AG, Jena, Germany) using the 24-2 Swedish Interactive Threshold Algorithms (SITA) standard strategy. Near addition was added to the subject's refractive correction. If fixation losses were >20% and false-positive or false-negative results were higher than 15%, the test was repeated. At least two reliable SAPs were performed to minimize the effect of learning.[39] Typical glaucomatous defects considered were those with a pattern standard deviation (PSD) significantly higher than the 5% level and/or a glaucoma hemifield test outside the normal limits.

Pattern electroretinogram

Both the SS-PERG and the RE-PERG were recorded with a commercial instrument (RETIMAX Advanced ver. 4.3; CSO, Florence, Italy).

The RE-PERGs were recorded using a method similar to the PERG for Glaucoma (PERGLA) paradigm,[21] with some minor changes made by our laboratories. We used a stimulus of horizontal bars with a spatial frequency of 1.7 cycles/degree, which was found in previous studies as the most sensitive in detecting RGCs dysfunction in early glaucoma,[40,41] modulated in counterphase at 15 reversals/second and electronically generated on a high-resolution ionized gas electrically charged

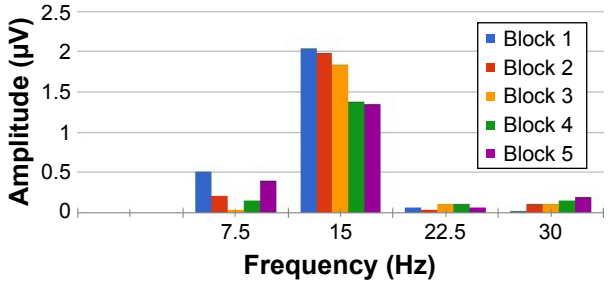

Figure 1 Example of five consecutive steady-state PERGs.
Note: At a frequency of the stimulus of 7.5 Hz, the second harmonic is observed at 15 Hz.
Abbreviation: PERGs, pattern electroretinograms.

plasma display (contrast: 90%; luminance: 80 cd/m^2; field size: 24° [width] ×24° [height]).

The subjects had undilated pupils of size between 3 and 4 mm, with an appropriate correction for the working distance (57 cm). The signals were recorded from a skin electrode 9 mm Ag/AgCl placed on the lower eyelid. A similar electrode placed on the lid of the unstimulated eye was used as a reference, as described in other studies.[36] In all cases, the impedance was below 5 k. The responses were amplified (gain of 100,000), filtered (bandwidth: 130 Hz), and sampled with a resolution of 12 bits. The analysis time was equal to the time period of the stimulus (133 ms).

An average of 650 events for SS-PERGs and 5 consecutive blocks of 130 events for RE-PERG was calculated, with automatic rejection of artifacts. The data were then exported to a text file. The amplitude (μV) and phase (πrad) of the second harmonic were then analyzed with the Fourier transform (Figure 1).

The repeatability of the amplitude and phase of the second harmonic was calculated as the standard deviation of amplitude (SD Ampl) and phase (SD Phase, Figure 2). The noise level obtained by recording a response to an occluded stimulus was ≤0.087±0.03 μV in both normal subjects and patients.

As described previously,[31] to avoid the inherent ambiguity for phase values around zero, which is typically associated with spurious variability, it is necessary to subtract multiples of 2π from the recorded value of the modulo (2 less than the recorded value).[25] So, phase values are consequently enclosed between 1 and 3 πrad without discontinuities. PERG signal was considered reliable only when the second harmonic was clearly visible in the spectrum of the frequencies.

Statistical analysis was performed using commercial software (MedCalc® 16.8.1.0). A *P*-value of ≤0.05 was considered statistically significant.

This study follows the tenets of the Declaration of Helsinki for human studies. The study was approved by both the ethical committees of the Brindisi Social Health District and the University of Bari. For this study, written informed consent was obtained from all subjects after the nature of the test and the possible risks were explained in detail.

Results

General demographics of the patients are summarized in Table 1; demographics of GP, NC, and CP are summarized in Tables 2–4, respectively.

We observed a significant reduction of PERG amplitude in GP compared to controls (PERG: 1.36 vs 1.68 μV, $P<0.001$).

