Electrophysiological Evidence for Heterogeneity of Lesions in Optic Neuritis

Alexander Klistorner,1 Stuart Graham,1 Clare Fraser,1 Raymond Garrick,1 Tan Nguyen,2 Michael Paine,2 Justin O’Day,2 John Grigg,1 Hemamalini Arvind,1 and Frank A. Billson1

PURPOSE. To examine the natural history of multifocal visual evoked potentials (mfVEPs) within 12 months of the first episode of optic neuritis (ON) in patients with possible multiple sclerosis (MS).

METHODS. Twenty-seven patients with a first episode of ON, no previous demyelinating events, and MRI lesions consistent with demyelination were examined with mfVEP. Changes in amplitude and latency of mfVEP were analyzed at 1, 3, 6, and 12 months after an acute attack.

RESULTS. Five of 27 patients had persistent loss of amplitude after 12 months of follow-up. This loss was most marked centrally. Amplitude recovered in the remaining 22 patients at 1 month, but delayed latency, which was also most marked centrally, persisted. Of these, two distinct subgroups were identified: six patients with no improvement in latency and 16 patients with significant latency recovery over the 12 months of follow-up, suggesting remyelination. Conversion to MS was highest in the group with severe amplitude loss, followed by the group with no latency recovery. The conversion rate was lowest in the group of patients with latency improvement.

CONCLUSIONS. Distinct patterns of disease evolution were identified using mfVEP in patients with first episode of optic neuritis and at high risk for MS, supporting the concept of heterogeneity of early lesions in MS. (Invest Ophthalmol Vis Sci. 2007;48:4549–4556) DOI:10.1167/iovs.07-0381

Optic neuritis (ON) is a frequent initial manifestation of multiple sclerosis (MS). In 40% to 75% of ON patients, ON eventually progresses to MS. The presence of typical lesions in the brain at the onset of ON, detected by magnetic resonance imaging (MRI), is a strong risk factor.1,2 In contrast to most brain lesions, the effects of disease on the optic nerve are often clinically apparent and potentially measurable.3 Thus, the recovery phase of ON presents an opportunity to examine the processes of myelin destruction and repair and possible axonal degeneration.

The visual evoked potential (VEP) was proposed as a means of assessing the integrity of the visual pathway in ON.4 It was suggested that the amplitude of the full-field VEP reflects the number of functional optic nerve fibers determined by the severity of optic nerve inflammation in the acute stage of ON and subsequent axonal degeneration in later stages.5 Delayed conduction of conventional full-field VEP recording has been found in a high proportion of patients with ON and is thought to reflect demyelination of the optic nerve fibers.4,6 The subsequent shortening of latency in some patients is thought to represent remyelination.5

More recently, the multifocal VEP (mfVEP) introduced the possibility of topographic study of optic nerve function with measurement of amplitude and latency from locally derived VEP responses.7–10 It can, therefore, identify focal defects and has been shown to detect glaucomatous field losses with high sensitivity and specificity.11,12 Responses from peripheral areas can be recorded even when the central field is predominantly affected with no signal on conventional VEP. In recent studies,13,14 we have shown the mfVEP to be not only a sensitive technique for identifying ON but a potential marker for the subsequent development of MS in patients with ON as clinically isolated syndrome (CIS). This is important because known markers do not fully explain the subsequent risk for MS. For example, the most valuable predictor for the development of MS is the presence of white matter lesions observed on MRI of the brain. However, in the Optic Neuritis Treatment Trial (ONTT), those in whom MRI showed no lesions had 22% risk for MS (at 10 years), and those with MRI lesions had 56% risk.15

The ability of the mfVEP to detect focal areas of neurologic dysfunction and possibly monitor recovery from demyelination in ON provides a potentially useful clinical tool.16 Amplitude of the response or improvement in the speed of conduction on serial mfVEP recording may permit assessment of the presence and topography of the lesion, extent of axonal loss, and rate of remyelination in different sectors of the optic nerve. In turn, this may serve as a surrogate marker for the different pathologic patterns of the disease.

The only published longitudinal study of the mfVEP in ON to date (which analyzes latency of response) is one by Yang et al.,17 who demonstrate that in 29% of patients enrolled in their study, latency did improve significantly during the follow-up period. However, only two tests were performed for each patient, with initial testing between 3 weeks and 12 months of the acute episode and with an intertest interval ranging from 6 to 56 months. As the authors themselves indicated, such variability of enrollment time and follow-up interval may lead to a considerable underestimation of latency recovery rate.

