Retinal architecture and mfERG

Optic nerve head component response characteristics in MS

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ABSTRACT

Objective: To describe a novel neurophysiologic signature of the retinal ganglion cell and to elucidate its relationship to abnormalities in validated structural and functional measures of the visual system.

Methods: We used multifocal electroretinogram-generated optic nerve head component (ONHC) responses from normal subjects ($n = 18$), patients with multiple sclerosis (MS) ($n = 18$), and those with glaucoma ($n = 3$). We then characterized the relationship between ONHC response abnormalities and performance on low-contrast visual acuity, multifocal visual-evoked potential-induced cortical responses, and average and quadrant retinal nerve fiber layer (RNFL) thicknesses, as measured by spectral-domain optical coherence tomography.

Results: Compared with the eyes of normal subjects, the eyes of patients with MS exhibited an increased number of abnormal or absent ONHC responses ($p < 0.0001$). For every 7-letter reduction in low-contrast letter acuity, there were corresponding 4.6 abnormal ONHC responses at 2.5% contrast ($p < 0.0001$) and 6.6 abnormalities at the 1.25% contrast level ($p < 0.0001$). Regarding average RNFL thickness, for each 10- μm thickness reduction, we correspondingly observed 6.8 abnormal ONHC responses ($p = 0.0002$). The most robust association was between RNFL thinning in the temporal quadrant and ONHC response abnormalities ($p < 0.0001$).

Conclusion: Further characterization of ONHC abnormalities (those that are reversible and irreversible) may contribute to the development of novel neurotherapeutic strategies aimed at achieving neuroprotective, and perhaps even neurorestorative, effects in disorders that target the CNS in general, and MS in particular. *Neurology*® 2014;82:1888-1896

GLOSSARY

AON = acute optic neuritis; **CI** = confidence interval; **GEE** = generalized estimating equation; **ICC** = intraclass correlation coefficient; **mfERG** = multifocal electroretinography; **mfVEP** = multifocal visual-evoked potential; **MS** = multiple sclerosis; **OCT** = optical coherence tomography; **ONHC** = optic nerve head component; **RGC** = retinal ganglion cell; **RNFL** = retinal nerve fiber layer.

The eye has been proposed as a “window” into the CNS. High-precision techniques have evolved and are accelerating scientific discovery, particularly regarding protective and restorative neurotherapeutic effects.^{1–4}

Whereas spectral-domain optical coherence tomography (OCT) has been of great utility in precisely characterizing the topography of retinal architecture, multifocal electroretinography (mfERG) is a technique that can yield information on discrete functional responses within the retina, following a stimulus with precisely defined characteristics.^{5–15} The retinal ganglion cell (RGC) contribution to the global retinal response is small, thereby limiting the utility of this technique for the purpose of examining retinal mechanisms of axonal and neuronal degeneration.¹⁶

Supplemental data
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In 1999, Sutter²³ developed a method to differentiate the global “retinal” response from a stereotyped, small-amplitude, and longer-latency response hypothesized to represent the optic nerve head component (ONHC) response, the timing of which is in keeping with the distance from the retinal patch of stimulation and the subsequent generation of the action potential, with a directional vector toward the lamina cribrosa.

Normal ONHC responses are contingent on the integrity of the RGC neuron and its processes and signify the transformation of the action potential from membrane (unmyelinated) to saltatory (myelinated) conduction mechanisms as the RGC axons traverse the lamina cribrosa. Herein, we examine the relationship between ONHC response abnormalities and corresponding changes in low-contrast letter acuity, multifocal visual-evoked potential (mfVEP) responses, and retinal nerve fiber layer (RNFL) thickness measures.

METHODS Study participants. Thirty-nine subjects participated in our cross-sectional study, including 18 normal subjects (n = 36 eyes) and 18 patients (n = 33 eyes; 3 eyes were not able to be analyzed because of technical factors) who were diagnosed with definite multiple sclerosis (MS) according to the modified McDonald diagnostic criteria (table 1).¹⁷ Each patient with MS within our cohort was enrolled consecutively upon consent to complete all of the required structural and functional assessments. All patients with MS had a documented history of acute optic neuritis (AON) (16 unilateral, 2 bilateral; AON n = 20 eyes; no history AON n = 13 eyes) with a required duration of >6 months from the inception of visual symptoms to the time of our study assessments. The testing procedures were performed over 2 days, given the extensive array of structural and functional assessments (on average, the range in total testing time required to complete all investigations was between 4 and 6 hours per patient). Furthermore, 3 patients with glaucoma were evaluated for mfERG-generated ONHC abnormalities to assess whether such derangements can occur secondary to pathology not exclusively predicated on inflammatory demyelinating mechanisms.

