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Optic nerve head component responses of the multifocal electroretinogram in MS

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ABSTRACT

Objective: To employ a novel stimulation paradigm in order to elicit multifocal electroretinography (mfERG)–induced optic nerve head component (ONHC) responses, believed to be contingent upon the transformation in electrical transmission properties of retinal ganglion cell axons from membrane to saltatory conduction mechanisms, as they traverse the lamina cribrosa and obtain oligodendrocyte myelin. We further sought to characterize abnormalities in ONHC responses in eyes from patients with multiple sclerosis (MS).

Methods: In 10 normal subjects and 7 patients with MS (including eyes with and without a history of acute optic neuritis), we utilized a novel mfERG stimulation paradigm that included interleaved global flashes in order to elicit the ONHC responses from 103 retinal patches of pattern-reversal stimulation.

Results: The number of abnormal or absent ONHC responses was significantly increased in MS patient eyes compared to normal subject eyes ($p < 0.001$, by general estimating equation modeling, and accounting for age and within-subject, intereye correlations).

Conclusion: Studying the relationship between ONHC abnormalities and alterations in validated structural and functional measures of the visual system may facilitate the ability to dissect and characterize the pathobiological mechanisms that contribute to tissue damage in MS, and may have utility to detect and monitor neuroprotective or restorative effects of novel therapies.
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GLOSSARY

AON = acute optic neuritis; **ERG** = electroretinography; **GEE** = generalized estimating equation; **mfERG** = multifocal electroretinography; **mfVEP** = multifocal visual evoked potential; **MS** = multiple sclerosis; **ONHC** = optic nerve head component; **RGC** = retinal ganglion cell.

Electroretinography (ERG) is a physiologic technique used to study intraretinal electrical responses to stimuli with well-defined characteristics.[1–4] The development of multifocal ERG (mfERG) has facilitated the transition from analysis of a consolidated global retinal response to a topographical mapping of normal and pathologic patterns of retinal activity. However, unlike multifocal visual evoked potential (mfVEP) responses, those derived from mfERG studies are highly stereotyped, both within and across normal subjects.[1–5]

Recognizing that the retinal ganglion cell (RGC) contribution to the mfERG is small, and overlaps with signals generated from other retinal sources (e.g., bipolar neurons), Sutter and colleagues[1,2] developed a modified high-precision mfERG stimulus paradigm to include global flash stimuli that are interleaved at specific intervals, in order to elucidate a discrete neurophysiologic response signature that corresponds to the normal electrical transmission mechanisms of RGC axons across the topographical landscape of the retinal nerve fiber layer. This induced component of the mfERG is referred to as the optic nerve head component (ONHC) response, and its presence signifies the normal electrical transformation from membrane to saltatory transmission properties, as unmyelinated.