In this study, we prospectively enrolled patients with newly diagnosed acute ON who had positive MRI findings and observed them with serial mfVEP in order to determine whether varying amplitude and latency patterns could be identified during follow-up after an attack. Only patients with initial MRI lesions (“possible MS” according to McDonald criteria18) were enrolled in the study because they were at higher risk for clinically definitive MS19,20 and therefore were more likely to be truly representative of the MS disease process. This report presents the interim (12-month) results of an ongoing larger investigation.

From the 1Department of Ophthalmology, Save Sight Institute, University of Sydney, Sydney, Australia; and the 2Center for Eye Research, Melbourne University, Melbourne, Australia.

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Corresponding author: Alexander Klistorner, PO Box 4337, Sydney, 2001, NSW, Australia; sasha@eye.usyd.edu.au.

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METHODS

Patients

Twenty-seven patients with clinically diagnosed acute unilateral ON and no previous diagnosis of MS were enrolled. All patients had demyelinating MRI lesions of the brain on initial MRI scan and were classified as having possible MS according to McDonald criteria.18 Patients with any other ocular abnormality were excluded. All patients had completed a 3-day course of intravenous methylprednisolone (1 g daily) and a 2-week oral taper of steroids.

To estimate between-test latency variability, mfVEP was recorded twice, at a 3-month interval, in a control group of 20 healthy subjects (mean age, 35.2 ± 6.1 years). Healthy subjects underwent complete ophthalmologic examination, including dilated funduscopy. Eligibility criteria for healthy subjects included 6/60 vision in both eyes, normal anterior segment and retinal evaluation, normal optic disc morphology, and ability to perform a reliable Humphrey 24 to 2 SITA-standard visual field test.

Procedure of mfVEP Testing

As described in detail elsewhere,21-23 mfVEP testing was performed with multifocal objective perimetry (Accumap; ObjectiVision Pty. Ltd., Sydney, Australia) under standard stimulus conditions. Briefly, the stimulus consisted of a corticaly scaled cardboard pattern of 58 segments (eccentricity up to 24°, with nasal step up to 32°) (Fig. 1a). Each segment contained a 4 × 4 grid of black-and-white checks (proportionately scaled to segment size) that reversed the pattern according to a pseudorandom sequence (4096 elements, 54 seconds long). A family of sequences was used to simultaneously drive all 58 segments. At the end of each run (54 seconds), a different sequence was assigned to the segment for the next run. Usually eight runs (up to a maximum of 10) were needed to achieve a good signal-to-noise ratio.

The visual stimulus was generated on a 21-inch high-resolution display (Hittachi Ltd., Tokyo, Japan) with a stimulation rate of 75 Hz. Luminance of the white check was 146 cd/m², and luminance of the black check was 1.1 cd/m² (Michelson contrast of 99%). Background luminance of the screen was 73.5 cd/m², and a dim room light was always on.

Subjects were seated 30 cm from the screen with refractive correction for near vision. They were asked to fixate on the small, randomly changing number at the center of the stimulus pattern, which was used as a fixation monitor. Numbers (or letters) were displayed in random order, and the subject was asked to respond by pressing a button when a particular number (or letter) appeared. This ensured good concentration throughout the recording; percentages of missed and incorrect responses were calculated automatically after each run (any run with more than 30% missed or incorrect responses was rejected). Pupils were not dilated. All recordings were performed monocularly.

Four gold cup electrodes (Grass, West Warwick, RI) were used for bipolar recording; two electrodes were placed 4 cm from either side of the onion, one electrode was placed 2.5 cm above the onion in the midline, and one electrode was placed 4.5 cm below the onion in the midline. Electrical signals were recorded along four channels as the difference between superior and inferior, left and right, and obliquely between horizontal and inferior electrodes. Visual evoked responses were amplified 1 × 10⁵ times and band-pass filtered 1 to 20 Hz.

Analysis

Opera (Oslo, Norway) software correlated the pattern reversal sequence with the electrical signals recorded, and a response for each segment was obtained. The largest peak-trough amplitude within a 60- to 180-ms interval was determined for each channel.

