The Photopic Negative Response of the Flash Electroretinogram in Multiple Sclerosis

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PURPOSE. To use the photopic electroretinogram (ERG) to evaluate retinal function in eyes of multiple sclerosis (MS) patients with and without a history of optic neuritis (ON) and to compare the functional and structural status of the inner retina.

METHODS. Full-field ERG responses to brief red flashes ($0.04\text{--}2.8\text{ cd} \cdot \text{s/m}^2$) on a rod-saturating blue background were recorded from 51 MS patients and 33 age-matched control subjects. In patients, perimetry was performed and peripapillary retinal nerve fiber layer thickness (RNFLT) was assessed by optical coherence tomography (OCT) and scanning laser polarimetry (SLP). MS eyes were separated into groups: "ON >6" months ($n = 25$), "ON <6" months ($n = 29$), and "no ON" ($n = 33$) based on positive or negative history of ON and time since the last episode. Thirteen ON<6 eyes were re-evaluated 1 year later.

RESULTS. PhNR amplitudes were lower in ON>6, ON<6, and no-ON eyes (mean $\pm$ SD, 17.3 ± 7.6 , 16.0 ± 6.5 , and $23.8 \pm 9.3\text{ }\mu\text{V}$, respectively), than in control eyes ($29.8 \pm 6.5\text{ }\mu\text{V}$; $P < 0.001$) for a standard stimulus of $1.42\text{ cd} \cdot \text{s/m}^2$; a- and b-wave amplitudes were unaffected. PhNR amplitudes correlated with visual fields mean deviation (MD) in ON>6 ($r^2 = 0.43$; $P < 0.001$) and no-ON eyes ($r^2 = 0.10$; $P < 0.05$), with similar results for weaker stimuli. PhNR amplitudes correlated with RNFLT in ON>6 eyes: OCT ($r^2 = 0.52$; $P < 0.0001$) and SLP ($r^2 = 0.51$; $P < 0.01$); and in no-ON eyes, OCT ($r^2 = 0.21$; $P < 0.01$) and SLP ($r^2 = 0.17$; $P < 0.05$). ON<6 amplitudes did not correlate significantly with other measures, but increased after 1 year by $5.1 \pm 3.1\text{ }\mu\text{V}$ ($P < 0.001$), visual fields MD increased by $1.8 \pm 2.3\text{ dB}$ ($P < 0.05$), and RNFLT loss persisted.

CONCLUSIONS. Photopic ERG PhNR amplitudes in MS patients are significantly reduced in eyes with and without a history of ON. (*Invest Ophthalmol Vis Sci.* 2012;53:1315–1323) DOI: 10.1167/iops.11-8461

Multiple sclerosis (MS) is a chronic autoimmune inflammatory disease of the central nervous system, characterized by demyelination, axonal dysfunction, and ultimately neuronal degeneration.¹ The afferent visual pathway, which extends from the retina to the primary visual cortex, is often affected. 30% to 70% of MS patients will have optic neuritis (ON) during the course of the disease, and visual problems are often the earliest symptoms of the disease.² Asymptomatic or subclinical involvement of optic nerves occurs in MS as well.^{3–5} The

retinal nerve fiber layer, which contains unmyelinated axons of retinal ganglion cells and glial cells, can be imaged readily and noninvasively, making it a useful structure for assessing pathologic damage due to MS on neurons and their axons.

Common tests to evaluate visual pathway abnormalities include a subjective test of visual sensitivity, often a Humphrey visual field test (HVF; Carl Zeiss Meditec, Dublin, CA), noninvasive structural evaluations of the retina using optical coherence tomography (OCT; Carl Zeiss Meditec) and scanning laser polarimetry (SLP; GDx; Carl Zeiss Meditec) and objective evaluation of function using visual evoked potentials (VEPs). In previous studies,^{1,6} MS eyes with a history of ON showed loss of both visual field sensitivity and retinal nerve fiber layer (RNFL). RNFL loss is complete in 3 to 6 months after an acute episode of ON in the most common type of MS, relapsing-remitting (RR)MS, whereas functional losses appear earlier and tend to recover over time, despite persistent RNFL loss.^{7–9} The discrepancy between structural and functional changes in the course of optic neuritis highlights the need for assessment of both aspects to better understand the pathophysiology of the disease. Most reports indicate that the RNFL thickness remains relatively constant once loss is established, until another ON attack,^{10,11} although losses between 6 and 12 months after an episode have been reported,⁷ and a gentle steady decline has been documented in a large multicenter sample of patients studied over several years.¹² After an attack of ON, the visual field sensitivity (HVF) and VEP amplitude and latency continue to recover past the time that nerve fiber layer loss is permanent.¹ The recovery of VEP latency is likely to occur as optic nerve or tract experience remyelination. However, recovery of function by remaining ganglion cells and their axons could be contributing, as well, to recovery seen in visual sensitivity and VEP amplitude.

VEP and HVF do not discriminate retinal from more central effects of the disease. To assess retinal function in MS patients, the electroretinogram (ERG) can be used. The full-field flash ERG is a noninvasive test in which massed electrical potentials from the entire retina are recorded, and signals from different layers of the retina can be evaluated. The flash ERG is widely used as a diagnostic tool for diseases affecting the photoreceptors and bipolar cells, whose signals are reflected by a- and b-wave, respectively. However, another component of photopic flash ERG, the photopic negative response (PhNR), informs about normal or reduced activity of retinal ganglion cells and their axons in humans and nonhuman primates,^{13–15} as well as about patency of K^+ currents in retinal glial cells in primates and rodents.^{16,17} The PhNR amplitude is reduced in macaque monkeys after pharmacologic blockade of inner retinal activity,¹³ as well as in macaque eyes with induced experimental glaucoma and patients with glaucoma^{14,15} or other optic neuropathies.¹⁸

Previous studies have reported reduced amplitude of the pattern ERG (PERG) in MS patients, particularly the N95 of the transient PERG.^{19,20} This signal is known to originate from retinal ganglion cells and their axons, and it has been useful for

From the College of Optometry, University of Houston, Houston, Texas.