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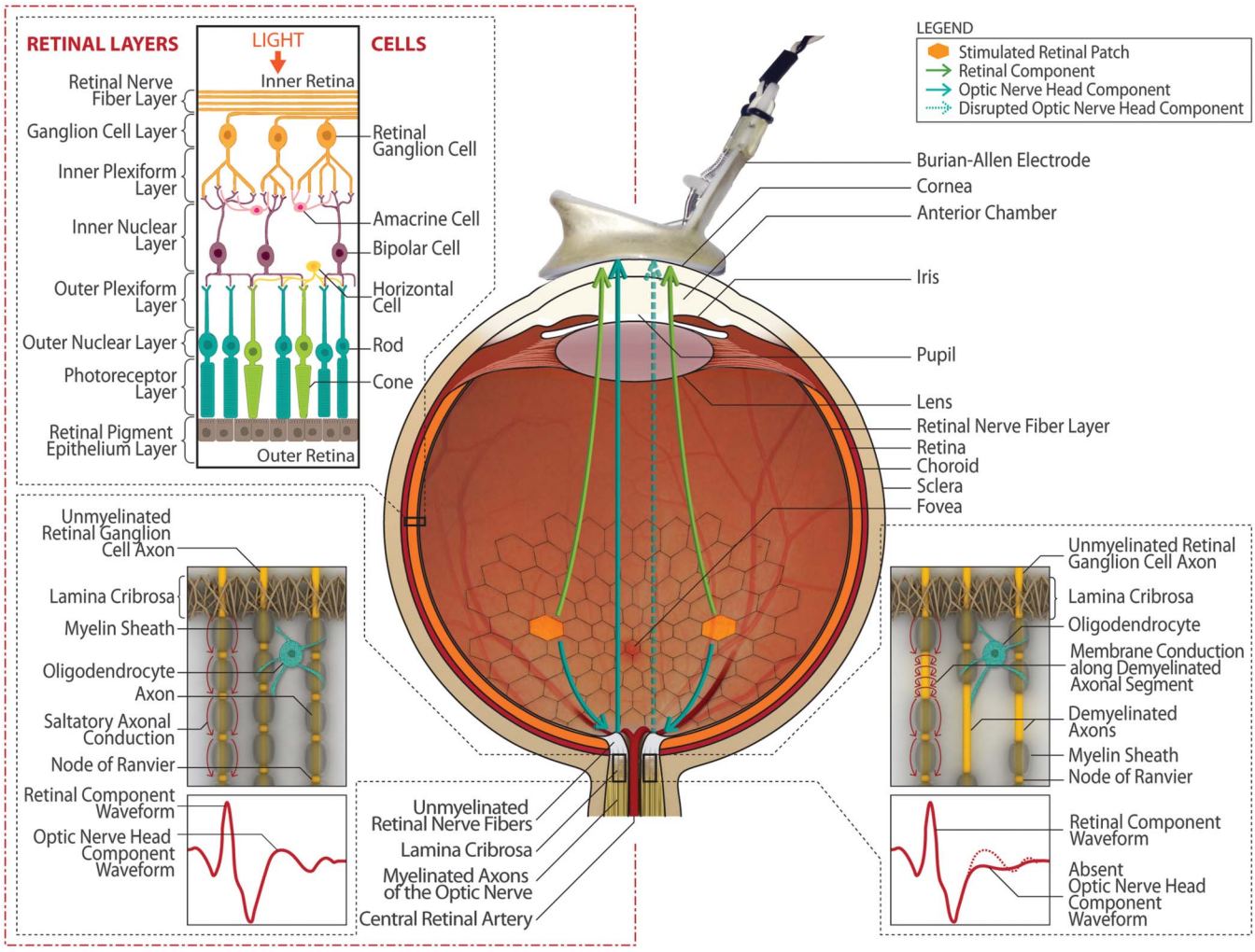
RGC axons traverse the lamina cribrosa, beyond which they are myelinated (figure 1).[1,2] We employed the global flash mfERG stimulation paradigm to demonstrate definitive abnormalities of ONHC responses in patients with multiple sclerosis (MS).

METHODS Our objective for this pilot investigation was to characterize the abnormalities of mfERG-generated ONHC responses in patients with MS and a history of acute optic neuritis (AON), when compared to the fellow eye, and with respect to eyes from normal subjects.

Patients. We examined 10 normal subjects (mean age 29.4 years, n = 20 eyes) and 7 patients with definite MS (mean age 41.9 years, n = 14 eyes) as confirmed using the McDonald modified criteria[6] and a history of AON (table). The patients with MS were recruited consecutively in the Clinical Center for MS at UT Southwestern Medical Center, and were excluded if they had any other ophthalmologic condition (e.g., glaucoma, macular degeneration), high myopia (>−5.0 D), or any major medical condition with impact upon the visual system other than MS. Further,

Figure 1 Generation of the multifocal electroretinogram–induced optic nerve head component response



