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The Relationship Between Stage of Leber's Hereditary Optic Neuropathy and Pattern Electroretinogram Latency

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Purpose: The purpose of this study was to compare the baseline steady-state pattern electroretinogram (SS-PERG) of patients with G11778A Leber hereditary optic neuropathy (LHON) with different stages of visual acuity (VA) loss before allotopic gene therapy (GT).

Methods: Patients ($n = 28$) were enrolled into groups (GT I: chronic bilateral VA $\leq$35 Early Treatment Diabetic Retinopathy Study [ETDRS]; GT II: acute bilateral VA $\leq$35 ETDRS; GT III: acute unilateral, VA $\leq$35 ETDRS, and better eye VA $\geq$70 ETDRS) and tested with SS-PERG together with 210 age-matched normal controls (NCs). SS-PERG amplitude (nV) and latency (ms) of each eye were averaged for groups GT I, GT II, and NC. Symptomatic eyes (GT III-S) and asymptomatic eyes (GT III-A) of group GT III were included separately and accounted for by using generalized estimating equation (GEE) methods.

Results: Compared to NC, SS-PERG amplitudes were reduced similarly by approximately 50% ($P < 0.001$) among all GT groups (NC > GT I, GT II, GT III-S, and GT III-A). SS-PERG latencies were shorter by $\geq$3.5 ms in all LHON groups and differed by disease stage (G III-A < NC, $P = 0.002$; GT III-S < GT III-A, $P = 0.01$; GT II < GT III-S, $P = 0.03$; GT I < NC, $P < 0.001$, but not different from other GT groups, all $P > 0.1$).

Conclusions: Although SS-PERG amplitude reduction did not distinguish between disease stages, SS-PERG latency shortening occurred in asymptomatic eyes and symptomatic eyes and distinguished between disease stages.

Translational Relevance: SS-PERG latency shortening is consistent with primary damage of smaller/slower axons and sparing of larger/faster axons and may provide an objective staging of LHON, which may be helpful to determine efficacy in LHON trials.

Introduction

Leber hereditary optic neuropathy (LHON) is a maternally inherited mitochondrial genetic disease characterized by rapid, severe, and irreversible loss of central vision that typically occurs during young adult life.[1–4] Visual loss is primarily due to dysfunction and death of retinal ganglion cells (RGCs) and their intraretinal axons with very high energy demand and vulnerability to insufficient energy metabolism.[5] Although visual symptoms usually begin in one eye first, loss of visual acuity and central visual field sensitivity may become bilateral over a few months. A test able to detect early RGC dysfunction preceding irreversible RGC death would help formulate predictions on disease course and inform timing of preventive treatment, including gene therapy (GT).

The pattern electroretinogram (PERG) is an electrophysiological test utilizing contrast-reversing checkerboards or gratings to measure RGC activity.[6–8] The PERG amplitude is reduced in patients with the LHON phenotype[5,9–11] and may also be reduced in some asymptomatic carriers with the G11778A LHON mutation.[12] This suggests that the PERG may be able to distinguish LHON phenotypes with different stages and time of onset. Although there are standard guidelines that define a single minimum stimulus and recording protocol for clinical transient PERG (Tr-PERG) in response to slow-reversing patterns,[8] other paradigms

for steady-state PERG (SS-PERG) in response to fast-reversing patterns have been adopted in a number of studies[13–16] as the periodic waveform can be advantageously analyzed in the frequency domain resulting in a precise, automated detection of response amplitude and phase (latency) that are highly reproducible.[10,17,18]

Here, we compare the baseline SS-PERG of patients with LHON with different stages and times of onset of visual symptoms before undergoing a phase I GT trial investigating the safety of a scAAV2 investigational product (IP) containing a wild-type synthetic nuclear encoded ND4 subunit for G11778A LHON.[19,20] We show that although the SS-PERG amplitude was much reduced in all LHON groups compared with normal controls (NCs), the SS-PERG latency progressively shortened with increasing disease stage. Latency shortening is consistent with LHON pathophysiology in which smaller/slower axons are primarily affected resulting in the residual response being dominated by larger/faster axons.