Standard protocol approvals, registrations, and patient consents. All participants provided informed and written consent before the beginning of the study procedures. Consent was obtained according to the Declaration of Helsinki. The protocol was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center.

Visual system assessments. Visual acuity testing. Visual performance was assessed on low-contrast (2.5% and 1.25%) Sloan letter acuity charts as previously described.¹⁰ Studies suggest that a meaningful and reproducible change in low-contrast acuity is between 7 and 10 letters.^{18–20}

Optical coherence tomography. High-speed, high-definition, spectral-domain OCT was performed using Spectralis (software version 5.4; Heidelberg, Germany) to assess alterations in retinal

Table 1 Demographics and clinical characteristics of the patients with MS

Subjects with MS, n (%)	18 (100)
Relapsing-remitting	16 (88.9)
Primary progressive	1 (5.6)
Secondary progressive	1 (5.6)
Optic neuritis, n (%)	18 (100)
Unilateral	16 (88.9)
Bilateral	2 (11.1)
Age, y, mean (SD)	44.4 (8.5)
Range	32–58
Sex, n (%)	
Female	14 (77.8)
Male	4 (22.2)
Average disease duration, y (SD)	8.9 (5.8)
Range	1–21
Average EDSS score (SD)	2.7 (1.8)
Range	1–6.5
Current treatments, n (%)	
Copaxone	6 (33.3)
Avonex	5 (27.8)
Tysabri	4 (22.2)
Other	3 (16.7)

Abbreviations: EDSS = Expanded Disability Status Scale; MS = multiple sclerosis.

architecture, including average and quadrant RNFL thickness, as previously described.⁹

Multifocal visual-evoked potentials. Multifocal pattern-reversal visual-evoked potentials were performed monocularly as previously described (6.3 software, VERIS; EDI, Redwood City, CA) to assess changes in timing and magnitude (i.e., amplitude) of visual cortical responses.^{13,21} We analyzed mean amplitude and timing response asymmetry across 60 sectors of cortical responses. To interrogate the intereye differences spanning the 60 sectors (organized as rings) of cortical responses, waveform metrics were transformed into pseudocolor 2-dimensional representations through the VERIS software, which utilized both root-mean-square and goodness-of-fit computations. Red sectors designate dominance of the right-eye waveforms, whereas blue constitutes left-eye dominance.

Multifocal electroretinography. For mfERG assessments, a scaled hexagonal array with a pattern-reversal stimulus was used to provoke responses that can be collected as corneal signals by an electrode placed within a contact lens.²² Eyes were anesthetized with proparacaine and then dilated with tropicamide and phenylephrine. We then used Burian-Allen bipolar contact lens electrodes for mfERG recordings. Corneal-derived signals directly reflect retinal stimulation-induced responses. For a quality recording, the impedance of the BA electrode, when measured at 30 Hz (the standard), should ideally be at ≤5 kΩ. For our purposes, we were quite stringent regarding quality recordings and thereby always attempted to achieve impedance levels of ≤2 kΩ.

For mfERG stimulation, stimuli were presented as integral multiples of a base interval. Furthermore, these stimuli transitioned

between 2 states (e.g., bright and dark hexagons) in a pattern-reversal manner (i.e., a binary-stimulation paradigm). Binary stimulation was performed in the context of pseudorandom sequences (so-called m-sequence stimulation) as previously reported.^{15,22,23} Eye fixation was confirmed via the use of a fundus camera.