For amplitude analysis, the wave of maximal amplitude from four channels was automatically selected by the software to create a combined topographic map.24

Latency analysis—four traces for each eye recorded for each individual segment—was performed. Amplitude of the traces from all four channels of each eye of all four tests from a single segment of the visual field was analyzed, as described (4 channels × 2 eyes × 4 tests = 32 traces), and the amplitude of the largest wave was recorded. The second peak of the largest wave was automatically determined for latency measurement by a specially designed algorithm. The same channel and the same peak (minimum or maximum) were then used for latency analysis for that particular segment in both eyes in all four tests (Fig. 1b). Latency asymmetry was calculated as the difference between the latencies of both eyes in milliseconds.

The signal was considered nonrecordable (and, therefore, latency was not analyzed) in segments in which amplitude of the response was less than 1.96 times the noise level (determined as standard deviation of the trace within the interval 400 to 1000 ms). Average latency was calculated and analyzed if at least two thirds (67%) of the segments gave identifiable responses.

mfVEP testing was performed 1, 3, 6, and 12 months from onset of ON.

Other measures of visual function, including Snellen visual acuity, Ishihara 24-plate color vision test, and relative afferent pupillary defect, were also recorded. MRI—including FLAIR, T1 and T2 sequences of the brain, and fat-saturating T2 sequences of the orbit—was performed on all patients within 1 week of the onset of ON and was repeated at the end of the follow-up period or at the time of relapse. All procedures were performed in accordance with the tenets of the Declaration of Helsinki, and informed consent was obtained from all participants.

Statistical analysis was performed (Statistica 4.1, StatSoft, Tulsa, OK).

RESULTS

Mean age of the 27 patients at the time of study enrollment was 32.6 (±8.5) years; 18 patients (67%) were women. Clinical and demographic data are presented in Table 1.

In four patients, mfVEP amplitude was nonrecordable in most (more than 67%) of the tested areas of the visual field at the 1-month visit; minimal recovery occurred in the periphery during the study period. Follow-up recordings from one patient are presented in Figure 2a. Another patient demonstrated recordable (though relatively reduced) mfVEP in most areas of the tested visual field at the 1-month visit. However, his subsequent test results demonstrated progressive amplitude deterioration; no responses were detected at the 6-month visit (Fig. 2b). This lack of response was accompanied by deterioration in visual acuity.

Amplitude recovery was significantly greater in the more peripheral parts of the field compared with the central regions. The ratio of average amplitude across each ring of eccentricity (Fig. 2b, inset) in the affected eye to that of the corresponding ring of the unaffected eye at 12 months was calculated and compared between the rings (Fig. 2b). This difference was statistically significant (P = 0.04; one-way ANOVA). In all five cases, visual acuity remained significantly reduced (6/18 at best) at 1 year.

The mfVEPs in these five patients were, therefore, characterized by extinguished waveforms at earlier or later visits and did not permit meaningful serial latency analysis. However, in all five patients, areas that had minor amplitude recovery at different stages of the follow-up period displayed considerably delayed latency (range, 15–40 ms) when compared with corresponding areas of the fellow eye.

In the remaining 22 patients, mfVEP demonstrated complete or near complete restoration of amplitude in most of the tested areas. When compared with the nonaffected eye, the amplitude average across the tested field increased from 71.0% (±17.1%) to 92.7% (±9%) during the follow-up period. A
A typical example of mfVEP amplitude recovery is presented in Figure 3. In all 22 cases, amplitude recovery was sufficient to permit serial latency analysis.

Average latency of the affected eye in this group was significantly delayed compared with the unaffected eye at 1 month (177.4 ± 12.1 ms vs 149.1 ± 8.4 ms; P < 0.00001; paired t-test). Mean latency asymmetry between affected and unaffected eyes at 1 month was 28.8 ± 8.1 ms (range, 18–46).

During the follow-up period, the mean latency of the affected eye demonstrated a clear tendency to shorten that was statistically significant (P < 0.0001; repeated-measures ANOVA; Fig. 4a). Post hoc analysis (Tukey-Kramer multiple-comparison test) revealed that the only nonsignificant difference occurred between 6 and 12 months. Average latency of the unaffected eyes, on the other hand, remained unchanged (P = 0.45; repeated-measures ANOVA). Asymmetry analysis based on average difference between all segments of affected and unaffected eyes also demonstrated good recovery of delayed latency (Fig. 4b) and was significant for all four tests (P < 0.0001; repeated-measures ANOVA; Tukey-Kramer multiple-comparison test).

However, detailed analysis of individual cases revealed marked heterogeneity in the course of latency recovery. When
the rate of latency asymmetry recovery during the follow-up period was plotted (latency asymmetry at each visit expressed as a percentage of the initial latency asymmetry), it demonstrated two distinct patterns. In one group (six patients), latency on follow-up visits fluctuated within ±10% of the initial value (3–4 ms), with no consistent improvement trend. In the other group (16 patients), significant and steady latency shortening (range of improvement, 40%–90%) was observed (Fig. 5). A difference between the two groups was clearly visible by 12 months.