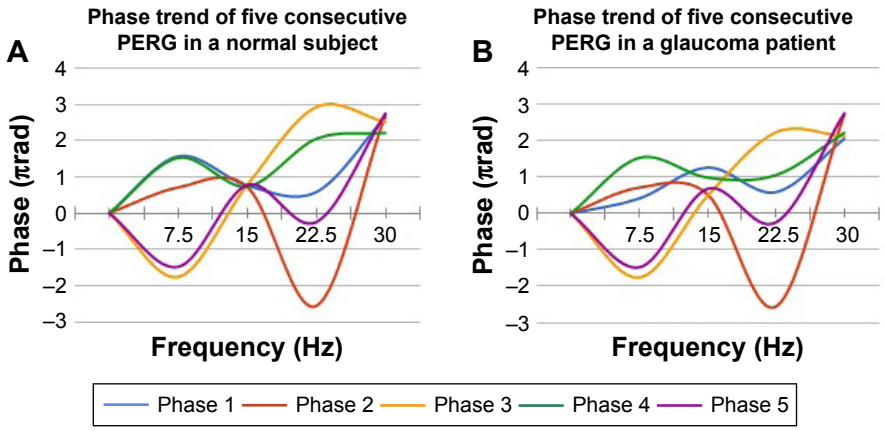

Figure 2 Example of phase trend of five consecutive PERGs.
Notes: Five consecutive tests of steady-state PERG in the same subject. The line chart shows the trend of the phase in the frequency domain from 7.5 to 30 Hz. In normal subjects (**A**), the phase always passes from the same point at 15 Hz that corresponds to the second harmonic of the signal in response to stimulus of 7.5 Hz. In glaucoma patients (**B**), there is, in the same point, higher variability of phase trend than in a normal subject.
Abbreviation: PERG, pattern electroretinogram.

Table 1 Demographic and relevant ocular characteristics of the study participants

	Patients (N=112 cases)					
	GP (n=50)	NC (n=35)	CP (n=27)	*P*-value*		
	Mean ± SD	Mean ± SD	Mean ± SD	GP vs NC	GP vs CP	NC vs CP
Age	67.4±11.1	65.2±9.96	65.9±7.7	0.358	0.622	0.827
Male, %	50	51	48	0.48**	0.44**	0.48**
MD, dB	−2.5±1.91	0.3±1.02	−1.54±1.48	<0.001	0.027	<0.001
PSD, dB	2.66±2.05	1.23±0.68	1.85±0.73	<0.001	0.051	<0.001
RNFL, μm	75.84±13.34	92.03±8.93	91.22±8.39	<0.001	<0.001	0.719
GCC, μm	67.26±11.70	80.14±4.94	83.04±6.36	<0.001	<0.001	0.048
PERG amplitude, μV	1.36±0.14	1.68±0.15	1.37±0.17	<0.001	0.5	<0.001
PERG phase, πrad	1.62±0.19	1.66±0.10	1.60±0.14	0.27	0.76	0.08
SD phase	0.30±0.14	0.10±0.05	0.12±0.04	<0.001	<0.001	0.4

Notes: *One-way analysis of variance (Bonferroni corrected); **chi-square.
Abbreviations: CP, cataract patients; GCC, ganglion cell complex; GP, glaucoma patients; MD, mean deviation; NC, normal controls; PERG, pattern electroretinogram; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SD, standard deviation; SD phase, standard deviation phase PERG.