Supported by National Eye Institute Grant EY007551 (LJF).

Submitted for publication August 22, 2011; revised December 30, 2011; accepted January 14, 2012.

Disclosure: J. Wang, None; H. Cheng, None; Y.-S. Hu, None; R.A. Tang, None; L.J. Frishman, None

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assessing their function in eyes with diseases that affect the optic nerve.²¹ However, studies in macaque have shown that the N95 of the PERG and the PhNR of the full-field flash ERG share common generators.²² The flash ERG has the advantage that it provides the opportunity to simultaneously test all layers of retinal processing, including the photoreceptors and bipolar cells whose signals are passed to the ganglion cells. PhNR amplitude correlates well with loss of retinal ganglion cell axons, as measured by imaging the RNFL in eyes with open-angle glaucoma.²³ Therefore, the PhNR could provide a useful functional biomarker of retinal ganglion cell and axon function after an episode of ON in MS patients.

The purpose of this study was to investigate the changes in retinal function and structure associated with ON in MS patients. We found that the PhNR amplitude was significantly reduced in eyes of patients with a history of ON, and interestingly, even in MS eyes that were never diagnosed with ON. Some of the findings in this study have been reported in abstract form (Wang J, et al. *IOVS* 2010;52:E-Abstract 5795).

METHODS

All procedures were reviewed and approved by the University of Houston Committee for the Protection of Human Subjects and complied with the Declaration of Helsinki. Informed consent was obtained from the subjects after explanation of the nature and possible consequences of the study.

Subjects

The subjects in this study included 51 patients (18–60 years of age) with MS of the relapsing–remitting type, with or without a history of ON, and 33 age-matched normal control subjects with normal corrected visual acuity (20/20) and good ocular health. The diagnoses of MS and ON were made by experienced neurologists and neuro-ophthalmologists, based on clinical criteria.^{24,25} Patients with diagnosed diseases other than MS that affect the central nervous system and/or cause retinal disease were excluded from the study. Eyes also were excluded if they had no light perception, or they were in the peak period of an acute attack of ON (<1 month after onset). Most of the patients had been treated with IV steroids to control symptoms of acute ON and were receiving immunomodulatory medication, such as interferon β -1a, to slow the course of the MS. All patients received a complete neuro-ophthalmic evaluation that included best corrected visual acuity, color vision, pupil assessment, intraocular pressure, dilated ophthalmoscopy, standard automated perimetry (HVF 30-2), peripapillary RNFL thickness measurement with OCT and SLP, and photopic flash ERG. For analysis, right eyes only were included if both eyes of one subject were assigned to the same group. Eyes of the 51 MS patients were classified into three groups: 33 eyes without a history of ON (No-ON group; five left eyes excluded), 29 eyes with an episode of ON less than 6 months and more than 1 month before ERG testing (ON<6; three left eyes excluded), and 25 eyes with an episode of ON more than 6 months before ERG testing (ON>6; seven left eyes excluded). Thirteen MS patients from the first visit (13 ON<6 eyes [right eye only was included for one subject who had ON<6 in both eyes], seven no-ON eyes, and five ON>6 eyes) who had at least one eye with ON<6 at that visit and had not experienced another episode of ON, were retested 1 year later. Patient demographics and clinical characteristics are summarized in Table 1.

ERG Recordings

Photopic full-field flash ERGs were recorded differentially between the two eyes using DTL fiber electrodes moistened with carboxymethyl cellulose 1% and placed underneath the lower eyelid. Pupils were fully dilated with tropicamide 1% and phenylephrine 2.5%. Recordings were made using stimulation of one eye at a time, during which the other eye was occluded. The stimuli were brief (<5 ms) red flashes (λ_{max} =

TABLE 1. Demographic and Clinical Characteristics

	MS	Control
Subjects at first visit, <i>n</i>	51	33
Subjects at second visit, <i>n</i>	13	—
Male:female ratio	1:5	1:3
Mean age $\pm$ SD, y	41 $\pm$ 10	39 $\pm$ 12
Duration, y, median (range)	3 (1–14)	—
Episode of ON, median (range)	1 (0–3)	—
MS patient groups: visual acuity, median (range)		
ON>6 eyes, <i>n</i> = 25	20/20 (20/20–CF)	
ON<6 eyes, <i>n</i> = 29	20/20 (20/20–CF)	
No-ON eyes, <i>n</i> = 33	20/20 (20/15–20/50)	

660 nm, Espion ColorDome; Diagnosys LLC, Lowell, MA) ranging from 0.04 to 2.84 $\text{cd} \cdot \text{s}/\text{m}^2$ presented on a rod-saturating blue (λ_{max} = 460 nm, 100 scotopic cd/m^2) background. This color combination provides a good stimulus for eliciting the PhNR.^{26,27} Responses to each flash strength were averaged over 20 trials. PhNR amplitude was measured from baseline at 65 ms after the flash. This time for measuring the PhNR was established by comparing response amplitudes between 60 and 80 ms, as described in the results. The a-wave amplitude was measured from baseline to trough of the first negative wave and its latency from flash onset to the trough. The b-wave amplitude was measured from a-wave trough to b-wave peak, and b-wave latency was measured from the time of the stimulus flash to the peak of the b-wave.

