Special Issue

The Photopic Negative Response: An Objective Measure of Retinal Ganglion Cell Function in Patients With Leber's Hereditary Optic Neuropathy

Rustum Karanjia,[1–4] Adriana Berezovsky,[5] Paula Yuri Sacai,[5] Nivea Nunes Cavascan,[5] Henry Yuheng Liu,[3] Samir Nazarali,[3] Milton Nunes Moraes-Filho,[6] Kirsten Anderson,[1,2] Jeffrey Show Tran,[1] Sung EunSong Watanabe,[5] Milton Nunes Moraes,[6] Federico Sadun,[7] Anna Maria DeNegri,[8] Piero Barboni,[9] Carolina do Val Ferreira Ramos,[10] Chiara La Morgia,[11,12] Valerio Carelli,[11,12] Rubens Belfort Jr,[5] Stuart Glenn Coupland,[3,4] Solange Rios Salomao,[5] and Alfredo A. Sadun[1,2]

[1]Doheny Eye Institute, Los Angeles, California, United States
[2]Doheny Eye Centers of UCLA, Department of Ophthalmology, David Geffen School of Medicine at University of California, Los Angeles, Los Angeles, California, United States
[3]Ottawa Eye Institute, University of Ottawa, Ottawa, Ontario, Canada
[4]Ottawa Hospital Research Institute, Ottawa, Ontario, Canada
[5]Departamento de Oftalmologia e Ciências Visuais, Escola Paulista de Medicina, Universidade Federal de São Paulo (UNIFESP), São Paulo, SP, Brazil
[6]Instituto de Olhos de Colatina, Colatina, ES, Brazil
[7]Department of Ophthalmology, Ospedale Parodi Delfino, Colleferro, Rome
[8]Azienda Ospedaliera San Camillo-Forlanini, Roma, Italy
[9]Studio Oculistico d'Azeglio, Bologna, Italy
[10]Hospital Federal da Lagoa, Rio de Janeiro, Brazil
[11]Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS), Institute of Neurological Sciences of Bologna, Bellaria Hospital, Bologna, Italy
[12]Unit of Neurology, Department of Biomedical and Neuromotor Sciences (DIBINEM), University of Bologna, Bologna, Italy

Correspondence: Rustum Karanjia, Suite 215, 800 Fairmount Pasadena, CA 91105, USA; Karanjia@jsei.ucla.edu.

Submitted: February 28, 2017
Accepted: July 22, 2017

Citation: Karanjia R, Berezovsky A, Sacai PY, et al. The photopic negative response: an objective measure of retinal ganglion cell function in patients with Leber's hereditary optic neuropathy. *Invest Ophthalmol Vis Sci*. 2017;58:BIO300–BIO306. DOI: 10.1167/iovs.17-21773

Purpose. The photopic negative response (PhNR) is a slow negative component of a flash photopic full-field ERG that has been shown to be specific for retinal ganglion cell (RGC) activity. Direct evaluation of RGC function is desirable in patients with Leber's hereditary optic neuropathy (LHON) in which the loss of central acuity can make it difficult to monitor patients with standard metrics. The purpose of this study was to evaluate the use of PhNR as an objective noninvasive clinical metric in LHON.

Methods. Full-field photopic ERG recordings were collected in subjects with the mt.11778G>A/ND4 LHON mutation using a red on blue stimulus. The PhNR was identified using a computer-based automated detection system, and data were manually examined to remove movement artifacts.

Results. The PhNR amplitude was compared between controls ($n = 13$), carriers ($n = 17$), and affected ($n = 6$). Mean PhNR amplitude decreased significantly across groups ($P < 0.0001$). Post hoc Tukey's test revealed a significant decrease in PhNR amplitude between carriers and controls ($P < 0.05$) and between carriers and affected ($P < 0.01$).

Conclusions. We are able to demonstrate that the PhNR amplitude is significantly decreased in patients affected by LHON compared to carriers in a well-described pedigree. Surprisingly, there was also a decrease in PhNR in carriers, suggesting potential subclinical RGC dysfunction in some carriers. This is important in patients affected with LHON who typically have a dense central scotoma. The PhNR may be a useful objective outcome measure for future clinical trials.

Keywords: LHON, PhNR, ERG

Leber's hereditary optic neuropathy (LHON) is a rare mitochondrial disease, preferentially affecting young males, and is characterized by a painless, subacute loss of central vision.[1,2] Over 95% of individuals with LHON carry one of three pathogenic mitochondrial DNA (mtDNA) mutations affecting complex I of the oxidative phosphorylation chain (i.e., m.11778G>A/ ND4, m.3460G>A/ ND1, and m.14484T>C/ ND6) that impair oxidative phosphorylation and increase reactive oxygen species.[2] Typically some carriers experience significantly diminished visual acuity, central or cecocentral scotoma, and dyschromatopsia, thereby becoming affected. Visual dysfunction in LHON is attributed to a selective degeneration of retinal ganglion cells (RGCs) and their axons.[3] LHON is characterized by incomplete penetrance, and only a

subset of individuals bearing a LHON mutation (carriers) manifest the disease (affected). The earliest signs of conversion include RGC dysfunction,[4] "pseudoedema" of the peripapillary retinal nerve fiber layer (RNFL) and retinal telangiectasia, ultimately leading to a cecocentral scotoma.[5]