Table 2 Clinical characteristics of glaucoma patients

No	Gender	Age, years	Amp (μv)	SD amp	Phase	SD phase	MD	PSD	RNFL	GCC	SS-PERG	RE-PERG
1	F	50	1.28	0.07	1.48	0.38	−2.3	2.4	86	68	a	a
2	F	44	1.38	0.07	1.47	0.37	−2.7	2.0	73	66	a	a
3	M	60	1.22	0.11	1.66	0.36	−4.0	2.8	61	53	a	a
4	F	73	1.35	0.07	1.33	0.33	−1.8	2.45	87	81	a	a
5	M	67	1.48	0.07	1.05	0.15	−1.51	1.69	97	87	a	n
6	M	81	1.29	0.06	1.56	0.46	−3.95	2.07	53	43	a	a
7	M	69	1.44	0.23	1.27	0.17	−0.63	1.53	90	74	a	a
8	M	46	1.65	0.12	1.06	0.16	−0.64	1.63	94	79	n	n
9	F	66	1.34	0.04	1.93	0.53	−7.0	12.0	54	49	a	a
10	M	81	1.27	0.07	1.7	0.4	−3.87	3.76	60	51	a	a
11	F	64	1.47	0.10	1.65	0.35	0.41	1.82	90	88	a	a
12	M	76	1.47	0.08	1.04	0.14	−3.06	3.22	78	79	a	n
13	M	39	1.25	0.07	1.83	0.53	−0.86	1.3	85	86	a	a
14	M	56	1.66	0.09	1.53	0.33	−1.13	1.38	87	77	n	a
15	F	59	1.32	0.04	1.48	0.28	−0.3	1.09	84	73	a	a
16	M	74	1.26	0.11	1.73	0.43	−1.32	1.18	98	66	a	a
17	F	66	1.44	0.09	1.37	0.17	−4.57	5.56	92	78	a	a
18	F	80	1.24	0.08	1.2	0.2	−2.5	2.9	63	71	a	a
19	F	82	1.42	0.07	1.86	0.46	−3.0	2.0	50	47	a	a
20	F	82	1.22	0.06	1.15	0.15	−3.5	3.2	50	47	a	a
21	M	57	1.24	0.09	1.58	0.38	−5.7	5.8	72	71	a	a
22	F	62	1.47	0.10	1.12	0.12	0.67	1.25	87	81	a	a
23	M	52	1.36	0.12	1.61	0.41	−2.37	2.55	79	55	a	a
24	F	65	1.34	0.05	1.45	0.35	−3.5	3.6	79	70	a	a
25	F	65	1.64	0.05	1.19	0.19	−0.55	1.7	75	68	n	a
26	F	62	1.43	0.13	1.1	0.1	0.67	1.25	74	65	a	a
27	M	75	1.17	0.09	1.59	0.49	−5.0	4.0	72	63	a	a
28	F	55	1.50	0.24	1.06	0.1	0.7	1.9	84	77	a	n
29	M	48	1.49	0.09	1.13	0.13	0.78	1.36	88	71	a	a
30	F	63	1.54	0.13	1.1	0.1	−0.07	1.43	96	79	a	a
31	F	62	1.74	0.08	1.15	0.15	−0.14	1.36	84	81	n	a
32	M	75	1.25	0.14	1.43	0.43	−1.78	2.4	71	70	a	a
33	M	77	1.45	0.11	1.88	0.48	−3.1	3.1	95	74	a	a
34	M	85	1.24	0.15	1.43	0.43	−5.25	3.4	60	55	a	a
35	F	76	1.29	0.06	1.15	0.15	−4.12	2.9	91	63	a	a
36	M	76	1.23	0.09	1.55	0.45	−3.2	2.8	76	83	a	a

(*Continued*)

Table 2 (*Continued*)

No	Gender	Age, years	Amp (μv)	SD amp	Phase	SD phase	MD	PSD	RNFL	GCC	SS-PERG	RE-PERG
37	M	74	1.23	0.08	1.3	0.3	−3.44	3.1	53	47	a	a
38	M	70	1.26	0.12	1.8	0.4	−5.0	4.2	70	57	a	a
39	F	74	1.30	0.16	1.74	0.44	−4.8	−4.8	55	52	a	a
40	M	66	1.24	0.06	1.84	0.44	−2.5	2.7	83	60	a	a
41	F	76	1.45	0.11	1.59	0.39	−2.5	2.6	84	75	a	a
42	M	77	1.17	0.09	1.74	0.44	−2.6	2.5	67	48	a	a
43	F	82	1.37	0.20	1.09	0.09	−2.2	1.9	68	65	a	a
44	F	72	1.33	0.06	1.93	0.43	−4.8	5.2	68	65	a	a
45	M	68	1.66	0.26	1.05	0.15	−0.4	1.7	76	69	n	n
46	F	76	1.35	0.04	1.03	0.13	−1.4	2.5	77	70	a	n
47	M	77	1.26	0.13	1.19	0.19	−2.8	3.8	76	69	a	a
48	F	61	1.41	0.08	1.12	0.22	−3.0	3.2	69	65	a	a
49	F	70	1.15	0.06	1.41	0.31	−4.5	3.5	65	64	a	a
50	M	56	1.25	0.08	1.11	0.16	−5.0	4.0	66	68	a	a

Abbreviations: a, abnormal; Amp, amplitude PERG; F, female; GCC, ganglion cell complex; M, male; MD, mean deviation; n, normal; PERG, pattern electroretinogram; phase, phase PERG; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SD amp, standard deviation amplitude PERG; SD phase, standard deviation phase PERG; SS-PERG, steady-state pattern electroretinogram.