Standard Automated Perimetry

A perimeter (Humphrey field analyzer model 750; Carl Zeiss Meditec, Inc.) with a SITA (Swedish interactive threshold algorithm) 30-2 protocol was used, with a Goldman size III (0.43°) stimulus on a 31.5-apostilb background. The mean deviation (MD) on a logarithmic (decibel) scale, the global metric that is used for HVF analysis, is defined as the mean of the difference of the measured sensitivity (in decibels) and normal values of age-matched controls in the built-in database. Another parameter that we used was a linear measure of visual sensitivity, expressed as the average unlogged visual sensitivity in 1/Lambert (1/L), which was calculated by dividing the original sensitivity in dB by 10 for each point, taking its antilog, and finally, averaging the unlogged values over the whole field.

Peripapillary RNFL Thickness Assessed by OCT and SLP

OCT (Stratus model 3000, software version 4.0.1; Carl Zeiss Meditec, Inc.) was used to acquire three standard 3.4-mm diameter circular scans centered on the optic disc (fast RNFL protocol). The overall RNFL thickness along the circumference was automatically calculated by the OCT software and compared with a built-in normative database of age-matched control subjects. The integrated area of the RNFL was calculated by multiplying the mean RNFL thickness by the circular scan circumference,²⁸ and the circumference was adjusted on the basis of the axial length and cornea effective power.²⁹ For 33 patients, macular thickness was also measured using the fast macular thickness protocol, to obtain six cross-sectional B-scans, 6 mm in length, at equally spaced angular orientations (30°) in a radial spoke pattern centered on the fovea. (For a small group of patients [*n* = 20] late in the study, Cirrus OCT [Carl Zeiss Meditec] was used as well, and results showed similar trends. Because of the small number of subjects, these data are not reported here.) Peripapillary RNFL thickness was also assessed using the scanning laser polarimeter (GDx-VCC; Carl Zeiss Meditec). The average temporal, superior, nasal, and inferior (TSNT) thickness was analyzed automatically by the polarimeter software.

Data Analysis

The PhNR stimulus-response relation from the smallest response to saturation was fit with the generalized Naka-Rushton function^{30,31}:

$$V = (V_{\max} * I^n) / (I^n + I_0^n) \quad (1)$$

where V is response amplitude, $V_{\max}$ is the maximum amplitude, I is stimulus strength, I_0 is the level of I that produces a response amplitude of one half $V_{\max}$, and n denotes the slope of the function where I is equal to I_0 . Curve fitting was performed using the Marquardt-Levenberg algorithm (SigmaPlot 10; Systat Software, Inc.), to find reasonable parameter values that minimized an equally weighted sum of squared differences between the data and the equation.

The procedure MIXED (SAS 9.2; SAS Institute, Inc., Cary, NC) was performed to determine whether ERG parameters (PhNR amplitude, a- and b-wave amplitude and latency) were significantly different across normal control, No-ON, ON<6, and ON>6 groups. Analysis with mixed model statistics (procedure MIXED) takes into account the possible correlations among groups because the two eyes of some subjects were in different groups. Procedure MIXED was used for responses to the standard stimulus strength ($1.42 \text{ cd} \cdot \text{s/m}^2$) and for repeated measurements for the seven flashes used to generate stimulus response functions. For the longitudinal comparisons for a single group, repeated-measures (two visits, seven flashes) ANOVA was used to compare PhNR stimulus response functions between the two visits. For the $1.42 \text{ cd} \cdot \text{s/m}^2$ flash, paired t -tests were performed to compare PhNR amplitude at the second visit to that at the first visit. Linear regression analysis was used to calculate Pearson's correlation coefficient (r ; r^2 reported in this article) to indicate the direction and strength of any linear association between PhNR amplitude and other functional/structural measurements (HVF MD on a logarithmic scale, for which an exponential curve also was fit, visual sensitivity on a linear scale, RNFL thickness measured by OCT and SLP).

RESULTS

Reduced PhNR of Photopic ERG in MS Patients' Eyes

The typical full-field brief flash ERG recorded from a normal control subject (Fig. 1, left) is composed of three main waves: an initial negative-going a-wave, a positive-going b-wave, and a slow negative-going PhNR after b-wave. The amplitudes of the three waves increased as stimulus strength was increased. Figure 1 (middle and right) also shows the ERG responses

FIGURE 1. Photopic full-field flash ERG responses recorded from a 43 year old RRMS patient with LE ON>6 (middle) and RE no-ON (right) and an age matched control subject (left), with stimulus strength increasing from bottom to top. Arrow: PhNR.

FIGURE 2. Stimulus response functions of PhNR amplitude in (A) control eyes ($n = 33$) and (B) MS eyes ($n = 54$) with a history of ON, regardless of whether it was more than or less than 6 months after the last episode. Measured from baseline at fixed times after the stimulus flash of 60, 65, 70, 75, and 80 ms.

recorded from a 43-year-old RRMS patient whose left eye had experienced one episode of optic neuritis 6 years earlier. The right eye had no history of ON. Compared with the control subject, both eyes of the patient maintained similar timing and amplitude for the a- and b-waves, but the PhNR amplitudes were markedly lower in the ON eye (LE) and slightly lower in the no-ON eye (RE). The patient showed reduced visual sensitivity (HVF) in the ON eye (MD, -4.91 dB), and in the no-ON eye as well (MD -3.66 dB). The ON eye showed peripapillary RNFL thinning in both OCT ($83.1 \mu\text{m}$) and SLP tests ($44.1 \mu\text{m}$), whereas the no-ON eye showed more normal peripapillary RNFL thickness of 99.4 and $47.7 \mu\text{m}$, respectively.