Methods

Participants

The study followed the tenets of the Declaration of Helsinki and was approved by the Institutional Review Board of the University of Miami. Informed written consent was obtained from all subjects Patients with LHON were recruited at the Bascom Palmer Eye Institute, as previously described,[19,21] to participate in an open-label, phase I, GT dose-escalation study to assess the safety of escalating doses of scAA-V2(Y444,500,730F)-P1ND4v23 vector to treat G11778A mutation that accounts for most LHON cases (NCT02161380). Best-corrected visual acuity was measured using the Early Treatment Diabetic Retinopathy Study (ETDRS) chart. Neuro-ophthalmic examination also included assessments of the pupils and anterior segment and ophthalmoscopy of the optic nerve head and retina. Visual field test results were obtained with the Humphrey 30-2 white-on-white standard automated perimetry. Genomic DNA was obtained from the patient's white blood cells obtained from venous blood. A polymerase chain reaction-based test using the amplification refractory mutation system was used to detect the presence or absence of three nucleotide substitutions known to cause LHON (3460G>A, 11778G>A, or 14484T>C). Three phenotypic groups of patients with LHON with molecularly confirmed G11778A DNA mutation were selected for the study and their eyes were classified into four groups with different stages of disease, based on visual acuity and time of onset of visual symptoms (Table 1) according to inclusion criteria detailed in previous reports of our team.[19] In brief, the three phenotypic groups were:

- Group GT I, Bilateral Chronic. Visual acuity loss of ≤35 ETDRS letters (Snellen ≤20/200) lasting ≥12 months in one eye and at least 6 months in the more recently affected eye.
- Group GT II, Bilateral Acute. Visual acuity loss of ≤35 ETDRS letters in both eyes for less than 12 months.
- Group GT III, Unilateral Acute. Visual acuity loss of <35 ETDRS letters for less than 12 months (GT III-S) in one eye and acuity ≥70 letters (Snellen = 20/40) in the contralateral eye (GT III-A).

For the purposes of this study, group NC, normal controls were defined as having Snellen Visual acuity ≥20/20 (≥85 ETDRS letters) with no history of ophthalmological or neurological diseases. Table 1 summarizes the demographic and phenotypic characteristics of the three groups.

Electrophysiology

The SS-PERG was simultaneously recorded from both eyes, as previously described,[10,13] from skin electrodes taped on the lower eyelids in response to horizontal gratings (1.7 cycles/degrees, 98% contrast) presented on a visual display placed at a viewing distance of 30 cm (Figs. 1A, 1B). Subjects were fitted

Table 1. Demographic and Phenotypic Characteristics of Study Participants

Patient Phenotype	Eye Group	Vision, ETDRS	Duration, Months	*N*	Age, Years	SD	Gender, M%
Bilateral-chronic	GT I	≤35	≥12	10	33.4	11.4	100
Bilateral-acute	GT II	≤35	<12	9	33.0	11.3	44
Unilateral-acute	GT III-symptomatic	≤35	<12	8	27.4	12.2	100
	GT III-asymptomatic	≥70	<12	8	27.4	12.2	100
Normal controls	NC	≥85	N/A	210	37.0	12.0	70

N/A, not applicable.