Table 3 Clinical characteristics of normal controls

No	Gender	Age, years	Amp (μv)	SD amp	Phase	SD phase	MD	PSD	RNFL	GCC	SS-PERG	RE-PERG
1	M	74	1.66	0.11	1.6	0.1	−1.69	1.54	103	87	n	n
2	M	73	1.57	0.08	1.79	0.1	−0.4	2.2	97	78	a	n
3	M	59	1.88	0.06	1.66	0.1	0.04	1.17	77	81	n	n
4	F	42	1.63	0.16	1.6	0.18	−1	1	107	76	n	n
5	M	64	1.59	0.08	1.64	0.17	−0.43	1.47	101	88	a	n
6	M	62	1.58	0.06	1.55	0.08	0.89	1.34	105	88	a	n
7	M	60	1.47	0.04	1.7	0.16	0.93	1.24	92	84	a	n
8	F	44	1.71	0.06	1.67	0.14	−0.78	1.28	96	79	n	n
9	M	69	1.68	0.09	1.7	0.02	−1.29	2.05	98	79	n	n
10	F	50	1.94	0.09	1.71	0.12	−0.68	1.9	110	83	n	n
11	F	73	1.71	0.03	1.67	0.06	0.47	1.37	84	74	n	n
12	M	55	1.65	0.09	1.8	0.06	1.46	1.4	103	84	n	n
13	F	65	1.77	0.04	1.5	0.1	0.71	1.39	99	79	n	n
14	M	73	1.5	0.07	1.63	0.04	1.51	1.43	86	85	a	n
15	M	55	1.3	0.05	1.5	0.16	1.51	1.43	89	78	a	n
16	F	74	1.74	0.06	1.57	0.04	0.23	1.8	79	72	n	n
17	M	63	1.71	0.06	1.71	0.08	0.5	1.8	96	82	n	a
18	F	65	1.86	0.05	1.65	0.08	1.02	1.45	89	77	n	n
19	M	59	1.65	0.17	1.67	0.12	−0.01	1.4	81	74	n	n
20	F	73	1.7	0.16	1.9	0.06	1.45	0.97	91	78	n	n
21	M	53	1.6	0.05	1.75	0.06	1.44	0.8	82	75	a	n
22	F	65	1.5	0.06	1.76	0.18	−0.75	1.22	106	87	a	n
23	M	70	1.71	0.05	1.43	0.1	−0.97	1	95	76	n	n
24	M	65	1.5	0.09	1.67	0.1	0.63	1.2	96	83	a	n
25	F	60	1.81	0.12	1.66	0.1	1.01	0.87	80	80	n	n
26	F	64	1.59	0.07	1.75	0.08	0.85	1.02	93	87	n	n
27	M	65	2.14	0.16	1.75	0.1	−0.23	1.1	79	70	n	n
28	F	66	1.75	0.13	1.54	0.16	0.44	0.96	91	81	n	a
29	F	76	1.67	0.08	1.7	0.1	1.2	1.0	81	72	n	n
30	M	66	1.69	0.04	1.73	0.1	1.01	1.4	88	74	n	n
31	M	77	1.75	0.08	1.7	0.16	1.81	1.41	82	80	n	n
32	F	86	1.65	0.09	1.5	0.1	−0.5	1.3	92	86	n	n
33	F	65	1.55	0.1	1.61	0.1	0.8	0.7	91	83	a	n
34	F	64	1.47	0.05	1.63	0.1	0.8	0.7	88	82	a	n
35	F	88	1.88	0.12	1.59	0.08	0.4	0.8	94	83	n	n

Abbreviations: a, abnormal; Amp, amplitude PERG; F, female; GCC, ganglion cell complex; M, male; MD, mean deviation; n, normal; phase, phase PERG; PERG, pattern electroretinogram; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SD amp, standard deviation amplitude PERG; SD phase, standard deviation phase PERG; SS-PERG, steady-state pattern electroretinogram.