PhNR amplitude was measured from the baseline to the PhNR trough. In both control subjects and MS patients, the largest PhNR amplitudes on average occurred at 65 and 70 ms after the flash (Fig. 2). Given this result, we elected to measure PhNR amplitude at 65 ms, a time also used in a previous study from this laboratory.²⁶ In Figure 3A, the PhNR amplitude is plotted as a function of the flash strength in the control, no-ON, ON>6, and ON<6 eyes. PhNR amplitude increased as flash strength increased and was at its maximum at the flash strength of $1.42 \text{ cd} \cdot \text{s/m}^2$ for all groups. These stimulus response relations (for responses to all seven flashes tested) were

FIGURE 3. Plots of PhNR amplitude measured at 65 ms after the stimulus flash in control subjects and MS patients. (A) PhNR amplitude is plotted as a function of stimulus strength with the Naka-Rushton fit in control subjects, MS no-ON, MS ON>6, and MS ON<6 eyes. Error bars, $\pm 1 \text{ SE}$. (B) Plots of PhNR amplitude in response to the $1.42 \text{ cd} \cdot \text{s/m}^2$ flash in control, no-ON, ON>6, and ON<6 eyes. The boundaries of each box indicate the 25th and 75th percentiles. The solid and dashed lines within the box mark the median and mean, respectively. Error bars below and above the box indicated the 10th and 90th percentiles, respectively. Filled circles: 5th and 95th percentiles.

TABLE 2. Photopic ERG Parameters in Control Subjects and MS Patients

	Control	No-ON	ON>6	ON<6	F	P
a-wave amplitude, μV	22.2 $\pm$ 7.6	22.8 $\pm$ 7.9	23.3 $\pm$ 8.1	20.2 $\pm$ 5.9	1.03	>0.05
a-wave implicit time, ms	14.4 $\pm$ 1.0	15.1 $\pm$ 1.3	14.7 $\pm$ 0.9	15.1 $\pm$ 1.3	2.86	0.04
b-wave amplitude, μV	73.5 $\pm$ 23.6	79.5 $\pm$ 25.2	81.8 $\pm$ 25.9	71.5 $\pm$ 25.3	1.15	>0.05
b-wave implicit time, ms	31.5 $\pm$ 1.2	32.1 $\pm$ 2.2	32.4 $\pm$ 2.1	32.2 $\pm$ 2.7	0.49	>0.05

Data are presented as the mean $\pm$ SD for responses to a standard stimulus of 1.42 cd $\cdot$ s/m².

significantly different ($F = 33.43$; $P < 0.0001$) across the four groups. Compared to the control subjects, amplitudes were lower in the ON>6 ($P < 0.0001$), ON<6 ($P < 0.0001$), and no-ON ($P < 0.0001$) eyes. The ON>6 eyes were not significantly different from the ON<6 eyes ($P > 0.05$), but the no-ON eyes had larger PhNR amplitudes than the ON>6 ($P < 0.001$) and ON<6 ($P < 0.0001$) eyes. Figure 3B shows plots of the PhNR amplitude in response to a flash strength of 1.42 cd $\cdot$ s/m² in control, no-ON, ON>6, and ON<6 eyes. Responses to this stimulus will be used for comparisons with structural measures below. The differences between groups were similar to those for the whole stimulus response curves. Compared to control eyes (29.8 $\pm$ 6.5 μV [mean $\pm$ SD]), PhNR amplitude was significantly reduced in the no-ON (23.8 $\pm$ 9.3 μV ; $P < 0.001$), ON>6 (17.3 $\pm$ 7.6 μV ; $P < 0.001$), and ON<6 (16.0 $\pm$ 6.5 μV ; $P < 0.0001$) eyes. The no-ON eyes had larger PhNR amplitudes than the ON>6 ($P < 0.001$) and ON<6 ($P < 0.001$) eyes. PhNR amplitudes for the ON>6 versus the ON<6 eyes were not significantly different ($P > 0.05$).

As shown in Table 2, the amplitudes of a- and b-waves for the flash strength of 1.42 cd $\cdot$ s/m² were not significantly different across the control, no-ON, ON>6, and ON<6 eyes. The time from stimulus onset to the peak of the b-wave (b-wave latency) also was not significantly different across groups ($P > 0.05$). The time from stimulus onset to the trough of the a-wave (a-wave latency) was slightly longer (<1 ms) in the MS eyes than in the control eyes ($F = 2.86$, $P = 0.04$).

Correlation between PhNR Amplitude and Visual Sensitivity Measured by HVF

To compare the PhNR measured in MS patients to HVF sensitivity measures, we analyzed the data to determine whether sensitivity of the PhNR was changing. The PhNR stimulus-response relations, up to saturation, illustrated in Figure 3 were fit with the generalized Naka-Rushton function.^{30,31} Inspection of parameters of the fits in Table 3, show that saturated amplitude (V_{max}) differed among groups, consistent with the statistical analysis reported above. However, the other parameters were relatively stable across groups. The exponent, n , was fairly close to 1 in all cases, indicating a roughly linear rise of the response amplitude with increasing flash strength before the response saturated as previously reported for the PhNR.³¹ A measure of sensitivity, I_0 , (stimulus strength necessary for a response of one-half V_{max}), was also similar across groups, and

was not significantly correlated with either decibels or sensitivity (1/L) in any of the MS patient groups.