LHON starts by first affecting the fibers of the papillomacular bundle resulting in a central scotoma.[6] This loss of central vision leads to early and severe loss of visual acuity that rapidly runs into a floor effect. This floor effect, with most patients off-chart, renders visual acuity a poor metric for following affected patients. Visual field testing through automated perimetry is often used to determine visual function in patients with optic nerve disease. However, there is also a floor effect for this test as most patients have a mean deviation worse than −30 dB.[6] Visual fields also require patient input and can be challenging for the patients who have lost central visual acuity as this affects the ability of the patients to fixate.[1,7] Visual electrophysiological recordings, such as visual evoked potentials (VEPs) and pattern electroretinogram (PERG), have also been used to characterize patients with LHON and provide a more objective and reliable metric to assessing RGC function.[8–10] However, both techniques have limitations. VEPs, for example, are not a direct measure of the RGC function, while PERG utility is primarily restricted to the inner retina and necessitates refractive correction and precise foveal fixation, which present a challenge for patients with LHON.[11] Recently, it was discovered that RGCs also generate a slow negative wave response observable on the ERG immediately following the b-wave of the cone response. This component of the ERG is referred to as the photopic negative response (PhNR). Several studies have shown correlation between PhNR amplitude and the presence of RGC pathologies such as in glaucoma, idiopathic intracranial hypertension, diabetic optic nerve atrophy, optic neuritis, and retinal vascular diseases.[12–16] As a full-field test, the PhNR does not have the refractive and fixational constraints of the PERG, making it easier for the patient.[17]

In the present study, we investigated whether PhNR was affected in the full-field ERG of patients with LHON. The results are intended to provide a foundation for the use of PhNR as a potential objective noninvasive clinical metric of visual function in LHON patients.

Methods

The PhNR amplitude and timing were recorded in LHON affected (from herein "affected"), LHON unaffected mutation carriers (from herein "carriers"), and off-pedigree controls of the previously investigated Soave-Brazil (SOA-BR) LHON pedigree,[18] carrying the m.11778G>A/ ND4 mutation on a haplogroup J background of mtDNA. Collection of data was carried out in Colatina ES, Brazil and at the Federal University of Sao Paulo (UNIFESP) in Sao Paulo, Brazil. The collection of the data was in accordance with the World Medical Board Declaration of Helsinki and approved by the Committee on Ethics in Research of the Universidade Federal de Sao Paulo, Escola Paulista de Medicina.

Visual acuity and automated visual field data were collected as previously reported. Briefly, Early Treatment Diabetic Retinopathy Study (ETDRS) visual acuity charts were used to assess best-corrected visual acuity; Humphrey Field Analyzer (Humphrey Systems, Inc., Dublin, CA, USA) was used for visual field examination with the SITA threshold strategy program 30-2. The RNFL was also measured using a Cirrus optical coherence tomography (OCT) machine (Carl-Zeiss Meditech, Dublin, CA, USA) for affected and carrier patients.

Table 1. Patient Demographics

	Number of Subjects	Age, y	Sex, % male	Best-Corrected Visual Acuity
Controls	13	31 ± 9	46%	20/20
Carriers	17	47 ± 15	64%	20/20
Affected	6	50 ± 18	66%	20/400 or worse

ERG Recordings

The pupils were maximally dilated to approximately 8 mm in diameter following topical application of 1% tropicamide and 10% phenylephrine. Patients were light adapted for a minimum of 10 minutes before any recordings were obtained. ERGs were recorded with DTL-Plus (Diagnosys LLC, Lowell, MA, USA) microconductive thread electrodes secured on the temporal and nasal canthus with the fiber positioned at the lower limbal margin of iris after topic anesthesia with proxymetacaine 0.5% (Anestalcon, Alcon, Fort Worth, TX, USA). Gold cup electrodes were used on the temple for reference and Fz for ground. The full-field PhNR stimulus conditions were produced by a LED-based ColorBurst (Diagnosys LLC) handheld stimulator. Red (640 nm) stimulus flashes of 4 milliseconds (msec) duration were presented at a 2-Hz rate on a blue (470 nm) rod saturating background. Red flash stimulation was presented at 1, 5, and 7 cd·s/m^2, while the blue background remained at 10 cd/m^2. An Espion e^2 (Diagnosys LLC) was used to record PhNR waveforms. Three sets of 50 sweeps of 150-msec duration were recorded with bandpass filtering between 0.3 and 300 Hz at the three stimulus flash intensities. Each of the three repetitions was edited to remove eye blink and other artifacts, and a grand average was determined for each eye at each of the three stimulus intensities. Recordings were obtained from both right and left eyes. Only data from the left eye (the second recorded eye) were used for analysis. PhNR waveforms were visually inspected, and the a-wave, b-wave, and PhNR components were determined. The amplitude of the PhNR was identified as the first negative deflection after the b-wave, and the amplitude was recorded relative to baseline (0 μV).