Table 4 Clinical characteristics of cataract patients

No	Gender	Age, years	Amp (μv)	SD amp	Phase	SD phase	MD	PSD	RNFL	GCC	SS-PERG	RE-PERG
1	M	67	1.37	0.07	1.77	0.16	−0.5	3.35	90	86	a	n
2	M	78	1.26	0.11	1.65	0.2	1.14	1.78	81	97	a	a
3	F	66	1.3	0.14	1.52	0.1	−2.0	1.7	87	82	a	n
4	M	68	1.39	0.1	1.58	0.16	−0.43	1.47	81	76	a	n
5	M	54	1.31	0.13	1.49	0.1	−0.78	1.28	105	91	a	n
6	M	76	1.3	0.16	1.72	0.08	−0.78	1.28	79	71	a	n
7	F	71	1.2	0.07	1.62	0.12	−4.0	2.4	98	89	a	n
8	M	74	1.6	0.18	1.59	0.18	−6.0	3.24	95	83	n	n
9	F	60	1.26	0.07	1.35	0.16	−1.2	1.77	90	78	a	n
10	M	67	1.27	0.11	1.53	0.18	−0.09	1.37	88	81	a	n
11	M	65	1.37	0.06	1.64	0.1	−2.2	1.7	81	76	a	a
12	M	55	1.28	0.06	1.64	0.12	−2.4	1.9	81	75	a	n
13	F	63	1.31	0.03	1.53	0.08	−1.37	1.31	88	78	a	n
14	F	62	1.33	0.07	1.53	0.06	−1.0	1.48	97	90	a	n
15	M	70	1.35	0.22	1.42	0.16	−3.9	3.75	91	76	a	n
16	F	67	1.3	0.04	1.31	0.12	−2.5	2.59	83	74	a	n
17	M	58	1.32	0.04	1.45	0.16	−1.01	2.88	96	86	a	n
18	F	61	1.68	0.03	1.72	0.06	1.21	0.77	86	81	n	n
19	F	55	1.66	0.06	1.76	0.1	−1.23	1.91	85	80	n	n
20	F	70	1.34	0.13	1.7	0.14	−2.2	1.55	100	84	a	n
21	F	70	1.25	0.07	1.74	0.08	−1.5	1.25	95	82	a	n
22	F	86	1.23	0.08	1.78	0.08	−2.19	1.5	99	84	a	n
23	M	63	1.18	0.08	1.68	0.1	−0.81	1.93	92	87	a	n
24	M	73	1.24	0.08	1.49	0.12	−1.07	1.48	94	84	a	n
25	F	60	1.26	0.06	1.74	0.08	−1.45	1.01	115	92	a	n
26	F	55	1.6	0.15	1.77	0.08	−1.4	1.8	98	90	n	n
27	F	65	1.29	0.08	1.62	0.06	−2.0	1.4	88	89	a	n

Abbreviations: a, abnormal; Amp, amplitude PERG; F, female; GCC, ganglion cell complex; M, male; MD, mean deviation; n, normal; PERG, pattern electroretinogram; phase, phase PERG; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SD amp, standard deviation amplitude PERG; SD phase, standard deviation phase PERG; SS-PERG, steady-state pattern electroretinogram.

GP showed significantly different mean deviation (MD), PSD, RNFL, and GCC values from the control group (−2.5 vs 0.3, $P<0.001$; 2.66 vs 1.23 dB, $P<0.001$; 75.84 vs 92.03 μm, $P<0.001$; and 67.26 vs 80.14 μm, $P<0.001$, respectively).

As predicted, the reduction in PERG amplitude positively correlated to MD, RNFL, and GCC and negatively correlated to PSD (MD 0.57, $P<0.0001$; RNFL 0.35, $P=0.0002$; GCC 0.31, $P=0.0008$; and PSD −0.29, $P=0.0021$; Table 5).

To better understand the influence of comorbidities on the electrophysiologic diagnosis of glaucoma, we evaluated SS-PERG amplitude and RE-PERG phase variability (SD phase), respectively. In particular, we assigned a score of 1 or 0 to the pathologic or normal outcome of SS-PERG and RE-PERG. We considered a low amplitude in SS-PERG (<1.5 μm) and a high phase variability of PERG signal in RE-PERG (>0.15 SD) as pathologic.[36,41]

Figure 3 summarizes the SS-amplitude and the RE-PERG phase SD for each patient of the study. The scatter diagram shows that a significant reduction in SS-PERG amplitude (<1.5 μV) can be observed both in GP and CP with a consistent overlap among the groups; it is not the same for phase variability of PERG signal in RE-PERG >0.15 SD, which better discriminates GP from CP.