Variation of the latency asymmetry in patients from the first (latency nonrecovery) group did not exceed intertest variability in controls (2.8 ± 1.9 ms).

In the latency recovery group, the rate of latency improvement decreased with time from 2.3 ms per month between 1 and 3 months to 1.9 ms per month between 3 and 6 months and finally to 0.95 ms per month between 6 and 12 months.

To investigate the topographic distribution of latency delay across the visual field, latency asymmetry values were averaged at different eccentricities according to the rings of the stimulating pattern (Fig. 2, inset). It was established that signals derived from the central areas of the tested visual fields were more delayed at initial presentation than responses derived from the peripheral areas (Fig. 6). This difference was significant (P = 0.006; one-way ANOVA), and this tendency persisted throughout the follow-up period.

No significant correlations were observed between magnitude of latency recovery and patient age at the onset of ON (Spearman rank correlation, P = 0.3) or visual acuity at initial presentation (P = 0.07). Mean latency recovery for men was 60.4% ± 15.8%, and for women it was 55.2% ± 12.4%. The difference was not statistically significant (Mann-Whitney test; P = 0.3).

During the follow-up period, clinically definite MS, as defined by the McDonald criteria, was diagnosed in 12 patients. The diagnosis of MS was based on the second (usually sensory) neurologic episode, with corresponding MRI changes. All five patients with amplitude nonrecovery (100%) converted to clinically definite MS within 6 months of the initial episode. Seven of the 22 patients with significant amplitude recovery converted to clinically definite MS during the 12-month follow-up, 4 of 6 patients (67%) from the latency nonrecovery group and 3 of 16 patients (19%) with improved latency. The difference between the conversion rates of patients with amplitude nonrecovery and patients with amplitude recovery was statistically significant (P = 0.0028; Fisher exact test).

**DISCUSSION**

This study prospectively examined changes in amplitude and latency of mfVEP in patients with acute demyelinating ON and MRI changes typical of MS. It showed different electrophysiological patterns in the early stages of the disease. It also demonstrated the possibility of topographically tracking the degree of axonal loss and remyelination of the optic nerve after a first acute attack.

Demyelination and inflammatory changes during an attack of ON result in conduction block in the optic nerve fibers, which normally resolves within a few weeks. Restoration of conductivity occurs because of the redistribution of ion channels along the demyelinated membrane. Although conduction is still considerably slower and imposes some limitations, including reduced ability to transmit pairs or trains of impulses or hyperexcitability, it is thought to be sufficient to account for clinical recovery.

On the other hand, longstanding conduction block, accompanied by functional deficit, is most likely to be the result of permanent axonal damage. Until recently, it was believed that axonal degeneration is a feature of chronic MS lesions not
apparent at early stages of the disease. There has been, however, renewed interest in the problem of early axonal degeneration in newly formed demyelinating lesions. By using amyloid precursor protein, Ferguson et al. were able to demonstrate axonal damage within acute MS lesions. This was confirmed first by Trapp et al., who used confocal laser microscopy to study axonal transection in early MS lesions, and later by Kornek et al., who demonstrated that massive axonal injury may even occur during the first few weeks after onset of demyelination.

Our analysis of the mfVEP amplitude revealed a group of patients with extensive and persistent deterioration of the response after a first episode of acute ON. Those patients showed minimal, if any, amplitude recovery (and even massive amplitude deterioration in one case) during the follow-up period. In all five patients, visual acuity remained significantly reduced. Therefore, it seems reasonable to assume that extensive and long-lasting mfVEP amplitude loss detected in these patients may reflect axonal damage early in the course of the disease.

In all five patients with limited or no recovery of amplitude, the central area of the visual field was affected more severely. This is in agreement with a previously published study demonstrating that thin axons subserving the central visual field are more susceptible to damage than thick axons, possibly because of mitochondrial dysfunction within the MS plaques. Furthermore, in patients whose amplitudes recovered, the latency of the response was also more delayed in the center than in the periphery. This may indicate size-selective susceptibility of axons to demyelination.