Generation of the ONHC responses. For the generation of the ONHC responses, we used 103 hexagons with a period of 0.09 milliseconds between samples, 24 samples per frame at a measured frame rate of 75 Hz and 5 frames per m-step. The multifocal flash intensities were 204 candelas/m². In contrast to the 8 segments used for mfERG responses, here we used 16 segments.²² The acquisition at the amplifier level for the single channel of data was a gain of 20 K, low-cutoff filter at 10 Hz, high-cutoff filter at 300 Hz, and the notch filter in the out position. To parse out the ONHC, a novel stimulus protocol has been developed that applies global flash stimuli that are interleaved at specific intervals between the multifocal stimuli, thereby enhancing the ONHC potential and the inner retinal response.^{15,22,23}

ONHC analysis and rating. The VERIS 6.3 software was used for both recording and documenting abnormalities of ONHC responses. Our analysis program was predicated on scoring of the number of hexagonal retinal stimulus patches that generate normal or abnormal ONHC responses, with a total normal score of 102. The central 103rd hexagon is superimposed on the center of the fovea centralis and generates no response given the absence of ganglion cells in this region.

The ONHC responses are induced from site-specific stimulus patches, which provoke the transformation of ganglion cell axonal conduction mechanisms from membrane to saltatory conduction, as these fibers traverse the lamina cribrosa and are myelinated (figure 1). The stimulus patches are arranged in concentric circles around the fovea, and their corresponding waveforms were then aligned in vertical columns (figure 1). In the context of optic nerve demyelination (note segmental loss of myelin internodes behind the lamina cribrosa in the bottom panel of figure 1), the ganglion cell axons that are affected are compromised in achieving the transition from membrane to saltatory conduction properties at the lamina cribrosa and thereby leading to ONHC waveform abnormalities or complete abolishment of the detected axonal potential (note that in the right lower position of the figure, the dotted line designates where the ONHC waveform should have appeared if not for the presence of demyelination). On the right side of figure 1, we illustrate mfERG-generated responses after a sequence of retinal patch stimuli, organized as a concentric ring around the fovea. The large principal retinal component responses are all aligned with near-perfect coregistration in time, i.e., equal latency as for each response, given that the electrical potential for each has a vector whose trajectory is directly toward the corneal Burian-Allen electrode. Alternately, the smaller and longer-latency ONHC responses are observed to exhibit a latency profile dependent on the distance from the patch of retinal stimulation to the optic disc. This pattern of short-latency ONHC responses with prolongation, then followed by return of short-latency responses, is referred to as the Chevron timing pattern (depicted by the dotted line drawn to pass through the peak amplitude of the ONHC responses, or in between a bifid response), which signifies the latency differentiation for the 2 principal cell types that contribute to the mfERG-generated ONHC response (i.e., the magnocellular and parvocellular ganglion cells, respectively).^{22,23}

We used an analysis approach predicated on established evidence that each mfERG response waveform is composed of a larger retinal component (contributions from cells in all retinal layers) and a longer-latency ONHC waveform with a characteristic signature

(figure 1). Waveforms were aligned by their principal retinal response components, which occur at approximately the same latency, irrespective of the stimulus patch location (figure 1). As expected, the ONHC appears earlier when the corresponding stimulus patch is closer to the optic disc, later when further away from the disc, and earlier once again as the stimuli are again in juxtaposition to the disc (i.e., the ONHC response latency profile takes on the so-called Chevron pattern) (figure 1).

Each response across the topography of retinal patches of stimulation was manually assessed to determine the presence or absence of each ONHC waveform response. A blinded rater assessed each waveform and categorically designated the ONHC waveform response as normal or abnormal. Abnormal was the designation for scoring a principal retinal component response that was not followed by the stereotyped longer-latency, smaller-amplitude response that characterizes the ONHC; a completely absent response was included among the abnormal category. The total number of abnormal ONHC waveforms was calculated for each eye. All ratings were performed independent of other observations, including visual acuities, mfVEP responses, or OCT measures of RNFL thickness. A second blinded rater independently repeated these methods to confirm findings and to test for interrater reliability.

Statistical analysis. Statistical analyses were performed using Stata 12.0 software (StataCorp, College Station, TX). The total number of abnormal ONHC waveforms in MS eyes was compared with healthy control eyes using generalized estimating equation (GEE) models that account for age and within-patient, intereye correlations. An intraclass correlation coefficient (ICC) was used to test for interrater agreement between 2 independent assessments of the ONHC response results. GEE models were used to characterize the relationships between low-contrast letter acuity and RNFL thickness (average and across each of 4 quadrants) and the corresponding number of normal or abnormal mfERG-generated ganglion cell axon ONHC responses.