PhNR amplitude at 20% of V_{max} of the stimulus response function, based on the Naka-Rushton fits, was calculated for each subject. This calculation was made so that a small PhNR in the linear portion of the stimulus response function could be compared with HVF measures, MD, and visual sensitivity (1/L). The results are shown in Figure 4 and summarized in Table 4 for this and subsequent analyses. For comparison, results based on maximum amplitude responses to the standard stimulus of 1.42 cd $\cdot$ s/m² are shown in Figure 5. In the ON>6 eyes, the 20% V_{max} PhNR, and PhNR amplitude for the standard stimulus were correlated similarly with HVF MD exponentially ($r^2 = 0.45$, $P < 0.05$; $r^2 = 0.43$, $P < 0.001$, respectively) and linearly with visual sensitivity expressed as 1/L ($r^2 = 0.47$, $P < 0.05$; $r^2 = 0.46$, $P < 0.001$). In the no-ON eyes, weaker correlations, again similar for the two PhNR amplitude measures, were observed with HVF MD ($r^2 = 0.10$, $P < 0.05$; $r^2 = 0.10$, $P < 0.05$) and with visual sensitivity ($r^2 = 0.20$, $P < 0.05$; $r^2 = 0.26$, $P < 0.05$). No significant correlation was observed in the ON<6 eyes between PhNR amplitudes and HVF MD or visual sensitivity (1/L).

Figures 4A and 5A both show an exponential fit for the ON>6 eyes to the relation between PhNR amplitude and HVF MD. The PhNR amplitudes in both plots approached a minimum value when HVF MD was between -10 and -15 dB, but HVF still tracked functional losses beyond -15 dB.³² Because the number of eyes having large visual sensitivity losses was small, PhNR amplitudes, in both figures correlated linearly with HVF MD (in decibels) almost as well (20% V_{max} vs. VF MD: $r^2 = 0.40$, $P < 0.001$; PhNR vs. VF MD $r^2 = 0.38$, $P < 0.001$). For ON<6 eyes, HVF measures were generally more reduced in eyes with an episode 2 to 3 months earlier (Figs. 4, 5, open symbols), than 4 to 6 months (Figs. 4, 5, filled symbols) earlier.

Correlations between PhNR Amplitude and RNFL Thickness

PhNR amplitude in response to the standard 1.42-cd $\cdot$ s/m² flash is plotted against average peripapillary RNFL thickness, measured by OCT, in Figures 5C and 5G. The amplitude was correlated significantly with RNFL thickness ($r^2 = 0.52$, $P < 0.0001$) and similarly with integrated area of RNFL ($r^2 = 0.51$, $P < 0.001$, plot not illustrated) in the ON>6 eyes. In the no-ON eyes, PhNR amplitude correlated weakly with RNFL thickness

TABLE 3. Parameters of Naka-Rushton Function Fits to the PhNR Amplitude Data

	Control	MS no-ON	MS ON>6	MS ON<6	Re-evaluated ON<6*
V_{max} , μV	33.85 (2.27)	25.41 (1.90)	19.75 (2.31)	18.35 (3.58)	20.18 (1.58)
I_0 , cd $\cdot$ s/m ²	0.24 (0.03)	0.21 (0.04)	0.19 (0.06)	0.25 (0.13)	0.19 (0.4)
n	1.19 (0.15)	1.24 (0.22)	1.00 (0.25)	0.92 (0.28)	1.11 (0.28)
r^2	0.96 (0.01)	0.95 (0.01)	0.92 (0.02)	0.91 (0.02)	0.92 (0.02)

Data are presented as mean (standard error).

* Naka-Rushton fit to PhNR stimulus response functions of ON<6 eyes at the 1-year re-evaluation.

FIGURE 4. Relation between 20% $V_{\max}$ PhNR amplitude obtained from Naka-Rushton fits and HVF (A, C) MD and (B, D) visual sensitivity in MS ON>6 and no-ON eyes (A, B) and in MS ON<6 eyes (C, D). Black solid lines are fitted to the ON>6 data and green solid lines to the no-ON data. The gray reference lines represent the mean (solid lines) of 20% $V_{\max}$ and two standard deviations away from the mean (dashed lines) in control subjects. The r^2 values in black and green indicate the coefficient of determination in the ON>6 and no-ON eyes, respectively.

($r^2 = 0.21$, $P < 0.01$) and with RNFL area ($r^2 = 0.26$, $P < 0.01$), and the data could be fit with a line just slightly shallower than the one for the ON>6 eyes. However, for the ON<6 eyes, no significant correlation was observed. For 86% (25/29) of the ON<6 eyes, the ON attack was the first one for that eye, and thinning of the RNFL may have been incomplete at the time of ERG recording. In a couple of cases, in which RNFL was already thin, a previous episode or episodes of ON had occurred. In the case of three patients who had ON episodes within 2 to 3 months of testing, the RNFL was very thick, exceeding the range reported for normal subjects in previous studies,³³ marked for comparison on the top of the graph.