Two different retinal patches of stimulation (orange hexagons) will yield electrical responses that are detected at the corneal surface with a Burian-Allen electrode (at the top of the diagram). The large-amplitude retinal response stems from multiple cell types within the retina, with only a modest contribution made by the retinal ganglion cells and their associated axons. Note that the principal retinal responses will be detected nearly simultaneously by the corneal electrode (green arrows representing the principal retinal response) and—given that the distance from the 2 patches of stimulation to the point where the electrical potentials are captured—are nearly identical. A smaller and later response can be stereotypically induced through the application of the interleaved global flash method, and is designated as the optic nerve head component (ONHC) waveform. Note that the electrical propagation first travels from the retinal patches of stimulation (again here designated as orange hexagons), the response to which is propagated to the optic nerve head (note the teal arrows designating the electrical response of unmyelinated retinal ganglion cell (RGC) axons to the optic nerve head), and then the response transmission finally propagates to the corneal electrode (note the teal arrows representing the electrical potential generated by the RGC axons during the translaminar transformation from membrane to saltatory conduction mechanisms). In the context of optic nerve demyelination, the ganglion cell axons that are affected are compromised with respect to achieving the transition from membrane to saltatory conduction properties at the lamina cribrosa (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 pathology).

Table Abnormal or absent optic nerve head component responses in normal subjects and patients with multiple sclerosis with acute optic neuritis[a]

	NC1	NC2	NC3	NC4	NC5	NC6	NC7	NC8	NC9	NC10
ONHC OD, n abnormal or absent	0	0	4	0	3	0	0	0	0	0
ONHC OS, n abnormal or absent	0	9	8	0	0	3	0	0	0	0
	MS1	MS2	MS3	MS4	MS5	MS6	MS7			
AON history	AON	AON	AON	AON	AON	AON	AON			
Affected eye	OS	OD	OU	OD	OS	OS	OS			
ONHC OD, n abnormal or absent	37	38	9	41	20	54	3			
ONHC OS, n abnormal or absent	89	42	38	29	43	47	37			

Abbreviations: AON = acute optic neuritis; MS = multiple sclerosis; NC = normal control; ONHC = optic nerve head component.
[a] In this table we provide characterization of 10 normal control subjects (NC1–NC10) and 7 patients with multiple sclerosis with a history of AON.

we only included patients whose episode of AON was ≥6 months from the onset of visual symptoms.

mfERG methods. For mfERG assessments, a scaled hexagonal array with a pattern-reversal stimulus was utilized to provoke responses that can be collected as corneal signals by a Burian-Allen bipolar contact lens electrode, as previously described (figure 1).[1,2]

Briefly, subjects fixated on a centralized 2-mm red-cross marker within the stimulator. Fixation was ensured by continual fundus monitoring (VERIS; EDI, Redwood City, CA). A novel stimulus paradigm (the ONHC 103-hexagon global-flash mfERG VERIS protocol) with 5 frames per m-step was used.[1,2] This paradigm enhances the inner retinal responses, and hence, the generation of the ONHC response. The first frame contained focal flashes (128 cd/m^2) controlled by the VERIS pseudorandom m-sequence; the second and fourth frames contained global flashes (128 cd/m^2); and the third and fifth frames were dark (1 cd/m^2) (figure 2). No value of impedance greater than a 2-Hz threshold was considered acceptable. Upon completion, the Burian-Allen electrode was removed, and a slit-lamp examination was performed. None of our subjects sustained any corneal injuries.

mfERG response analysis. The mfERG responses were analyzed using VERIS software version 6.3.3d7. The response traces were organized as concentric rings around the fovea, and were then plotted in vertical columns (figure 2). The tracings are mathematical extractions of signals that are correlated with time. For the analysis of mfERG retinal patch stimulation sequences, 2 principal waveforms were identified—the direct component, which is dominated by the retinal component appearing early, and the induced component, which is dominated by the ONHC response waveform that appears later. We scored ONHC waveforms as being abnormal (waveform disorganization or absent) utilizing a colorized map (pink or red filled hexagons designate the abnormal retinal patches, whereas white unfilled hexagons designate normal responses) (figures 3 and 4).

Statistical analysis. Statistical analyses were performed using Stata 12.0 software. The total number of waveforms with abnormal ONHC responses in MS eyes with AON was compared to MS eyes without a history of AON and with respect to healthy control eyes using generalized estimating equation (GEE) modeling.