In the GP group, SS-PERG showed a specificity of 82.1% (95% confidence interval [CI]:[a] 66.5–92.5). In the CP group, SS-PERG showed abnormal results in 24 cases (85%). In both GP and CP groups, considered as a whole, total specificity of SS-PERG dropped to 54.5% (95% CI: 41.8%–66.9%) due to false-positive higher incidence. In the GP group, RE-PERG showed a specificity of 84.6% (95% CI:[a] 69.5%–91.1%; Table 6A and B). In the CP group, RE-PERG showed abnormal results in two cases (7%). In both GP and CP groups, considered as a whole, RE-PERG total specificity increased from 84.6% to 86.4% (75.7–93.6, Tables 6A and B).

Discussion

It was found that variations in the phase are little affected by lens opacities and deterioration of optics that instead cause a nonspecific reduction of PERG amplitude.[25] Other causes of nonspecific reduction of the PERG amplitude

Table 5 CC and SL-P between MD PSD, RNFL, GCC, PERG amplitude, PERG phase, SD phase in 50 glaucoma patients

	MD	PSD	GCC	RNFL	Amplitude (μv)	Phase	SD phase
MD							
CC		–0.61	0.54	0.48	0.57	–0.02	–0.60
SL-P		<0.0001	<0.0001	<0.0001	<0.0001	0.80	<0.0001
PSD							
CC	–0.61		–0.33	–0.33	–0.29	0.12	0.4
SL-P	<0.0001		0.0004	0.0003	0.0021	0.22	<0.0001
GCC							
CC	0.54	–0.33		0.82	0.31	–0.165	–0.59
SL-P	<0.0001	0.0004		<0.0001	0.0008	0.08	<0.0001
RNFL							
CC	0.49	–0.33	0.82		0.34	–0.11	–0.53
SL-P	<0.0001	0.0003	<0.0001		0.0002	0.26	<0.0001
PERG amplitude							
CC	0.57	–0.29	0.31	0.35		0.05	–0.4
SL-P	<0.0001	0.0021	0.0008	0.0002		0.56	<0.0001
PERG phase							
CC	–0.02	0.12	–0.16	–0.11	0.05		0.10
SL-P	0.80	0.22	0.08	0.25	0.56		0.27
PERG SD phase							
CC	–0.60	0.40	–0.6	–0.5	–0.5	0.10	
SL-P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.27	

Note: RNFL, GCC, and PERG amplitude were converted to a logarithmic scale (dB) in association with visual field indices.
Abbreviations: Amp PERG, amplitude PERG; CC, Pearson correlation coefficient; GCC, ganglion cell complex; MD, mean deviation; PERG, pattern electroretinogram; PSD, pattern standard deviation; RNFL, retinal nerve fiber layer; SL-P, significance level *P*-value; SD amp, standard deviation amplitude PERG; SD phase, standard deviation phase PERG in 50 glaucoma patients.

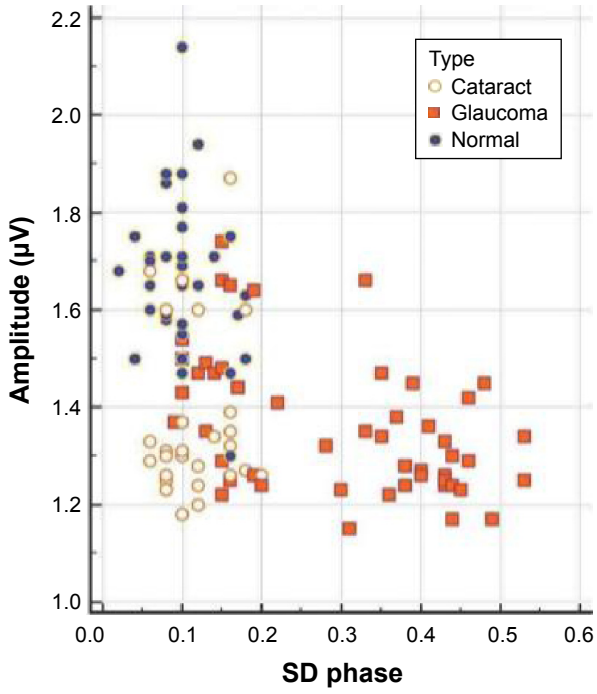

Figure 3 Scatter diagram shows distribution of phase standard deviation (SD) and PERG amplitude for each patient of the study.
Note: Amplitude of SS-PERG <1.5 μV and phase variability of PERG signal in RE-PERG >0.15 SD were considered abnormal.
Abbreviations: PERG, pattern electroretinogram; SS-PERG, steady-state pattern electroretinogram.