Subjects who had concomitant medical conditions other than LHON that affected the eyes were excluded from the study.

Statistical Analysis

ERG marker table values were exported as text files and opened in Microsoft Excel spreadsheet (Microsoft Corporation, Seattle, WA, USA). Results were analyzed using a 1-way analysis of variance (ANOVA) with post hoc Tukey's honestly significant difference (HSD) for multiple comparisons. Receiver operating characteristic (ROC) curve was constructed by varying a cutoff value between 5 and 40 μV and increasing by 5-μV intervals. Sensitivity and 1- specificity was computed for each cutoff point across the three pairwise comparison groups: affected versus control, carriers versus control, and affected versus carrier.

Results

PhNR recordings were obtained from off-pedigree control subjects ($n = 13$), carriers ($n = 17$), and affected patients ($n = 6$), and results recorded from the left eye were included for subsequent analysis. All off-pedigree controls had a best-

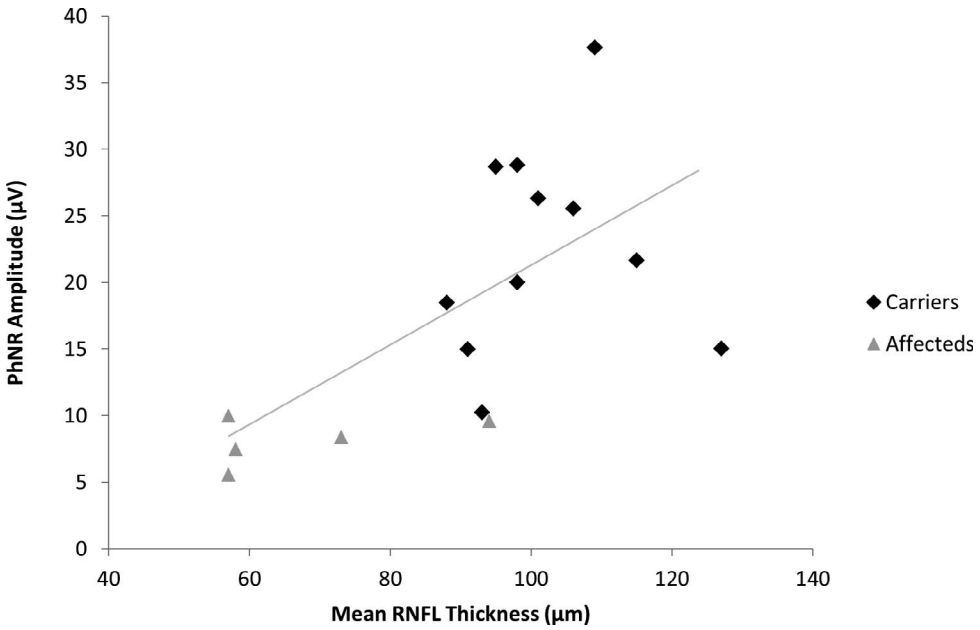

FIGURE 1. There was a significant correlation between the PhNR and documented OCT RNFL thickness for LHON carriers ($n = 11$) and affected ($n = 5$) ($P < 0.01$, $R^2 = 0.397$).

corrected visual acuity of 20/20 and had no history of any ocular disease. Patient demographics are shown in Table 1. The carriers also had a best-corrected visual acuity of 20/20 with a mean deviation (MD) of better than –6.13 dB. The average MD for the carriers was –2.16 ± 0.22 dB, and all affected individuals were severely affected with an average MD of –29.0 ± 1.15 dB. There was a significant difference in the RNFL for carriers (mean = 102.05, SD = 10.58) and affected individuals (mean = 67.8, SD = 16.58); $t(30) = 7.08$, $P < 0.0001$. Moreover, the OCT RNFL thickness was correlated with the amplitude of the PhNR in LHON affected and carriers ($P = 0.0003$, $r^2 = 0.355$).

The recorded PhNR amplitudes for representative controls, carriers, and affected patients are shown in Figure 2. Mean PhNR amplitude decreased significantly across groups ($F[2,33] = 23.85$, $P < 0.0001$). Post hoc Tukey's test revealed a significant decrease in PhNR amplitude between carriers and controls (mean carrier = –22.6 ± 1.7 µV, mean control = –30.5 ± 1.8 µV, $P < 0.05$) and between carriers and affected (mean affected = –8.8 ± 0.7 µV, $P < 0.01$). These findings were consistent across all three stimulus intensities: 1 cd·s/m^2, 5 cd·s/m^2, and 7 cd·s/m^2 (Fig. 3). Overall, increasing stimulus intensity did not significantly affect the recorded stimulus signals across conditions ($P > 0.05$; Figs. 1, 2).