The PhNR includes a contribution from the macula where ganglion cell density is greatest.³⁴ Macular scans (OCT) were also performed in 33 of the MS patients, and the results supported a macular contribution to the PhNR, even without segmentation to isolate the ganglion cell and innerplexiform layers. PhNR amplitude correlated significantly with the macular volume in the ON>6 eyes ($n = 29$, $r^2 = 0.35$, $P < 0.01$) but not in the ON<6 eyes ($n = 16$). The PhNR amplitude in the

no-ON eyes ($n = 21$) followed the trend in the ON>6 eyes, although the correlation was not significant (data not shown).

In an effort to avoid the effect of swelling on measurements of RNFL thickness in the ON<6 eyes, we also used SLP TSNIT to quantify thickness. SLP measures the birefringence of retinal ganglion axons in the RNFL. Microtubules in the axons make a significant contribution to the birefringence, with additional contributions from neurofilaments and axonal membranes.³⁵ SLP TSNIT values should not increase in patients with disc edema, despite increased RNFL thickness measured by OCT, because axonal swelling is not thought to affect the birefringent elements.^{36,37} As shown in Figure 5D, a significant correlation ($r^2 = 0.51$, $P < 0.01$) was observed between PhNR amplitude and average RNFL thickness for the ON>6 eyes. The results were similar to those for OCT, and in the ON<6 eyes, again there was no significant correlation, even though the three eyes with very thick RNFL in the OCT plot (Fig. 5G) were no longer obvious outliers in the SLP TSNIT plot (Fig. 5H).³⁸ RNFL thicknesses were 62, 51.3, and 59.7 μm , in eyes with 155, 208, 239 μm for OCT, respectively. These data suggest that swelling alone cannot explain the lack of significant correlation between structure and function in the ON<6 eyes. In the no-ON eyes, a weak correlation with SLP TSNIT ($r^2 = 0.17$, $P < 0.05$) was observed, and again, the line fit to the no-ON data was similar to the line fit to the ON>6 data.

Longitudinal Change in Function and Structure of the Inner Retina in ON <6 Eyes

Thirteen patients (13 eyes) in the ON<6 group were re-evaluated 1 year after the first session. The PhNR stimulus response functions were significantly different ($F = 10.9$, $P < 0.01$) between the two visits. PhNR amplitudes for the standard stimulus from the 13 eyes at the first visit are plotted against amplitudes at the second visit in Figure 6. In 92% of the eyes, PhNR amplitude in response to the standard stimulus was larger at the second visit, with an increase of $5.1 \pm 3.1 \mu\text{V}$ (mean $\pm$ SD; $P < 0.001$, paired t -test) compared with the first visit ($14.7 \pm 5.3 \mu\text{V}$; Fig. 6A), which was an increase by 17% of the average PhNR amplitude in the normal control subjects. These significant changes were not large, but were all in one direction, rather than both directions as would occur for normal variability from test to test. To assess the variability to be expected from repeated ERG testing, we examined test-retest repeatability for the PhNR in 15 control subjects and 20 MS patients with ON. Both recordings for a given subject were performed in the same session, 30 minutes apart, and data from the two eyes were included for all subjects. The 90% limit of agreement was $4.6 \mu\text{V}$, as determined by 1.64 times the standard deviation of the intrasession amplitude difference. The 95% limit of agreement was $5.5 \mu\text{V}$ (1.96 SD).

HVF MD also showed mild improvement in 85% of the eyes and by $1.8 \pm 2.3 \text{ dB}$ ($P < 0.05$) on average in all eyes (Fig. 6B).

TABLE 4. Correlation between PhNR Amplitude and Other Measures

	no-ON		ON>6		ON<6		Re-evaluated ON<6	
	r^2	P	r^2	P	r^2	P	r^2	P
PhNR vs. VF MD	0.10	<0.05	0.43	<0.001	0.008	>0.05	0.48	<0.05
20% $V_{\max}$ vs. VF MD	0.10	<0.05	0.45	<0.05	0.007	>0.05	0.45	<0.05
PhNR vs. VF VS	0.26	<0.05	0.46	<0.001	0.009	>0.05	0.31	<0.05
20% $V_{\max}$ vs. VF VS	0.20	<0.05	0.47	<0.05	0.007	>0.05	0.35	<0.05
PhNR vs. OCT RNFL	0.21	<0.01	0.52	<0.0001	0.009	>0.05	0.26	<0.05
PhNR vs. OCT Area	0.26	<0.01	0.51	<0.001	0.002	>0.05	0.31	<0.05
PhNR vs. GDx TSNIT	0.17	<0.05	0.51	<0.01	0.002	>0.05	0.25	<0.05
PhNR vs. macular volume	0.11	>0.05	0.35	<0.01	0.062	>0.05	—	—

FIGURE 5. Relationship between PhNR amplitude and HVF and between PhNR amplitude and RNFL thickness in MS ON>6 and no-ON eyes (A–D) and in MS ON<6 eyes (E–H). PhNR amplitude for a flash of 1.42 $\text{cd} \cdot \text{s}/\text{m}^2$ plotted as a function of (A, E) HVF MD and (B, F) visual sensitivity. PhNR amplitude was also plotted as a function of average peripapillary RNFL thickness obtained from (C, G) OCT and (D, H) SLP. Gray reference lines: the mean (solid lines) of PhNR amplitudes and two standard deviations from the mean (dashed lines) in control subjects. The inward ticks on the top axes (C, D, G, H) indicate the mean (middle tick) and 2 SD from the mean of RNFL thickness from machine norms for Stratus OCT³³ and SLP VCC.³⁸ Black solid lines are fitted to the ON>6 data and green solid lines to the no-ON data. The r^2 values in black and green indicate the coefficients of determination for the ON>6 and no-ON eyes, respectively.