are myopia and diabetic retinopathy.[32,33] Because of the variability of PERG amplitude due to nonspecific causes, the implication for glaucoma is that a worsening of the quality of the visual stimulus, as in CP, may display nonspecific PERG amplitude reductions due to stimulus deterioration, but not PERG phase delays, which remain related only to the disease.

Starting from the evidences provided by Porciatti about the little influence of the optic media on the phase delay, we decided to study not its absolute value, but its retest variability. In a previous study,[29] we found that this parameter was significantly different among healthy subjects and GP. In particular, we found that coefficient of variability of the phase was significantly increased in early GP (8.97%±2.52%) and glaucoma suspects (7.30%±2.51%) compared to healthy subjects (3.54%±1.13%; $P<0.0001$); in addition, it was correlated with PSD ($P=0.0009$), GCC ($P=0.028$), and RNFL ($P=0.0078$) exclusively in early GP. The great advantage of using this parameter was that, for analyzing the intrapatient, intratest variability, it did not need a normative database (Figure 2).

We suppose that the SD phase, not based on the absolute value of the phase, is not influenced by optic media opacities.

Table 6 ROCs without (**A**) and with (**B**) cataract patients

A

	AUC	SE[a]	95% CI[b]	Specificity	95% CI[b]
MD	0.83	0.05	0.738–0.906	71.79	48.72–89.74
PSD	0.78	0.05	0.683–0.867	62.56	33.33–84.62
GCC	0.79	0.05	0.685–0.868	54.36	28.45–77.55
RNFL	0.77	0.05	0.664–0.853	64.10	47.20–78.80
SS-PERG amplitude[c]	0.86	0.04	0.765–0.924	82.10	66.50–92.50
RE-PERG SD phase	0.93	0.04	0.857–0.976	84.60	69.50–94.10

B

MD	0.75	0.05	0.664–0.831	50.01	33.33–74.24
PSD	0.74	0.05	0.647–0.817	49.09	24.24–74.25
GCC	0.82	0.05	0.741–0.890	60.91	40.91–82.73
RNFL	0.79	0.05	0.699–0.858	59.09	34.85–74.76
SS-PERG amplitude[d]	0.68	0.04	0.592–0.771	54.50	41.80–66.90
RE-PERG SD phase	0.93	0.04	0.869–0.971	86.40	75.70–93.60

Notes: [a]DeLong et al;[42] [b]binomial exact. [c]mean amplitude of second harmonic of SS-PERG in GP; [d]mean amplitude of second harmonic of SS-PERG in GP and CP groups considered as a whole.

Abbreviations: AUC, area under the curve; CI, confidence interval; CP, cataract patients; GCC, ganglion cell complex; GP, glaucoma patients; MD, mean deviation; RE-PERG SD phase, SD of second harmonic phase of SS-PERG in GP; RNFL, retinal nerve fiber layer; ROCs, receiver operating characteristics; PSD, pattern standard deviation; SS-PERG, steady-state pattern electroretinogram; SE, standard error.

Therefore, the aim of this study was to verify the specificity of this new paradigm (called RE-PERG) in the presence of cataract.

Our results show that SS-PERG and RE-PERG have the same specificity in GP; but by mixing GP and CP, the specificity of SS-PERG drops to 54.5%, whereas the specificity of RE-PERG remains high (86.4%).

Based on the outcome of this study, we suppose that increased phase variability in RE-PERG could be the expression of the lower ability to follow stimuli due to RGCs' preapoptotic synaptic dysfunction in glaucoma.

Further studies are required: first, the procedure should be validated in other laboratories also to confirm our results; second, its reliability should also be verified in other conditions potentially able to bias the results (diabetic retinopathy, low myopia, etc); third, longitudinal studies should be carried out to verify its predictive value in ocular hypertensive patients; finally, it would be also helpful to verify the variations of the SD phase under therapy (both topical hypotensive and neuroprotective).

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Disclosure

The authors report no conflicts of interest in this work.

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