ROC curve analysis revealed a PhNR amplitude of 20 µV to be the optimal cutoff yielding a positive predictive value (PPV) of 100% for affected versus controls and a sensitivity and specificity of 100% (Fig. 4; Table 2). Comparing affected versus carriers at 20 µV yields a PPV of 50% with a sensitivity and specificity of 100% and 65%, respectively. Lastly, comparing carriers versus controls at 20 µV gives a PPV of 100% and a sensitivity and specificity of 35% and 100%, respectively (Table 2).

DISCUSSION

This is the first study assessing the clinical utility of the PhNR in patients with LHON. We found a significant reduction in PhNR amplitude in both affected LHON patients (74.4%) and asymptomatic carriers (26.1%) relative to off-pedigree controls ($P < 0.05$ for both). Remarkably, there was also a decrease in PhNR in carriers, suggesting potential subclinical RGC dysfunction in some of the carriers (as indicated by mild RNFL losses as well). This observation further corroborates the findings from Guy et al.[10] who found in a serial evaluation of 45 asymptomatic carriers of the mt.11778G>A/ND4 LHON mutation that the PERG declined significantly and in a progressive manner. Furthermore, one carrier was observed to have a normal PERG when asymptomatic, but later experienced a 50% reduction in amplitude prior to loss of vision. In the present study, there was a clear distinction in PhNR amplitude between affected and controls of –20 µV with no affected patients having an amplitude below –10 µV and none of the controls having an amplitude of greater than –20 µV when the 1 cd·s/m^2 intensity was used. This gives a PPV of 100% for PhNR between controls and affected. This is reduced to a sensitivity of 67% and a specificity of 100% between carriers and affected.

It has been hypothesized that LHON carriers may, in part, maintain compensation of RGCs thorough an increase in mtDNA copy.[19] Thus, it is reasonable to hypothesize that individuals who are able to upregulate mitochondria through biogenesis would be able to compensate for the effects of the mutation and would, thus, have normal PhNR amplitude. Conversely, those who are unable to increase the amount of mitochondria might have lower PhNR amplitude and be more susceptible to conversion. Prospective studies in carriers, which correlate the PhNR to their mtDNA copy number, would help to clarify this point. We also demonstrated an increase in the SD of the amplitude of PhNR in the carriers when compared to the controls or affected. This idea is consistent with the findings of the wide variability of the mtDNA copy number in carriers found by Giordano and colleagues.[19]

The PhNR was also assessed at three stimulus intensities 1, 5, and 7 cd·s/m^2. There was a gradual increase in amplitude with higher intensities, but this did not reach statistical significance. Of note, the distinction between carriers, affected, and controls was present at all three intensities (Fig. 3). Prior literature has examined the optimal recording conditions for eliciting the PhNR and found that flash