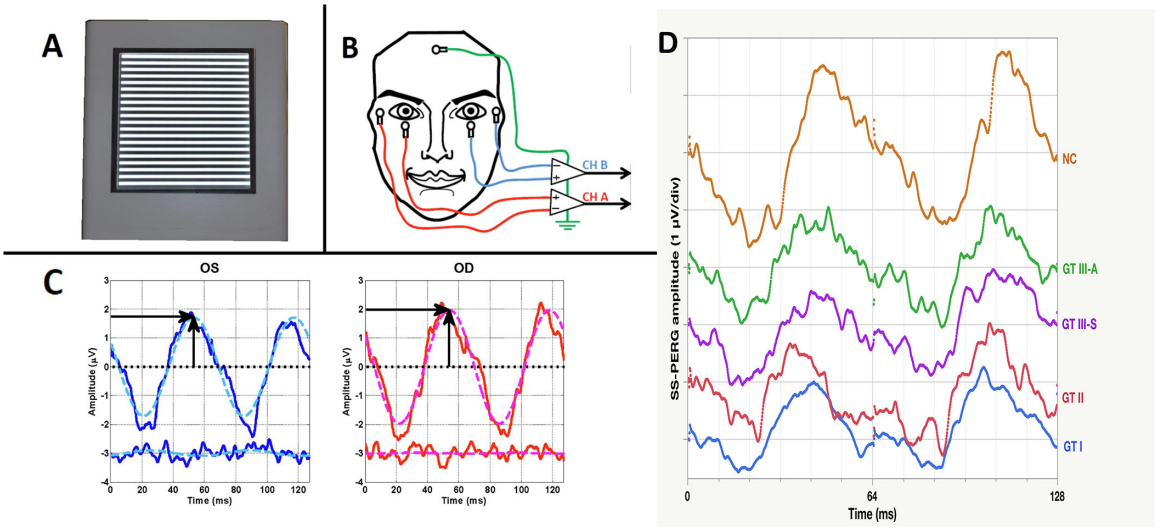

Figure 1. Outline of SS-PERG setup. (**A**) Pattern stimulus. (**B**) Surface electrode placement. (**C**) The SS-PERG and noise waveforms for each eye (continuous lines) in response to one stimulus cycle (two pattern reversals). The dashed sinusoidal waveforms superimposed to the SS-PERG waveforms represent the frequency-domain component that is measured. The vertical arrows represent the SS-PERG amplitude (zero-to-peak amplitude of the isolated sinusoidal component) and the horizontal arrow represents the SS-PERG latency (time-to-peak of the isolated sinusoidal component). (**D**) Examples of SS-PERG waveforms recorded in representative subjects of study groups (NC = normal controls; GT I = bilateral-chronic; GT II = bilateral-acute; GT III-A = unilateral asymptomatic; and GT III-S = unilateral symptomatic). Note in **D** that waveforms of all LHON groups, compared to controls, have reduced amplitude, and are shifted to the left (shortened latency).

with the appropriate add for the viewing distance and were instructed to fixate on a target at the center of the display. The operator controlled the quality of fixation over the recording period (approximately 2.30 minutes). Subjects did not receive dilating drops and were allowed to blink freely. During signal acquisition, sweeps contaminated by eye blinks or gross eye movements were automatically rejected over a threshold voltage of 25 μV. SS-PERG recording was initiated with a commercial instrument (Glaid, Lace Elettronica, Rome, Italy) that during the study was replaced by a second-generation commercial instrument (Jorvec, Miami, FL, USA). The two instruments had similar characteristics, except the type of display (Glaid: Cathode Ray Tube; Jorvec: Light-Emitting Diodes) and a slight difference in reversal rate (Lace: 16.28 reversals/s; Jorvec: 15.63 reversals/s. SS-PERGs have been previously recorded in the same subjects with either instrument and conversion factors for amplitude and phase established.[13] SS-PERG results reported in this study are expressed as Jorvec response amplitude in nanoVolts (nV) and phase shift in degrees. The SS-PERG waveform results from the overlapping of Tr-PERG waveforms generated at high reversal rate. The SS-PERG phase shift represents the integrated processing delay, which corresponds to the response latency.[22] The phase shift (degrees) can be expressed in latency time units of milliseconds (ms) as follows. As one reversal period (360 degrees) corresponds to 1/15.63 reversals/s = 0.064 s, SS-PERG latency (ms) is calculated as ([360 – phase shift [deg]]/360) *64 ms. The SS-PERG latency coincides with the time-to-peak of the first positive wave of the SS-PERG periodic waveform (example in Figs. 1C, 1D). Previous studies[23,24] demonstrated that latency of the SS-PERG reflects the time-to-peak of the P50 wave of the Tr-PERG. Both P50 and N95 amplitudes of the Tr-PERG contribute to the overall SS-PERG amplitude.