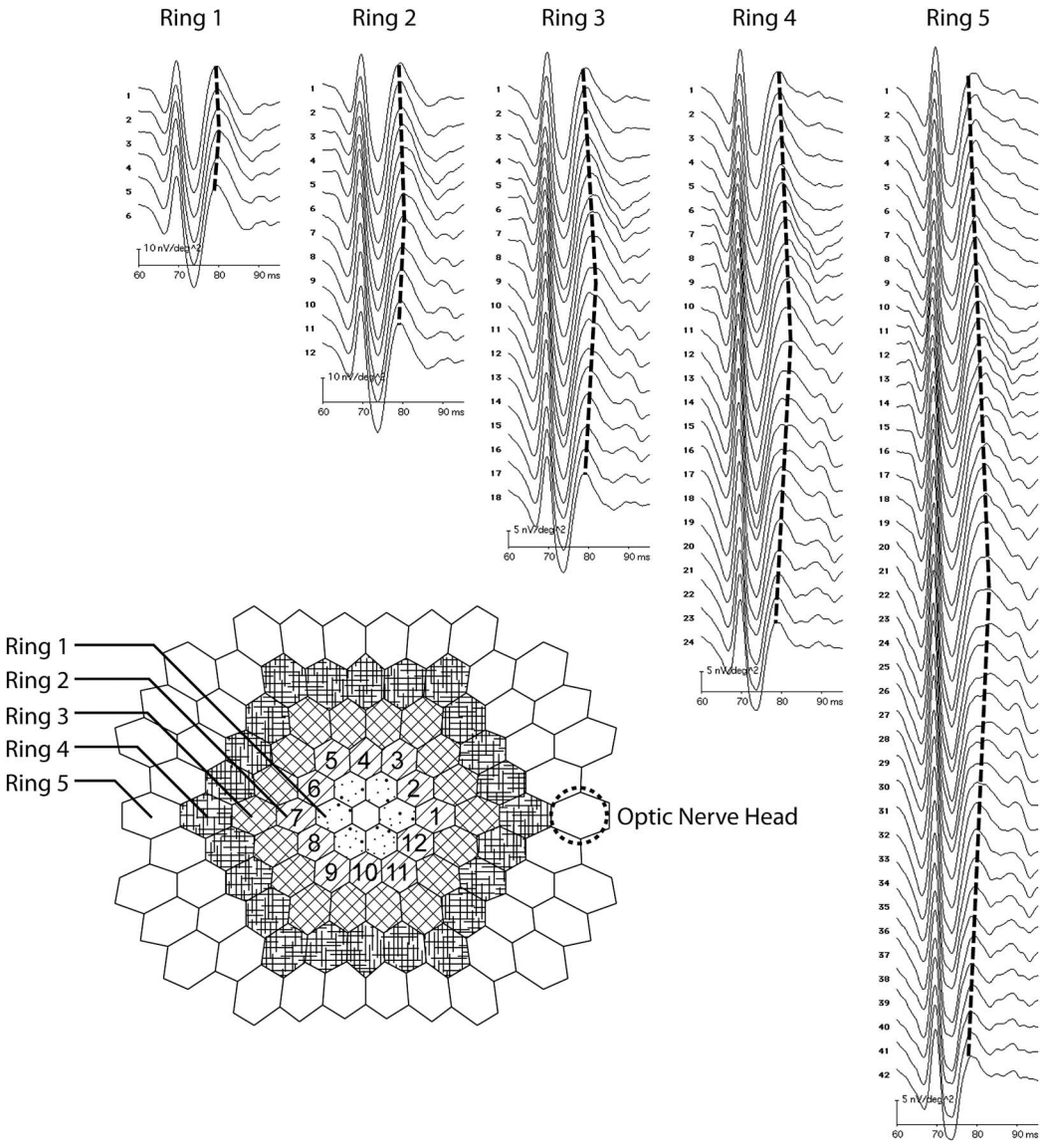
Standard protocol approvals, registrations, and patient consents. All participants provided informed and written consent prior to the beginning of study procedures. Consent was obtained according to the Declaration of Helsinki. The protocol was approved by the Investigational Review Board of UT Southwestern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