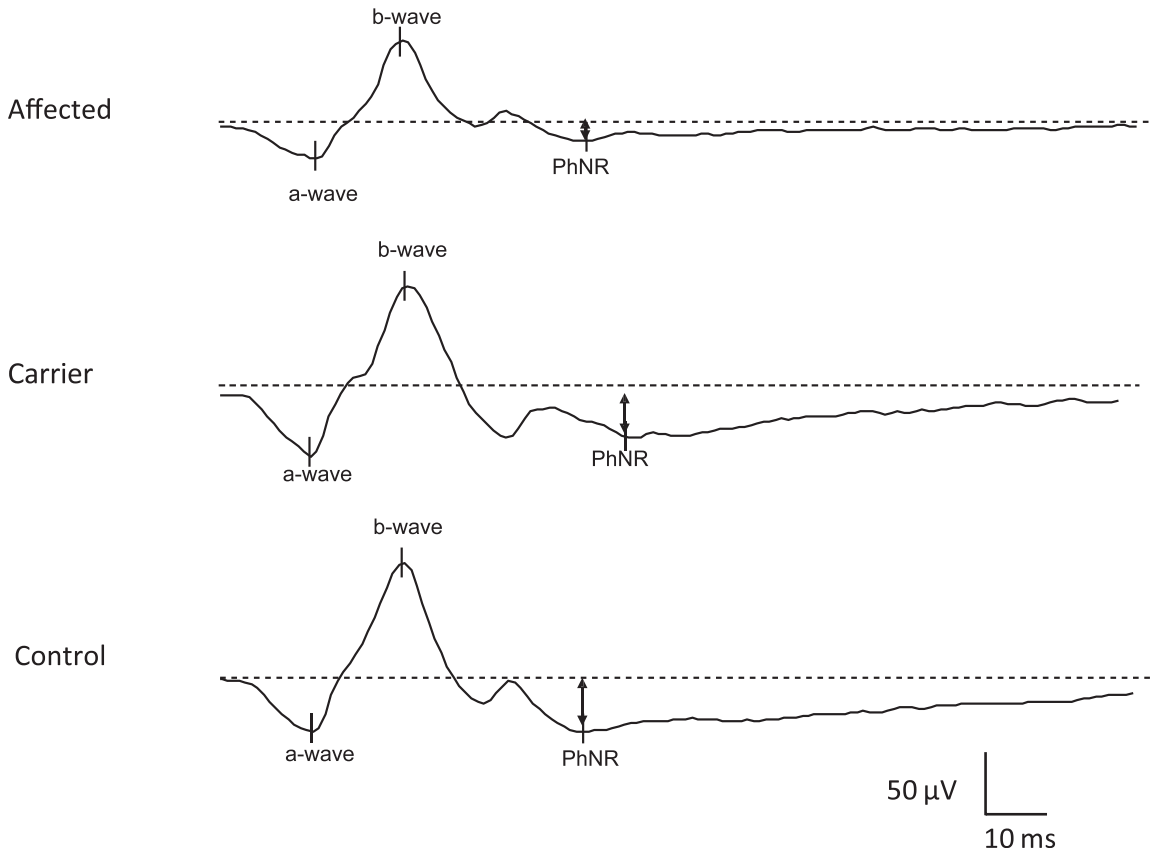

Figure 2. Typical PhNR recordings, at 1 cd·s/m^2, from controls, carriers, and affected. The amplitude of the PhNR was identified as the first negative deflection after the b-wave. PhNR amplitude was significantly smaller in affected when compared to carriers or controls.

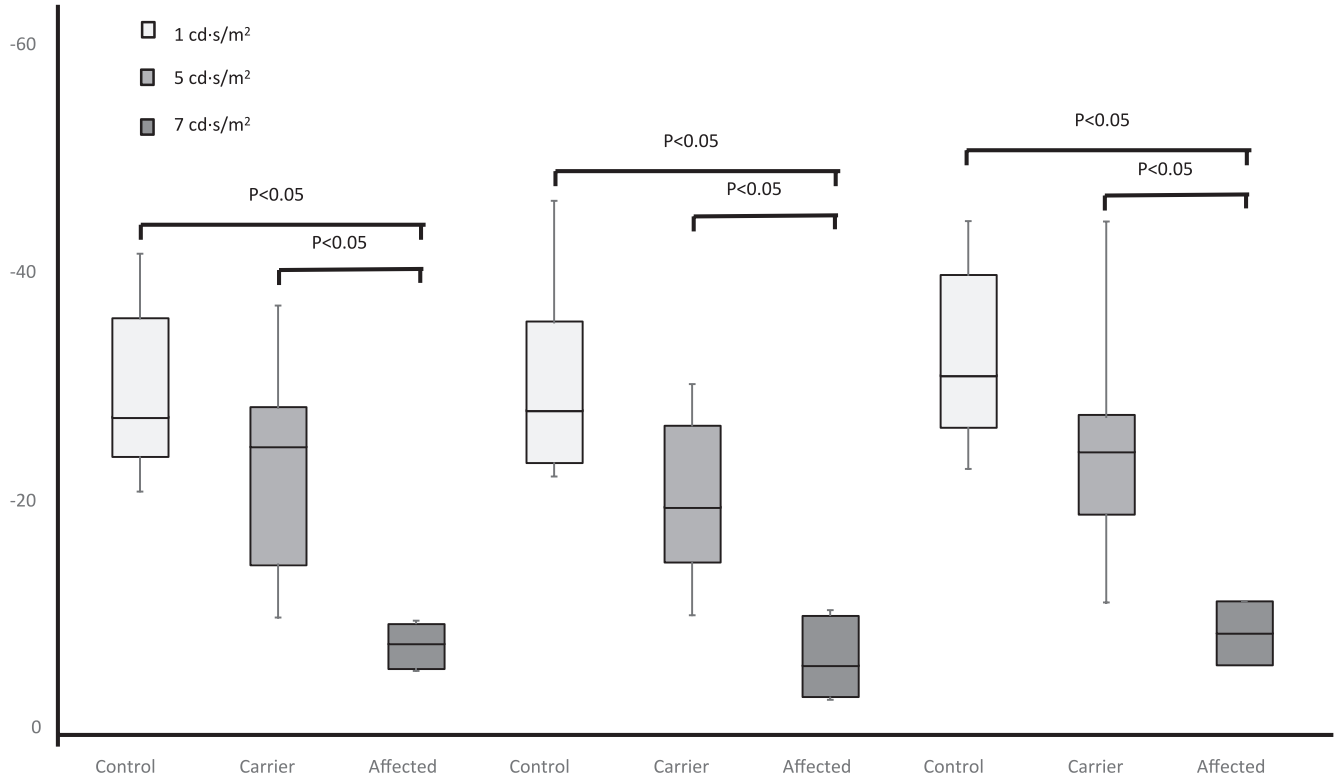

Figure 3. **(A)** PhNR amplitudes were plotted for controls ($n = 13$), carriers of the mt.11778 mutation ($n = 17$), and affected LHON patients ($n = 6$). **(B)** PhNR amplitudes at three different stimulus intensities 1, 5, and 7 cd·s/m^2. Increasing stimulus intensity did not affect the recorded stimulus signals across conditions ($P > 0.05$). The *boxes* represent the 25% to 75% interquartile ranges, and *horizontal lines* represent mean values.