Statistics

The present study compares baseline SS-PERG amplitude and latency values recorded in the three LHON groups before GT treatment and in a group of similarly aged normal controls. LHON participants received two baseline measurements for each eye that were averaged. Then measurements from the two eyes of groups GT I, GT II, and NCs were averaged yielding one measurement per participant. This was done to promote signal averaging among these three groups for which both eyes of each patient fell under the same eligibility requirements. Thus, each patient contributed one averaged measurement for analysis. For group GT III, measurements from asymptomatic (GT III-A) and symptomatic (GT III-S) eyes were analyzed separately. Thus, each patient contributed two measurements for analysis, one for each eye. Mean amplitudes and laten-

cies were compared with the generalized estimating equation (GEE)[25,26] methods followed by post hoc least significant difference (LSD) tests to account for inclusion of two measurements of patients with GT III.

Results

Representative examples of SS-PERG waveforms recorded in NCs and patients with LHON are shown in Figure 1D. It can be appreciated that waveforms of all LHON groups, compared with controls, have reduced amplitude, and are shifted to the left (shortened latency).

Group data are summarized in Figure 2 and corresponding estimates of marginal means analyzed with GEE statistics are detailed in Table 2. The SS-PERG amplitude (see Fig. 2A) was much reduced (≥50%) in all LHON groups compared with controls (post hoc LSD comparisons: controls > all LHON groups: all $P \leq 0.001$), including the asymptomatic eyes of the acute unilateral group. There were no significant differences in SS-PERG amplitude among LHON groups (all $P > 0.35$).

The SS-PERG latency (see Fig. 2B) displayed a significant shortening of 3 to 5 ms in all LHON groups that tended to increase with increasing LHON stage (GEE, $P < 0.001$). Post hoc LSD comparisons were, controls > all LHON groups (all $P \leq 0.002$); and unilateral asymptomatic > unilateral symptomatic, acute ($P \leq 0.011$); unilateral symptomatic > acute ($P = 0.030$); chronic not different from acute or unilateral