RESULTS **Optic nerve head component response: Patients with MS vs normal subjects.** In 16/20 normal eyes, we did not identify any ONHC waveform abnormalities (table and figure 2), whereas in 4/20 normal eyes, there were occasional ONHC response abnormalities (range of 3–9 abnormal waveforms per eye out of 103 hexagonal patches of stimulation) that tended to be localized to the outermost ring of stimulation (ring 5) where the density of RGC axons is sparse (table).

The number of abnormal or absent ONHC responses was significantly associated with MS eyes vs those from control subjects (table, figures 3 and 4). On average, we observed 34 more abnormal or absent ONHC responses from MS eyes when compared to eyes from healthy individuals ($p < 0.001$ by GEE and accounting for age and within-subject, intereye correlations). Alternately, among MS eyes, and irrespective of positive or negative history of AON, the loss of ONHC responses was not significantly different ($p = 0.34$). If corroborated in larger future studies, this observation may represent one of the most interesting and conspicuous aspects of our investigation. In particular, the magnitude of the severity of intraretinal pathology that ultimately compromises the fidelity in the transition from membrane to saltatory axonal conduction mechanisms at the lamina cribrosa may be affected similarly by manifest episodes of AON vs those mechanisms that contribute to the occult subclinical damage sustained by tissue elements that culminate in abnormal or abolished mfERG-induced ONHC responses.

DISCUSSION In this pilot investigation, we underscore the application of a novel mfERG interleaved global flash stimulation paradigm to demonstrate loss or abnormality of ONHC responses in MS eyes. These findings are in keeping with a cardinal pathophysiologic principle in MS-associated optic neuropathy: translaminar demyelination (either secondary to AON or as a

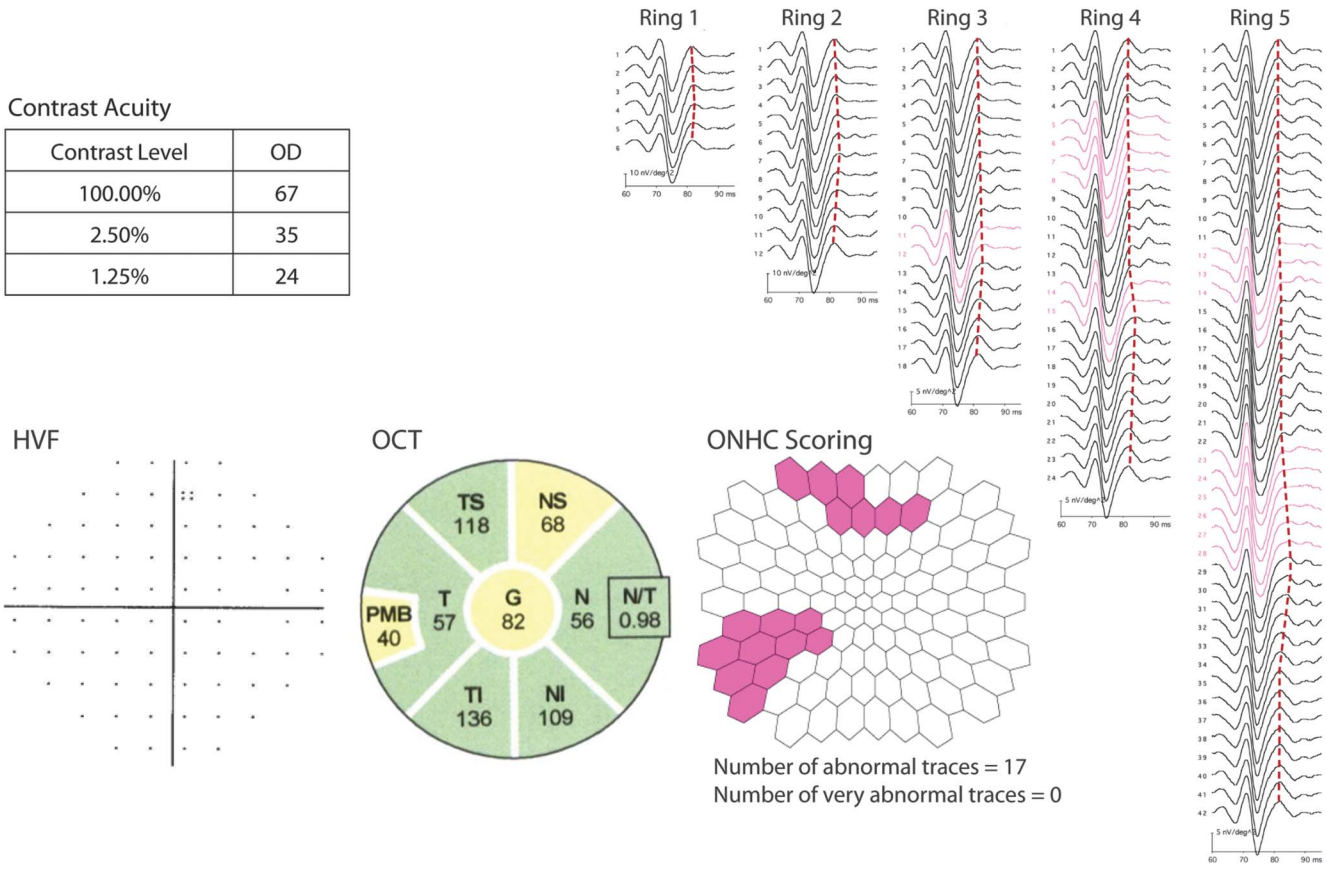
Figure 2 Characterization of the optic nerve head component responses in normal eyes