Investigative Ophthalmology & Visual Science

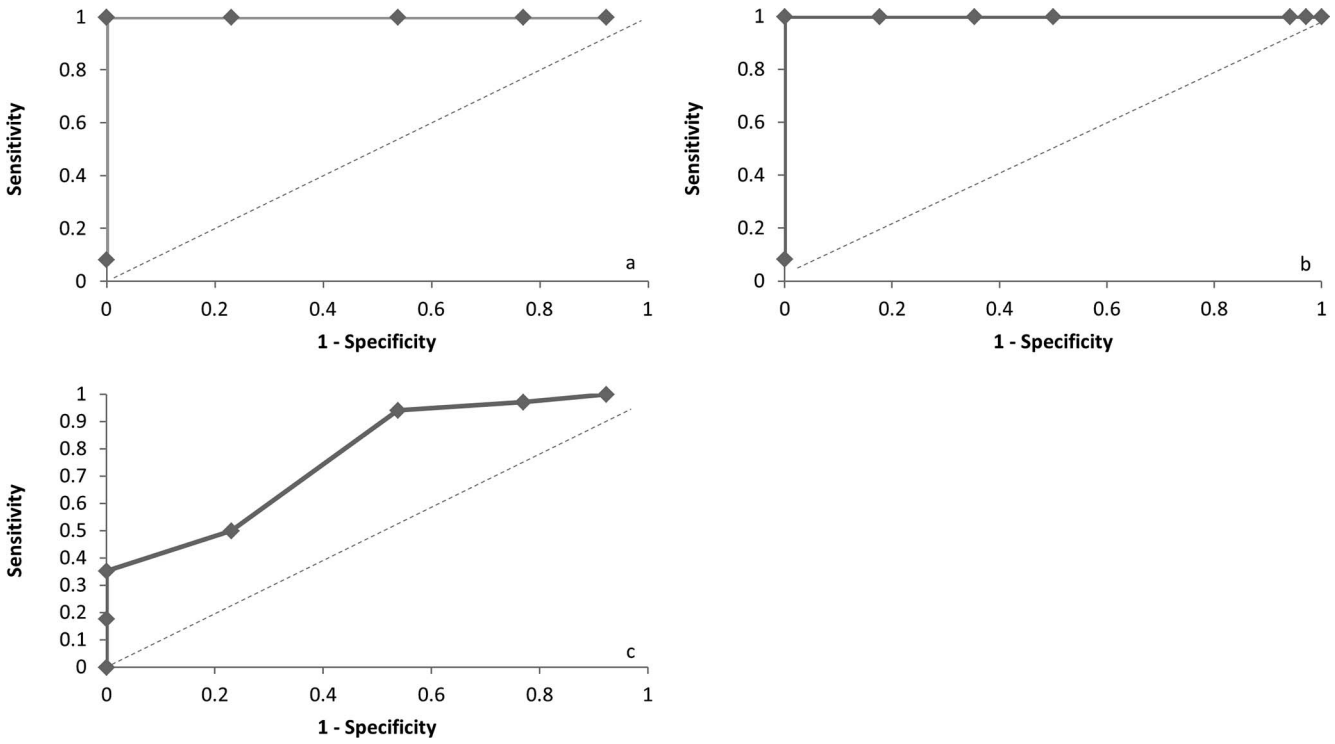

FIGURE 4. ROC curve for (**a**) affected versus controls, (**b**) affected versus carriers, and (**c**) carriers versus controls plotted at cutoff thresholds between 5 and 40 μV. *Dashed line* represents the reference line of zero predictive power.

intensities of 5 cd·s/m^2 resulted in the most stable and reliable recordings.[20] However, we found that stronger stimulus intensities were more difficult to tolerate for patients and resulted in higher intertest variability.

This study evaluated the PhNR in a single clinically characterized Brazilian pedigree of LHON patients carrying the mt.11778/ND4 mutation.[18] This single pedigree approach decreases confounders and provides a uniform study population. Affected members of our large pedigree were severely impacted with MD values of −25 dB or worse. This may explain why all the affected patients demonstrated such markedly reduced PhNR amplitudes. It will be important to verify this reduction in PhNR amplitude in a more heterogeneous population of LHON patients carrying different mtDNA mutations.

To our knowledge, this is the first study assessing the PhNR in patients with LHON and extends the existing literature on the effectiveness of measuring the PhNR in various optic neuropathies including optic neuritis, optic atrophy, and glaucoma.[16,21,22] Moss and colleagues[17] found that the PhNR was associated with a clinical measure of visual function in 10 patients with idiopathic intracranial hypertension. In their study, the PhNR amplitude was significantly reduced compared to visually normal controls. Ganglion cell complex volume, an indicator of RGC loss, was correlated with PhNR amplitude (r = 0.77). However, multivariate linear regression models accounting for optic nerve structure improved the correlation between RGC loss and PhNR (r = 0.94). Machida et al.,[7] and subsequently Shen et al.,[23] demonstrated that PhNR amplitude could be used to differentiate diseased eyes from normal eyes. Their investigation in patients with primary open-angle glaucoma found that the reduction in PhNR amplitude not only correlated with the degree of visual field defect, but also with the RNFL assessment of neural loss. Coupled together with the work of Kaneko et al.,[24] and Wilsey et al.,[25] who demonstrated the use of mfERG PhNR as a reliable measurement of PhNR for comparisons with visual field defects and tomographic loss in glaucoma, shows the utility of PhNR in clinical ophthalmology.