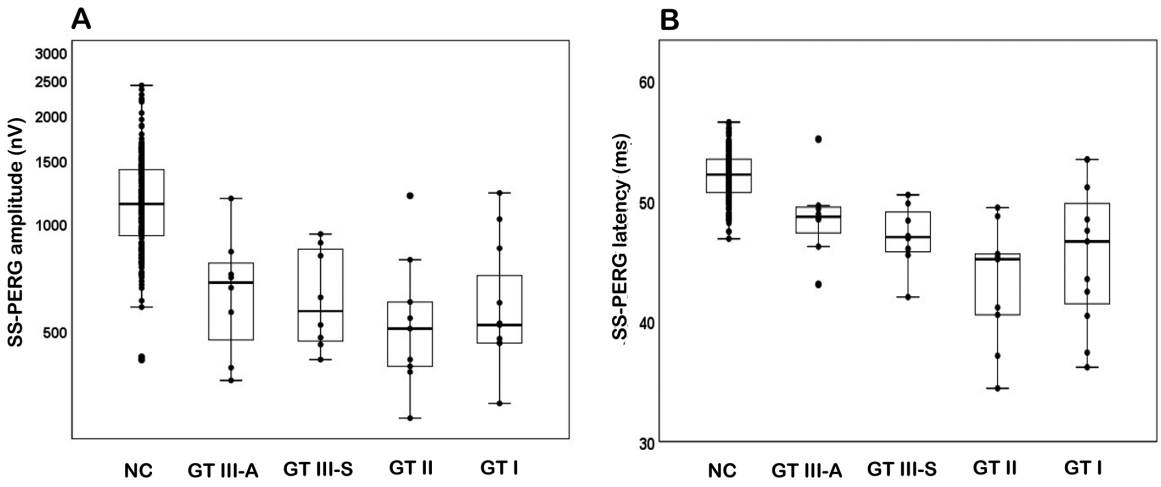

Figure 2. Changes in SS-PERG amplitude and latency with increasing stage of LHON symptoms. Distribution of amplitude (**A**) and latency (**B**) data in different study groups (NC = normal controls; GT I = chronic bilateral; GT II = acute bilateral; GT III-A = acute unilateral asymptomatic eyes; and GT III-S = acute unilateral symptomatic eyes). Note in **A** and **B** that interquartile ranges (boxes) of all LHON groups do not overlap with corresponding interquartile ranges of normal controls, and the medians tend to decrease with increasing stage except for the chronic bilateral LHON group.

Table 2. Mean SS-PERG Amplitude and Latency Values in Study Groups

Group	Mean Log Amplitude (nV)	SE	Lower 95% CI	Upper 95% CI
NC	3.0507	0.00897	3.0331	3.0683
GT I	2.7360	0.00612	2.6161	2.8560
GT II	2.7172	0.05826	2.6030	2.8313
GT III-A	2.8028	0.05496	2.6951	2.9105
GT III-S	2.7867	0.04568	2.6972	2.8762
Group	Mean Latency (ms)	SE	Lower 95% CI	Upper 95% CI
NC	52.1	0.1	51.8	52.3
GT I	45.5	1.7	42.1	48.9
GT II	43.0	1.6	39.9	46.2
GT III-A	48.6	1.1	46.4	50.8
GT III-S	47.0	0.9	45.3	48.7

CI, confidence interval.

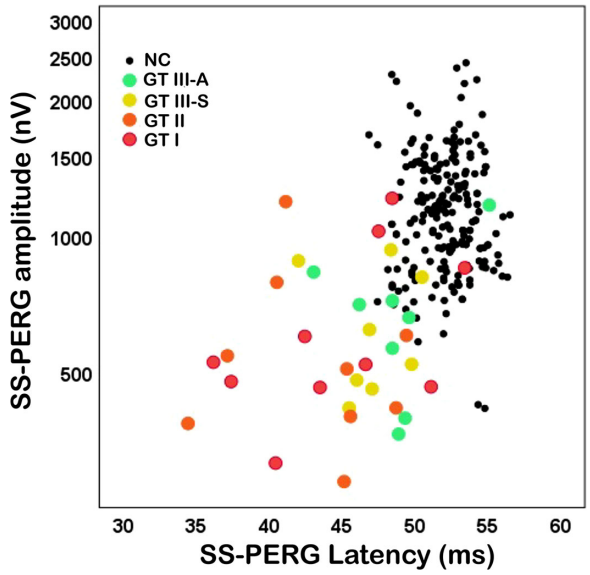

Figure 3. Correlation between SS-PERG amplitude and SS-PERG latency. The scattergram includes data of both normal controls (*smaller black symbols*) and the patients with LHON groups with different stages of the disease (larger symbols with different color). NCs, normal controls; GT I, chronic bilateral; GT II, acute bilateral; GT III-A, acute unilateral asymptomatic eyes; GT III-S, acute unilateral symptomatic eyes. Note that both amplitude and latency are reduced in patients with LHON ($r = 0.40$, $P < 0.001$), and the magnitude of reduction depends on the stage of condition.

LHON groups (all $P > 0.1$). Pairwise post hoc least significant difference tests, performed after finding a significant main effect in the GEE analysis, are shown in the Supplementary Table.

Figure 3 illustrates the relationship between SS-PERG amplitude and SS-PERG latency in individual NCs and patients with LHON. In NCs, there was no correlation between amplitude and latency ($r = -0.05$, $P = 0.52$). However, after including patient data in the analysis, the correlation became highly significant ($r = 0.40$, $P < 0.001$). There was little overlap between NCs and patients with LHON, including the asymptomatic eyes of the unilateral acute group.