RESULTS Thirty-nine subjects participated in the study: 18 normal subjects (mean age 31.1 ± 10.9 years) and 18 patients with definite MS (mean age 44.4 ± 8.5 years) (table 1). Furthermore, we analyzed data derived from 3 patients with glaucoma, provided to us by Dr. Erich Sutter. The mean Expanded Disability Status Scale score of our MS cohort was 2.7 (with a range of 1–6.5). We did not identify a significant relationship between disease severity and the number of abnormal ONHC responses ($p = 0.2710$). Compared with normal subject eyes, MS eyes exhibited a significant reduction in the number of detected or normal mfERG-generated ONHC waveforms, the distribution of which spanned across all sectors of the retina (table 2, figure 2). Specifically, the average number of abnormal ONHC waveforms per eye, of 102 total possible responses, was significantly higher in MS eyes (31.9 ± 19.1 sectors) compared with controls (2.6 ± 3.6 sectors; $p < 0.0001$, GEE models accounting for age and within-patient, intereye correlations) (table 2). In figure 2, note the marked and diffuse thinning (average or quadrant measures that are colored red indicate that the thickness is below 1% of predicted magnitude when compared with a matched population) affecting the left eye,

Figure 1 mfERG-induced ONHC response

This figure is a modification of an illustration of the technique we published as part of our pilot investigation into the methods by which the ONHC can be generated.²² Briefly, during the process of mfERG, the patient is exposed to continuous pattern-reversal stimuli in the configuration of 103 hexagons. Note that the 2 different retinal patches of stimulation (yellow hexagons) will ultimately provoke electrical responses that are detected at the corneal surface with a Burian-Allen corneal electrode. The composite large-amplitude retinal responses (yellow arrows) are derived from multiple cell types, with only a modest contribution made by the retinal ganglion cells and their axons. Alternately, a second, smaller and later response can be stereotypically induced through the application of the interleaved global flash method and is designated as the ONHC waveform (see the upper right portion of the figure), given that the observed potential is generated by a retinal patch of stimulation that leads to the action potential being propagated along the retinal ganglion cell axons and their acquisition of myelin at the translaminar zone. At this anatomical demarcation, the retinal ganglion cell axonal conduction properties are transformed from membrane to saltatory conduction. mfERG = multifocal electroretinography; ONHC = optic nerve head component; RC = retinal component. Reprinted and modified with permission.²²

while RNFL metrics are all within normal limits on the right. The presence of ONHC response abnormalities on the right, in the absence of a history of AON and with normal RNFL thicknesses, suggests that in this patient, occult damage has initially affected physiology on the right (likely because of demyelination) but is not yet sufficient to produce changes in retinal architecture (e.g., axonal and/or neuronal loss). In figure 2E, we show the mfVEP from this same patient after pattern-reversal, high-contrast (black and white) stimulation. Note the broad landscape of cortical responses being generated with right-eye stimulation (waveforms in red), whereas those from the left are markedly attenuated and

prolonged (i.e., delayed) in timing responses. In figure 2F, to interrogate the intereye differences spanning the 60 sectors (organized as rings) of mfVEP-induced cortical responses, waveform metrics were transformed into pseudocolor 2-dimensional representations, as previously reported.^{13,21} Gray coloring of the rings indicate that both eyes are in the noise level of the device, and therefore no analytical conclusions can be rendered. White represents visual cortical response waveforms that are strong and equally balanced between the 2 eyes (expected in normal subjects). Red sectors designate dominance of the right-eye waveforms (as in our patient), whereas blue constitutes left-eye dominance (subordinated in our

Table 2 Structure-function visual system measures differentiate MS and control eyes

	Patients with MS	Normal controls	<i>p</i>
Age, y, mean (SD)	44.4 (8.5)	31.1 (10.9)	
Abnormal ONHC waveforms (SD)	31.9 (19.1)	2.6 (3.6)	<0.0001
Average RNFL thickness, μm (SD)	78.1 (13.9)	95.1 (7.8)	<0.0001
Temporal RNFL thickness, μm (SD)	50.1 (13.8)	67.3 (9.6)	<0.0001
Nasal RNFL thickness, μm (SD)	65.1 (14.7)	70.5 (10.2)	0.2116
Superior RNFL thickness, μm (SD)	94.7 (17.3)	117.1 (16.6)	0.0001
Inferior RNFL thickness, μm (SD)	102.3 (21.8)	125.6 (13.2)	<0.0001
Letters on normal VA chart (SD)	57.9 (16.7)	58.8 (10.0)	0.1814
Letters on VA chart 2.5% (SD)	25.3 (14.4)	31.7 (8.4)	<0.0001
Letters on VA chart 1.25% (SD)	10.0 (8.8)	14.9 (8.8)	0.0001

Abbreviations: MS = multiple sclerosis; ONHC = optic nerve head component; RNFL = retinal nerve fiber layer; VA = visual acuity.