TABLE 2. Sensitivity and Specificity of PhNR in the SOA-BR LHON Pedigree

	Sensitivity	Specificity	PPV
Affected vs. control	1	1	1
Affected vs. carrier	1	0.65	0.5
Carrier vs. control	0.35	1	1

We found that the amplitude of the PhNR was correlated with the OCT RNFL thickness (Fig. 1). We note, despite this being a significant correlation (p = 0.003), the r^2 value of 0.355 is not high. This is in keeping with other studies that have looked at patients with glaucoma and optic nerve gliomas.[14,26] Further supporting the notion that there is a component of optic nerve dysfunction in the residual optic nerve fibers that can differ between patients.

The PhNR is an objective and patient-independent test that provides a significant advantage over visual field perimetry, which necessitates active patient collaboration. Therefore, clinical use of the PhNR may be valuable in young children, cognitively impaired adults, and in patients with visual field abnormalities that are affected by RGC and non-RGC lesions of the visual pathway. In patients with severe central vision loss, the test also has the benefit of not requiring fixation, which makes it easier for patients when compared to PERG.[27]

There are several noteworthy limitations to the present study. The small sample size of affected LHON patients makes it difficult to draw conclusions to the overall LHON population. As well, affected LHON patients were all severely affected and we could not subdivide the group by disease severity. This limited the ability to correlate PhNR function with HVF MD or OCT RNFL thickness. Lastly, the PhNR was only evaluated at a

single time point. Therefore, future studies may evaluate larger samples of patients and PhNR measurements taken during regular scheduled clinical follow-up. Long-term PhNR data may facilitate the study of the effectiveness of the PhNR as a potential predictor of clinical outcome in affected patients as well as a diagnostic and possibly predictive tool as carrier patients become affected. Furthermore, it will be of interest to test prospectively if PhNR may have a predictive value for those LHON patients prone to recover visual function spontaneously, or after treatment. This study still represents the largest data set of PhNR data in such a rare disease.

This study demonstrates that the PhNR can be an objective metric that directly reflects RGC physiology in patients with LHON. This is an important finding as we embark on various treatment strategies for the LHON. There is a paucity of objective outcome metrics that consider the function of the eye independent of anterior and posterior visual pathways. Ultimately, this limits the ability to accurately identify subjects at risk of conversion, and monitor, particularly early improvements, patients with LHON. In conclusion, the present findings demonstrated that the PhNR was significantly reduced in affected patients with LHON compared to carriers and healthy controls, but also carriers may show some degrees of impairment. Moving forward, the PhNR may be used as an adjunctive objective test for LHON, and further investigation is warranted to evaluate its clinical utility.

Acknowledgments

Supported in part by an unrestricted research grant from Stealth BioTherapeutics, and grant funding from the International Foundation for Optic Nerve Disease (IFOND).

Disclosure: **R. Karanjia**, Stealth BioTherapeutics (R), Genesight Biologics (R), Edison Pharmaceuticals (R); **A. Berezovsky**, None; **P.Y. Sacai**, None; **N.N. Cavascan**, None; **H.Y. Liu**, None; **S. Nazarali**, None; **M.N. Moraes-Filho**, None; **K. Anderson**, None; **J.S. Tran**, None; **S.E. Watanabe**, None; **M.N. Moraes**, None; **F. Sadun**, None; **A.M. DeNegri**, None; **P. Barboni**, Santhera Pharmaceuticals (R); **C. do Val Ferreira Ramos**, None; **C. La Morgia**, Santhera Pharmaceuticals (R), Genesight Biologics (R); **V. Carelli**, Santhera Pharmaceuticals (R), Genesight Biologics (R); **R. Belfort Jr**, None; **S.G. Coupland**, Diagnosys (C); **S.R. Salomao**, **A.A. Sadun**, Stealth BioTherapeutics (R), Genesight Biologics (R), Edison Pharmaceuticals (R)