The improvement in HVF is more obvious in the visual sensitivity plot in Figure 6C ($P < 0.01$). However, for OCT results, RNFL thinning was sustained, with increased loss in about half of the eyes. RNFL thickness was decreased, mean $\pm$ SD for all eyes, by $7.7 \pm 8.3 \mu\text{m}$ ($P < 0.01$). However, the SLP average TSNIT thickness did not show a statistically significant additional loss ($P > 0.05$). The relationships between PhNR and HVF visual sensitivity (1/L) and between PhNR and RNFL were improved sufficiently that the correlations became significant (Fig. 7, Table 4): $r^2 = 0.35$ ($P < 0.05$) for 20% V_{max} versus visual sensitivity, $r^2 = 0.31$ ($P < 0.05$) for PhNR (standard 1.42 $\text{cd} \cdot \text{s}/\text{m}^2$) versus visual sensitivity, and $r^2 = 0.26$ ($P < 0.05$) for PhNR versus RNFLT. The one eye with an RNFL thickness of $\sim 120 \mu\text{m}$ in the re-evaluated group was excluded from the correlation analysis. The RNFL thickness measured with SLP in this eye was in the clinically normal range, but the clinical examination report indicated the presence of pseudoedema in optic nerve head at the 1-year follow-up.

As shown in Figure 8, the amount of increase in PhNR amplitude at the second visit also correlated significantly with the RNFL thickness measured by OCT ($r^2 = 0.48$, $P < 0.01$) and SLP ($r^2 = 0.55$, $P < 0.01$) at the first visit. This correlation suggests that the PhNR amplitude recovery was greatest in eyes with the least loss of RNFL and did not recover as much if RNFL loss was already substantial. The improvement in PhNR amplitude did not correlate with initial HVF MD.

Finally, in the patients that were re-evaluated, in addition to the 13 ON<6 eyes, 5 ON>6 eyes and 7 no-ON fellow eyes were tested. No significant changes were observed in either functional or structural measures in these small samples.

DISCUSSION

In this study, we recorded the photopic flash ERG using a stimulus that facilitated eliciting a PhNR, and compared results with HVF and peripapillary RNFL thickness in patients with RRMS. We found a significant reduction in PhNR amplitude over the entire stimulus

response function in eyes of MS patients, both with and without a history of ON. Amplitudes of a- and b-waves were not affected. We also found significant reductions in PhNR amplitude in response to a standard stimulus of 1.42 $\text{cd} \cdot \text{s}/\text{m}^2$, which elicited a nearly saturated maximum response. In the eyes in which the ON episode had occurred more than 6 months before they were tested, PhNR amplitude in response to the standard stimulus also correlated well with HVF MD, HVF visual sensitivity. PhNR amplitude correlated well with RNFL thickness. For eyes with no history of ON, the correlations were weaker, but data were generally collinear with data from the ON>6 eyes. In eyes with ON less than 6 months before testing, PhNR amplitudes were not consistently related either to the HVF measures or RNFL thickness measures. The functional tests, PhNR and HVF (MD and visual sensitivity) showed a small but significant recovery a year later in the MS ON eyes that were initially tested within 6 months of an episode of ON, even though the eyes had persistent or even increased loss of RNFL.

The relation of PhNR amplitude to measures of sensitivity was examined using a response in the linear stimulus response range of the PhNR, in addition to the standard stimulus. This test was performed to ensure that results were not distorted by comparing nearly saturated PhNR amplitudes elicited with the standard stimulus with measures of visual sensitivity. In fact, in the present study, the results were very similar, whether PhNR amplitudes were in the linear range or nearly saturated. Although correlations were strong, there were instances both in Figures 4A and 5A in which PhNR amplitude was at or above the average control values, whereas HVF MD was down to -3 to -4 dB. It is possible that these patients and others in the study had experienced the damaging effects of MS episodes more centrally in the visual pathways.

The Photopic Negative Response as a Readout of Ganglion Cell Function

The PhNR has been shown in numerous studies to reflect the activity of ganglion cells and their axons.^{13–15} The finding that

FIGURE 6. Changes in functional and structural measures between two visits, a year apart, in 13 re-evaluated ON<6 eyes. The plots show a comparison of the first visit results (x-axis) and the second visit results (y-axis) for (A) PhNR amplitude, (B) HVF MD, (C) visual sensitivity, and average peripapillary RNFL thickness by (D) OCT and (E) SLP. The closer the data points are to the top left corner of the plot, the greater the improvement.

FIGURE 8. Plots of the change in PhNR amplitude (measured at 1.42 $\text{cd} \cdot \text{s}/\text{m}^2$) between the two visits, a year apart, as a function of average peripapillary RNFL thickness obtained from (A) OCT and (B) SLP at the first visit in 13 re-tested ON<6 eyes.

PhNR amplitude was significantly reduced in MS eyes with a history of ON indicates the presence of functional defects in retinal ganglion cells and their axons after an episode of ON. This finding agrees with previous pattern ERG studies³⁹ in which MS patients with a history of ON had reduced N95 amplitude. The result is also in agreement with a recent study showing persistent changes in PhNR in eyes of patients not diagnosed with MS, but with an isolated episode of ON.⁴⁰ Interestingly, that study also reported global reduction in the amplitude of a- and b- waves during an acute episode of ON.