The groups were highly significantly differentiated in the number of abnormal or absent multifocal electroretinography-generated ONHC responses ($p < 0.0001$) and in average and quadrant RNFL thicknesses ($p \leq 0.0001$; with the exception of the nasal quadrant, $p = 0.2116$).

patient as evidenced by amplitude attenuation and time-response prolongation.

In table 2, the groups were highly significantly differentiated regarding the number of abnormal or absent mfERG-generated ONHC responses ($p < 0.0001$) and regarding average and quadrant RNFL thicknesses ($p \leq 0.0001$; with the exception of the nasal quadrant, $p = 0.2116$). While no differences were detected across the 2 groups regarding normal visual acuity measures (i.e., at 100% contrast; $p = 0.1814$), a highly significant reduction in low-contrast letter acuity (at both the 1.25% and 2.5% levels; $p \leq 0.0001$) was detected in MS eyes. ICCs of ONHC markings between 2 independent graders indicated a high level of interrater agreement for both the analysis of patients with MS (ICC 0.84, 95% confidence interval [CI] 0.60–1.08) and the aggregate analysis of patients with MS and controls (ICC 0.94, 95% CI 0.89–0.99).

In the MS cohort ($n = 33$ eyes), a reduced number of correctly identified letters on low-contrast letter acuity was significantly associated with the number of abnormal ONHC responses. Specifically, a reduction in 7 letters of vision on low-contrast charts was associated with an average increase of 4.6 abnormal ONHC waveforms at 2.5% contrast ($p < 0.0001$) and 6.6 abnormal waveforms at 1.25% contrast ($p < 0.0001$; GEE models accounting for age and within-patient, intereye correlations) (table 3).

Coupling structure and function analyses, we observed a significant correlation between average RNFL thickness and the number of abnormal ONHC responses, with an average increase of 6.8 abnormal ONHC waveforms for every 10- μm reduction in

average RNFL thickness ($p = 0.0002$; GEE models) (table 3). Furthermore, our OCT regional analyses (by quadrants) revealed that the most significant structure-function correlation was between RNFL thickness in the temporal quadrant (the composition of RGC axons in this region of the retina includes the papillomacular bundle, which contains fiber tracts that transmit information important for vision, pupillary light reflexes, and for hypothalamic-mediated activities such as sleep-wake cycle transitions and neuroendocrine reflex arcs) and the magnitude of abnormal ONHC responses, with an average increase of 8.0 abnormal ONHC waveforms for every 10- μm reduction in the temporal quadrant thickness ($p < 0.0001$; GEE models) (table 3). Similarly, both the superior ($p = 0.0028$; GEE models) and inferior ($p = 0.0007$; GEE models) quadrant RNFL thicknesses correlated significantly with number of ONHC abnormalities (with an average of 4.6 and 4.9 abnormal waveforms, respectively, per 10- μm reduction in RNFL thickness) (figure 2). However, we did not identify significant correlations between the nasal quadrant RNFL thickness measures and abnormal ONHC responses ($p = 0.1033$; GEE models).

In the patients with unilateral optic neuritis, for whom technically acceptable recordings were achieved from both eyes ($n = 13$ patients), mfVEP derangements in the affected eye (timing response prolongation and amplitude attenuation) were compared with the fellow eye. Specifically, we assessed for response dominance by analyzing intereye differences across the 60 sectors containing spatially discrete visual cortical responses (organized as rings), and then used the VERIS software to convert them into pseudocolor 2-dimensional map representations as previously reported.^{13,21} We then assessed for the relationship between cortical response dominance by mfVEP pseudocolor maps and abnormalities in mfERG-generated ONHC responses, as previously described by our group.¹³ As such, there was dominance in the cortical responses generated from the unaffected (or at least less-affected) eye, which corresponded to a greater number of abnormal ONHC responses on the same side in 12 of the 13 patients (92% concordance, with a CI for one proportion with continuity correction at 62%–100%) (figure 2).