References

1. Yu-Wai-Man P, Griffiths PG, Brown DT, Howell N, Turnbull DM, Chinnery PF. The epidemiology of Leber hereditary optic neuropathy in the North East of England. *Am J Hum Genet*. 2003;72:333–339.
2. Meyerson C, Van Stavern G, McClelland C. Leber hereditary optic neuropathy: current perspectives. *Clin Ophthalmol*. 2015;9:1165–1176.
3. Sadun AA, Win PH, Ross-Cisneros FN, Walker SO, Carelli V. Leber's hereditary optic neuropathy differentially affects smaller axons in the optic nerve. *Trans Am Ophthalmol Soc*. 2000;98:223–232; discussion 232–225.
4. Balducci N, Savini G, Cascavilla ML, et al. Macular nerve fibre and ganglion cell layer changes in acute Leber's hereditary optic neuropathy. *Br J Ophthalmol*. 2016;100:1232–1237.
5. Carelli V, d'Adamo P, Valentino ML, et al. Parsing the differences in affected with LHON: genetic versus environmental triggers of disease conversion. *Brain*. 2016;139:e17.
6. Hwang TJ, Karanjia R, Moraes-Filho MN, et al. Natural history of conversion of Leber's hereditary optic neuropathy: a prospective case series. *Ophthalmology*. 2017;124:843–850.
7. Machida S. Clinical applications of the photopic negative response to optic nerve and retinal diseases. *J Ophthalmol*. 2012;2012:397178.
8. Ziccardi L, Sadun F, De Negri AM, et al. Retinal function and neural conduction along the visual pathways in affected and unaffected carriers with Leber's hereditary optic neuropathy. *Invest Ophthalmol Vis Sci*. 2013;54:6893–6901.
9. Sacai PY, Salomao SR, Carelli V, et al. Visual evoked potentials findings in non-affected subjects from a large Brazilian pedigree of 11778 Leber's hereditary optic neuropathy. *Doc Ophthalmol*. 2010;121:147–154.
10. Guy J, Feuer WJ, Porciatti V, et al. Retinal ganglion cell dysfunction in asymptomatic G11778A: Leber hereditary optic neuropathy. *Invest Ophthalmol Vis Sci*. 2014;55:841–848.
11. Viswanathan S, Frishman LJ, Robson JG, Harwerth RS, Smith EL III. The photopic negative response of the macaque electroretinogram: reduction by experimental glaucoma. *Invest Ophthalmol Vis Sci*. 1999;40:1124–1136.
12. Tamada K, Machida S, Yokoyama D, Kurosaka D. Photopic negative response of full-field and focal macular electroretinograms in patients with optic nerve atrophy. *Jpn J Ophthalmol*. 2009;53:608–614.
13. Machida S, Gotoh Y, Tanaka M, Tazawa Y. Predominant loss of the photopic negative response in central retinal artery occlusion. *Am J Ophthalmol*. 2004;137:938–940.
14. Kim HD, Park JY, Ohn YH. Clinical applications of photopic negative response (PhNR) for the treatment of glaucoma and diabetic retinopathy. *Korean J Ophthalmol*. 2010;24:89–95.
15. 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;41:2205–2211.
16. 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;42:514–522.
17. Moss HE, Park JC, McAnany JJ. The photopic negative response in idiopathic intracranial hypertension. *Invest Ophthalmol Vis Sci*. 2015;56:3709–3714.
18. Sadun AA, Carelli V, Salomao SR, et al. A very large Brazilian pedigree with 11778 Leber's hereditary optic neuropathy. *Trans Am Ophthalmol Soc*. 2002;100:169–178; discussion 178–169.
19. Giordano C, Iommarini L, Giordano L, et al. Efficient mitochondrial biogenesis drives incomplete penetrance in Leber's hereditary optic neuropathy. *Brain*. 2014;137:335–353.
20. Chen H, Wu D, Huang S, Yan H. The photopic negative response of the flash electroretinogram in retinal vein occlusion. *Doc Ophthalmol*. 2006;113:53–59.
21. Nakamura H, Miyamoto K, Yokota S, Ogino K, Yoshimura N. Focal macular photopic negative response in patients with optic neuritis. *Eye*. 2011;25:358–364.
22. Gotoh Y, Machida S, Tazawa Y. Selective loss of the photopic negative response in patients with optic nerve atrophy. *Arch Ophthalmol*. 2004;122:341–346.
23. Shen X, Huang L, Fan N, He J. Relationship among photopic negative response, retinal nerve fiber layer thickness, and visual field between normal and POAG Eyes. *ISRN Ophthalmol*. 2013;2013:182021.
24. Kaneko M, Machida S, Hoshi Y, Kurosaka D. Alterations of photopic negative response of multifocal electroretinogram in patients with glaucoma. *Curr Eye Res*. 2015;40:77–86.
25. Wilsey L, Gowrisankaran S, Cull G, Hardin C, Burgoyne CF, Fortune B. Comparing three different modes of electroreti-

nography in experimental glaucoma: diagnostic performance and correlation to structure. *Doc Ophthalmol*. 2017;134:111–128.

26. Abed E, Piccardi M, Rizzo D, et al. Functional loss of the inner retina in childhood optic gliomas detected by photopic negative response. *Invest Ophthalmol Vis Sci*. 2015;56:2469–2474.
27. Preiser D, Lagreze WA, Bach M, Poloschek CM. Photopic negative response versus pattern electroretinogram in early glaucoma. *Invest Ophthalmol Vis Sci*. 2013;54:1182–1191.

Investigative Ophthalmology & Visual Science