Discussion

This study assessed the SS-PERG in patients with different stages of visual acuity loss and times of onset of G11778A LHON as a measure of baseline RGC function before undergoing GT. Results shows that both SS-PERG amplitude and SS-PERG latency were much reduced, compared with NCs, in patients with LHON with disease stages ranging from the asymptomatic eye in acute disease to both symptomatic eyes in chronic disease. SS-PERG amplitude and latency changes were correlated in patients with LHON but not in NCs. Previous studies have also shown that the standard Tr-PERG amplitude may be reduced in patients with LHON.[5,9,11] However, previous studies were performed in patients with severe LHON stages, and reports of shortened Tr-PERG latency were anecdotal. It is possible that changes of Tr-PERG latency were not fully appreciated as the measurement of peak latency in Tr-PERG waveform is subjectively determined and not precise due to noise intrusion.[8] The SS-PERG originates from the overlapping of Tr-PERG, and deconvolution analysis[23,24] demonstrated that the SS-PERG amplitude includes both P50 and N95 waves of the Tr-PERG, whereas the SS-PERG latency corresponds to the time-to-peak of the P50 wave of the Tr-PERG. Compared to Tr-PERG, the SS-PERG has the advantage that the response waveform can be analyzed in the frequency domain, thereby allowing isolation of the sinusoidal component at the reversal rate from noise and precise objective measurement of its amplitude and phase shift (latency) in an automated way.

That SS-PERG latency is shortened in LHON rather than delayed is intriguing and a hypothesis has been discussed in previous studies of our group[27] as follows. Physiological studies[14] have shown that the SS-PERG in response to low spatial frequencies (primarily subserved by larger RGCs with thicker/faster axons) has a much shorter latency than the SS-PERG in response to high spatial frequencies (primarily subserved by smaller RGCs with thinner/slower axons). As LHON preferentially alters RGCs with smaller/slower axons including those of the maculopapillary bundle,[28] surviving RGCs with larger/faster axons primarily contribute to the SS-PERG, resulting in a response with a shorter latency, consistent with LHON pathophysiology. Shortened SS-PERG latency has also been reported to occur in optic neuritis.[27,29]

In summary, whereas baseline SS-PERG amplitude was much reduced already in asymptomatic eyes of patients with LHON and did not distinguish between disease stages, SS-PERG latency was shorter in all LHON groups and differed between groups with different stage and time of onset of visual acuity loss. A caveat of our findings was that chronic eyes were not different from the other LHON stages. We think this was due to the large variability in the chronic group compared with the other groups, which was likely associated to heterogeneity of time since diagnosis with a span of up to 20 years prior to enrollment.

The SS-PERG abnormality in asymptomatic eyes without obvious loss of retinal fiber layer thickness suggests that there is a manifest RGC dysfunction preceding cell demise, and perhaps there is a

therapeutic time window of opportunity for rescuing RGCs from death with treatment including GT in these eyes. LHON is a rare disease. Although the present results have been obtained in a relatively small group of patients, the statistical differences between groups were highly significant. Overall, results suggest that assessment of SS-PERG latency in patients with LHON provides an objective staging of LHON, which may be helpful in the analysis of LHON trials to determine efficacy. Our group is currently studying the relationship between SS-PERG and best-corrected visual acuity and the visual field in a longitudinal study of the same patients undergoing a phase I Gene Therapy trial investigating the safety of a scAAV2 vector containing a wild-type synthetic nuclear encoded ND4 subunit for G11778A LHON.[19,30]

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Meeting presentation: A preliminary version of this study has been presented at the 2021 ARVO annual meeting in a paper form: Porciatti et al., Progressive shortening of Steady-State Pattern ERG latency with increased LHON severity. Abstract ID #3536913.

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