Multifocal electroretinographic studies performed on 3 patients with glaucoma were provided to us by Dr. Erich Sutter. The first of these exhibited 28 and 14 abolished ONHC responses in the left and right eyes, respectively. The second patient had 37 and 10 lost responses, whereas the third patient had 16 and 40 absent ONHC responses in the left and right eyes, respectively. These findings suggest that optic neuropathy in general, including optic neuropathy unrelated to demyelination, may contribute to abnormalities in this neurophysiologic signature of

Figure 2 Abnormal ONHC responses in MS eyes with and without AON

Testing results from a patient with MS who had a history of left acute optic neuritis. (A, B) The mfERG responses to our protocol of stimulation, which included the interleaved global flash component. Note the near-uniform appearance of the principal retinal components derived from both eyes, along with the near-uniform coregistration of the waveforms. Alternately, note the detection of ONHC response abnormalities (or their absence) from the right eye (the so-called unaffected side, without a history of AON) designated in red, either in the waveform itself or as filled-in hexagons within the map of the retinal topography. From the left eye, we can appreciate a severe pattern of diffuse loss of the ONHC responses across the retinal landscape of hexagonal stimulation. (C, D) Measures of the retinal nerve fiber layer thickness (both average and quadrant analysis). (E) The mfVEPs from this same patient after pattern-reversal, high-contrast (black and white) stimulation. (F) To interrogate the intereye differences spanning the 60 sectors (organized as rings) of mfVEP-induced cortical responses, waveform metrics were transformed into pseudocolor 2-dimensional representations. AON = acute optic neuritis; G = global retinal nerve fiber layer thickness; mfERG = multifocal electroretinography; mfVEP = multifocal visual-evoked potential; MS = multiple sclerosis; N = nasal; NI = nasal inferior; NS = nasal superior; OD = right eye; ONHC = optic nerve head component; OS = left eye; PMB = papillomacular bundle; T = temporal; TI = temporal inferior; TS = temporal superior.

the ganglion cell axon (figure e-1 on the *Neurology*[®] Web site at Neurology.org).

DISCUSSION It has been suggested that the ganglion cell contribution to the principal retinal component response of the ERG is negligible and electrically subordinated by larger, overlapping signals generated from other retinal sources, such as bipolar neurons.^{24–26} As such, the ability to characterize the properties of the ONHC responses provides for the ability to topographically map ganglion cell neuronal architecture in the retina and to detect pathophysiologic changes that are associated with MS and other disorders that target the anterior visual system.²⁷

The loss, delay, or disorganization of ONHC responses could be a consequence of one of the cardinal pathologic features of the MS disease process: demyelination. Retrolaminar demyelination leads to conduction slowing or block, and could result in a transition failure from membrane to saltatory conduction, thereby resulting in ONHC response abnormalities. However, axonal transection, culminating in the dying-back of RNFL axons, and later degeneration of RGCs, would also result in impaired conduction and disruption of the ONHC response.

When MS targets the anterior visual system, it typically produces optic neuropathy, due to either AON or occult/subclinical optic neuropathy.^{2,18,28–30}

Table 3 Relationships among RNFL thickness, low-contrast letter acuity, and corresponding mfERG-generated ONHC response abnormalities in MS eyes

Predictor variable	Coefficient	95% CI	p
Per 7 letters missed on VA chart 2.5%	4.6	2.4-6.7	<0.0001
Per 7 letters missed on VA chart 1.25%	6.6	3.4-9.8	<0.0001
Per 10 μ m lost in average RNFL thickness	6.8	3.2-10	0.0002
Per 10 μ m lost in temporal RNFL thickness	8.0	5.2-11	<0.0001
Per 10 μ m lost in superior RNFL thickness	4.6	1.5-7.7	0.0028
Per 10 μ m lost in inferior RNFL thickness	4.9	2.0-7.7	0.0007

Abbreviations: CI = confidence interval; mfERG = multifocal electroretinography; MS = multiple sclerosis; ONHC = optic nerve head component; RNFL = retinal nerve fiber layer; VA = visual acuity.

Note that for every 7 letters lost on each chart, there was a significant loss of ONHC responses ($p < 0.0001$); in addition, for every 10- μ m reduction in temporal quadrant RNFL thickness, we observed the most significant impact on ONHC response abnormalities ($p = 0.0001$; with the application of generalized estimating equation models accounting for age and within-patient, intereye correlations).