Here we present the multifocal electroretinography–induced optic nerve head component (ONHC) responses from the right eye of a normal subject. The retinal patch stimulation sequence is organized as concentric rings centered upon the fovea centralis. The initial patch of retinal stimulation commences, with the hexagon adjacent to the superotemporal aspect of the peripapillary optic disc. Subsequently, the stimulation sequence moves superotemporally, temporally, inferiorly, and culminates inferotemporally, adjacent to the optic disc. The corresponding ONHC response latency progressively lengthens and then shortens in keeping with the changes in distance of the patch of retinal stimulation to the ONHC response at the translaminar zone where the retinal ganglion cell axons transform from membrane to saltatory conduction mechanisms. This pattern is referred to as the Chevron pattern, and it represents a nearly stereotypic neurophysiologic signature across individuals without pathology in the anterior visual system. To visually appreciate the Chevron pattern associated with the ONHC latency profile, we simply placed each interrupted line segment through the peak of the ONHC amplitude, or in between the appearance of 2 amplitude peaks (thought to represent the peaks affiliated with the magnocellular and parvocellular contributions to the ONHC response).

derivative of occult optic neuropathy) and the loss of the normal transformation of membrane to saltatory electrical transmission properties of RGC axons as they traverse the lamina cribrosa.[7] Notwithstanding this hypothesis, the mechanisms responsible for abnormalities in ONHC responses are likely manifold. For instance, persistently abolished ONHC responses may also occur in the context of fixed damage to RGCs or their axons (e.g., as in glaucoma).[8] Alternately, ONHC may also be reversibly disorganized or absent in the context of AON, under circumstances of transient inflammation, edema, and ion channel perturbations, and with subsequent reconstitution of normal RGC axonal physiology. The limitations of a pilot investigation such as ours include the small sample size, lack of age matching, and the variability in the epoch of time from symptom onset to the time of the experimental assessments. Moving forward, the careful, systematic, and longitudinal investigation of the mfERG-induced ONHC responses

Figure 3 **Characterization of the optic nerve head component responses in a multiple sclerosis unaffected eye**