The significant correlation of PhNR amplitude with RNFL (in the ON>6 and no-ON eyes) in the present study and in studies of glaucoma patients by other investigators^{23,41} suggests that PhNR amplitude is related to the number of ganglion cells and their axons that are functional. It is in the literature that RNFL loss occurs mostly within 3 to 6 months after an attack of ON.^{9,10} Retrograde degeneration of axons and ganglion cell bodies after a demyelinating event more proximally in the optic nerve or tract is likely to be an important factor in the reduction of PhNR amplitude seen in ON>6 eyes.

PhNR as an Early Detector of Ganglion Cell Dysfunction and Recovery

In ON<6 eyes, a significant reduction of PhNR amplitude was observed, but the amplitude was not consistently related to RNFL thickness measured by either OCT or SLP. This is not surprising as the RNFL loss was not complete at the time of the

FIGURE 7. Relationship between PhNR amplitude and visual sensitivity and between PhNR amplitude and RNFL thickness in re-evaluated ON<6 eyes. (A) 20% V_{max} PhNR amplitude derived from Naka-Rushton function fit to PhNR amplitudes at the second visit is plotted as a function of visual sensitivity. (B, C) PhNR amplitude measured at 1.42 $\text{cd} \cdot \text{s}/\text{m}^2$ is plotted as a function of (B) visual sensitivity and (C) OCT average peripapillary RNFL thickness at the second visit. One eye (gray filled circle) was excluded from regression analysis due to the clinical presence of pseudoedema in the optic nerve head. Gray reference lines: the mean (solid lines) of PhNR amplitudes and two standard deviations from the mean (dashed lines) in control subjects.

recordings. In fact, at times closest to the ON episode, there may have been residual swelling (e.g., Fig. 5G). The reduced PhNR amplitude at that point could also have been a result of neuronal dysfunction due to inflammation,^{40,42–44} and/or dysfunction of Müller glia cells or radial astrocytes in the optic nerve head.^{13,16,17} The PhNR response to a brief flash is slow to reach a trough, compared to the peak time expected for retinal ganglion cells, and is thought to be mediated by potassium (K^+) currents in retinal glia.^{13,16,17} These currents are set up as the glia move K^+ , released into the extracellular space during spiking activity, from regions of high extracellular concentration to regions of lower extracellular concentration, by a process called spatial buffering.⁴⁵ Clinically, measuring the PhNR may assist early detection of optic nerve disease when RNFL thickness measured by OCT or SLP is normal or borderline due to overt or subclinical edema and/or delayed neuronal atrophy.

RNFL thickness decreased in the MS eyes that were retested 1 year later, when the initial OCT scan had been performed within 6 months of an ON attack, both in the present study and in other studies with similar timelines.^{7,10} In contrast, visual sensitivity and electrodiagnostic tests in the present study and others^{7,46} showed improved function, even when there was persistent or increased loss of RNFL. As described above, the reduced PhNR soon after an ON episode could be due to impaired neuronal and/or glial function. Thus, improvement in either could have contributed to functional improvements seen a year later. The increased visual sensitivity, as well, a year later, suggests that neuronal signaling sent to the visual cortex became less abnormal. A comparison of the percentage increase in PhNR amplitude in this study with increases in mfVEP and VEP amplitude in similar longitudinal studies by other investigators supports this suggestion. The mfVEP amplitude in one study showed an increase over 12 months after an ON episode by an amount that we calculated to be 19% of the average mfVEP amplitude in normal control eyes reported in a related study by the same investigators.^{5,7} In another study, the VEP amplitude also recovered by 19%, based on the average amplitude of control eyes.⁴⁶ The percentage of PhNR amplitude increase observed in the present study was similar: 17% of the average amplitude in normal control eyes.

MS Eyes without a History of ON

Significant reduction in PhNR amplitude was found even in unaffected fellow eyes of MS ON eyes and in MS patients with no history of ON in either eye in this study (Figs. 4, 5, crossed open circles). Other studies have also reported functional and structural damage of the visual pathway, such as loss of RNFL and abnormal VEP latency and amplitude, in eyes with no history of demyelinating ON.^{5,47} Thus, chronic damage to retinal neurons and their axons in MS patients is also caused by subclinical pathologic changes associated with the disease, not just by manifest episodes of ON.

Outer Retinal Integrity in MS Patients

In the present study, a- and b-wave amplitudes were not significantly different in the MS eyes than in the control eyes, as reported recently in standard clinical testing.⁴⁸ As noted above, distal retinal function, documented by ERG a- and b-waves, can be affected in the acute phase of ON.⁴⁰ However, in the present study, in which recordings were made after an acute episode, there was only a hint of lower a- and b-wave amplitudes in ON<6 eyes, especially when compared to the eyes without a history of ON. Neuronal loss in the inner nuclear layer has been described in a histologic study of a population of postmortem eyes, mainly from secondary and primary progressive MS patients who had long-term disease.⁴⁹ Atrophy of

the inner nuclear layer in RRMS patients with shorter disease duration was less common.⁴⁹ Recently, however, the retina distal to the ganglion cells was observed to be permanently affected in a small set of MS patients, mainly of the RRMS type, from a large population.⁵⁰ Segmentation analysis of spectral-domain OCT macular scans showed thinning of both inner and outer nuclear layers in 10% of the MS patients, and multifocal ERGs showed reduced signals from retina distal to ganglion cells.⁵⁰ It is likely that these subgroups represent variants of the disease that we have not encountered.

Acknowledgments

The authors would like to thank Nalini V. Rangaswamy, Chad M. Melancon, and Chris Rhea for help in collecting some of the data and Nimesh B. Patel for advice and help with calculating integrated area of the RNFL.

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