The retina is unique in that it is an extension of the CNS and is endowed with all of the circuit elements reminiscent of other brain pathways, except for the absence of myelin on the axons of the ganglion cell neurons, at least until these fibers traverse the lamina cribrosa.

Perhaps most salient in the current investigation was our observation that abnormalities in low-contrast letter acuity, in conjunction with thinning in the RNFL (particularly within the temporal quadrant) and prolongation of mfVEP timing responses with cortical response amplitude attenuation, significantly correlated with abnormalities in mfERG-generated ONHC response characteristics.

In the current study, we confirmed strong agreement (92.3%) between mfVEP- and mfERG-generated ONHC response characteristics when attempting to confirm the more affected eye in patients with MS who had a history of unilateral optic neuritis. However, notwithstanding the observation that these 2 techniques are predicated on distinctive neurophysiologic principles and yet yielded similar capability in differentiating the magnitude of intraindividual, interocular pathology secondary to optic neuropathy, important technical differences may subordinate the utility of the application of mfVEP techniques for the establishment of normative physiologic data across control subjects and for the purpose of analyzing pathophysiologic signatures of ocular disease across patients with the same disease. The establishment of a normative dataset of mfVEP responses represents a formidable challenge given the widely recognized visual cortical anatomical heterogeneity (principally related to the complexity and broad diversity of cortical folding patterns), which serves to confound and limit the ability to generalize mfVEP response characteristics across subjects.³¹ Conversely, mfERG-

generated ONHC responses are recorded from an electrode placed directly on the eye, and do not appear to be influenced by any known variation, such as that observed with visual cortical anatomy.

A cardinal feature of the neurophysiologic principles of cable theory is that propagation velocity depends on fiber diameter ($v = k/\sqrt{d}$), which varies negligibly across the retinal topography among human subjects. Therefore, ONHC responses may be a more reliable physiologic measure for distinguishing abnormalities regarding population normative data, particularly in circumstances of bilateral disease, which is common in MS.

Notwithstanding some important limitations, changes in the ONHC response characteristics across the retina could represent an important early marker of demyelination; for example, during the early phase of inflammatory optic neuritis. Additional work is imperative in order to differentiate changes that reflect potentially reversible loss and reconstitution of the ONHC response, most likely in the context of an intact RGC neuron and its processes. In this setting, we would not necessarily expect to observe strong correlations with abnormal retinal architecture because validated measures of injury, such as the thickness of the RNFL and ganglion cell and inner plexiform layers, may still be normal. Alternately, in those circumstances in which better correlations are observed (loss of ONHC response with OCT thickness measures), we might expect the pathologic substrate of the abnormal or absent ONHC response to be on the basis of a broader set of pathobiological underpinnings (e.g., RGC injury or loss, axonal dysfunction or transection, and demyelination). Such insights into retinal pathology and visual system pathophysiology could prove useful in the assessment of novel therapies aimed at neuroprotection and repair by ensuring that we are enrolling those patients with evidence of potential response and, equally important, that we are not enrolling patients who are, because of their pathologic substrate, destined not to exhibit therapeutic responses due to false-negative effects.

AUTHOR CONTRIBUTIONS

Zane Schnurman was involved in the formulation, design, and execution of the study. He participated in the analysis of the data, and preparing the manuscript and its final revision. Teresa Frohman is the Director of the Eye Testing Laboratory at the University of Texas Southwestern MS Program, and Neuro-Ophthalmology Research Manager. She contributed to all aspects of the study, and prepared the manuscript. Shin Beh was involved in the formulation of the study, execution of the studies on the patients and control subjects, and was involved in data analysis and preparation of the manuscript. Amy and Darrel Conger contributed to the study through data collection and analysis, and assisted with editing and revision of the manuscript. Shiv Saidha contributed to all aspects of the data analysis, and assisted with editing and revision of the manuscript. Steven Galetta contributed to the analysis of the data, and formulation and editing of the manuscript. Peter Calabresi contributed to all aspects of the study. Ari Green contributed to the analysis of the data, as well as the formulation and editing of the manuscript. Laura Balcer and Elliot Frohman contributed to all aspects of the study.

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DISCLOSURE

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