Here we present data from the unaffected (historically) right eye from a patient with multiple sclerosis with a history of left acute optic neuritis. The upper left text box indicates the number of correct letters identified on contrast acuity charts (at 100%, 2.5%, and 1.25% levels). Below we show the normal pattern-deviation plot from Humphrey automated perimetry, using the 30-2 test. In the left lower aspect of the figure we present the retinal nerve fiber layer (RNFL) thickness analysis by high-speed, high-definition, spectral-domain optical coherence tomography (OCT; Spectralis, Heidelberg, Germany). The average RNFL thickness is mildly reduced (at 82 microns for the "unaffected" right eye), suggesting the presence of occult disease activity. On the right aspect of the figure, we present the concentric rings of retinal patch stimulation, with the multifocal electroretinography (ERG) responses aligned vertically. The multifocal ERG responses with greatest conspicuity to each patch of retinal stimulation constitute the principal response (which constitutes a composite physiologic signature, with contributions from cells across all retinal layers). Alternately, the optic nerve head component (ONHC) response waveforms emerge following the principal retinal response, with a delayed latency, albeit with a characteristic signature. Specifically, the ONHC responses are detected 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 once again in juxtaposition to the disc (i.e., the Chevron response pattern). The waveforms traced in red are those where the ONHC is either abnormal or absent. The retinal patch tomography map (bottom middle part of the figure) indicates the location of the abnormal or absent responses.

in MS, and the relationship to validated structural (e.g., optical coherence tomography) and functional measures (e.g., contrast acuity, visual field analysis, mfVEP, and pupillometry) of the visual system, will ultimately determine the validity (both face and construct) and the utility of the ONHC response to detect and monitor neuroprotective or restorative effects of novel therapies.

AUTHOR CONTRIBUTIONS

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 our patients and control subjects, and was involved in the data analysis and preparation of the manuscript. Zane Schnurman was involved in the formulation, design, and execution of the study. He participated in the analysis of the data, preparing the manuscript, and its final revision. Amy and Darrel Conger contributed to the study through data collection and analysis and with respect to assistance with the editing and revision of the manuscript. Shiv Saidha contributed to all aspects of the data analysis and with respect to assistance with the editing and revision of the manuscript. John Ratchford contributed to all aspects of the data analysis and with respect to assistance with the editing and revision of the manuscript. Carmen Lopez contributed to the acquisition of the data, coordinating patient enrollment, and assisted in all aspects of the experimentation on all MS and normal subjects at the Center. 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. Laura Balcer 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. Elliot Frohman is the senior author and contributed to all aspects of the study.

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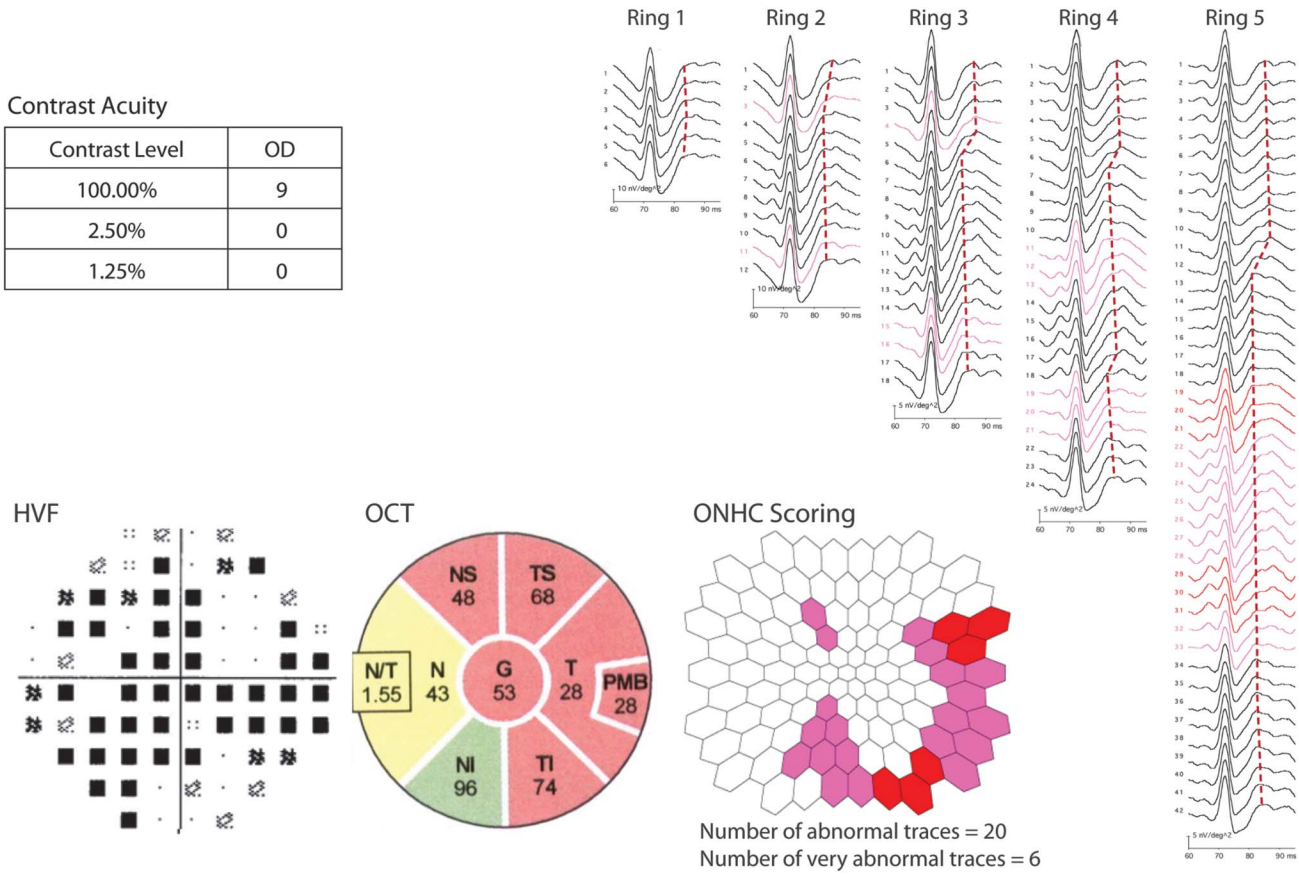
Figure 4 Characterization of the optic nerve head component responses in a multiple sclerosis affected eye



Here we present data from the same patient in figure 3, but derived from the eye with a history of acute optic neuritis (i.e., the left). Note the severe loss of acuity (in both high- and low-contrast acuity levels), the broad suppression of the corresponding Humphrey visual field, and the optical coherence tomography (OCT) retinal nerve fiber layer (RNFL) topography map demonstrating diffuse thinning of the RNFL (both average and several sectors; red indicates RNFL thickness levels below 1% of predicted for a matched population). On the right aspect of the figure, note the more diffuse nature of the abnormal or absent optic nerve head component (ONHC) responses. Compared to the right eye, the waveforms are more poorly defined or absent. Bedside examination revealed a severe left relative afferent pupillary defect. Funduscopically, there was diffuse optic disc pallor (signifying chronic changes, compositionally most consistent with astrogliosis, a cardinal histopathologic feature of chronic neuropathies, including those associated with multiple sclerosis).

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This Week's *Neurology*® Podcast

Optic nerve head component responses of multifocal electroretinogram in MS **(See p. 545)**

This podcast begins and closes with Dr. Robert Gross, Editor-in-Chief, briefly discussing highlighted articles from the August 6, 2013, issue of *Neurology*. In the second segment, Dr. Beau Bruce talks with Drs. Teresa and Elliot Frohman about their paper on optic nerve head component responses of multifocal electroretinogram in MS. Dr. Adam Numis then reads the e-Pearl of the week about Terson syndrome. In the next part of the podcast, Dr. Alberto Espay focuses his interview with Dr. John Trojanowski on progressive accumulation of tau pathology in patients with Alzheimer disease and how it occurred in a stereotypical manner. Disclosures can be found at www.neurology.org.

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