The other main finding of this study was the discovery of two distinct patterns of mfVEP latency change after an episode of ON. Thus, among the patients in whom amplitude recovered sufficiently for latency to be analyzed (22 patients), there was a clear separation with regard to latency improvement: Although most patients (63%) demonstrated significant latency shortening during the follow-up period, the remainder (37%) showed practically unchanged (except for small intertest variation) latency delay. It is thought that the latency of VEP mainly reflects the speed of conductivity along the optic nerve, and, though a small improvement after clinical recovery may result from the resolution of inflammation and edema, most of the improvement probably results from subsequent remyelination.

It is now widely accepted that the partial remyelination of MS plaques is not rare. Remyelination of MS lesions may have different important functions such as lesional repair, protection of axons, and restoration of conduction velocity. Recent studies suggested that myelin plays an important role in providing trophic support to axons and protects them from in-
flammatory mediators and immune effector cells. Histologic and experimental studies have demonstrated that restoration of myelin can start shortly after a demyelinating event, and its extent varies from complete absence to partial or full remyelination of the lesion. This is consistent with the finding of significant latency improvement in a considerable proportion of patients in our study, which most likely is a reflection of various degrees of early remyelination.

The dichotomy of mfVEP latency recovery presented in this report resembles histopathologic patterns of early MS lesions that have been described recently. It was reported that approximately 70% of early MS lesions have type 1 or type 2 patterns of demyelination, which are normally associated with preserved oligodendrocytes (OLGs) and extensive remyelination (OLG category 1 lesions), whereas the remaining 30% of lesions represent type 3 or type 4 patterns, which are characterized by primary OLG damage and little or no remyelination and are linked to OLG category 2 lesions. The authors suggest that the pathogenic mechanisms of the former lesions are similar to those described in experimental autoimmune encephalomyelitis and that those of the latter are similar to those described in viral or toxin-induced oligodendrogliopathy. It was demonstrated that although immunopathologic patterns of demyelination do not correlate with the clinical types of MS, all lesions from a single patient exhibited the same pattern of demyelination. Therefore, it was proposed, though not without controversy, that "fundamentally different mechanisms and targets of demyelination as well as tissue destruction and repair underlie distinct pathologic subgroups, independent of clinical features." Consequently, it was suggested that the finding of surrogate markers, which reliably distinguish between pathologic subtypes, might guide future MS therapies targeting the distinct pathogenic processes. Although direct comparison between electrophysiological and histopathologic patterns was not possible in the present study (no biopsy was performed on any patient), the quantitative distribution of remyelinating and non-remyelinating cases is similar. The character of mfVEP changes after an episode of ON may therefore indicate the general pattern of early disease development in patients and may play a role in differentiating histopathologic types of MS.

Few longitudinal latency studies of full-field VEP after an episode of ON have previously been published. Although some studies report a tendency for VEP latency to shorten during the first few years after an attack, others conclude that VEP latencies, once delayed, remain unchanged. This discrepancy can be partly attributed to the nature of conventional full-field (or any large-field) VEP because it provides a summed response of all neuronal elements stimulated and because it is greatly dominated by the macular region given its cortical overrepresentation. As a result, the shape of the full-field VEP waveform can change depending on the part of the nerve/visual field

**FIGURE 3.** Examples of mfVEP trace arrays, amplitude deviation, and amplitude asymmetry plots recorded from patient with good amplitude recovery during follow-up period.

**FIGURE 4.** (a) Mean latency of the ON and fellow eyes during follow-up period. (b) Average latency asymmetry during follow-up period. Error bar represents SD (a,b).
affected. Halliday et al.4 pointed out that delay detected on full-field VEP can sometimes be more apparent than real. Moreover, study groups were often heterogeneous with respect to the cause of ON.51,53 Including patients without MRI changes introduces the possibility of bias toward more benign disease with possibly less risk for progression to MS.

The study by Yang et al.17 provided the first indication of the possible use of a multifocal technique to monitor remyelination. The present study is the first longitudinal study of mVEP in ON performed at certain intervals after the first episode of acute inflammation and using similar intertest intervals for all patients. All recruited patients had MRI lesions typical of demyelination, and in a significant proportion of them ON converted to MS during the 12-month follow-up period. Conversion was highest in the group with severe amplitude loss (5 of 5), followed by the group with no latency recovery (4 of 6). Finally, conversion was lowest in the group with latency improvement (3 of 16). Therefore, patients with profound and sustained amplitude loss may have a more severe form of the disease.

Although it is not possible to correlate the electrophysiological subtypes with clinical types of MS with such short duration of follow-up, the study does lend support to the idea of histopathologic heterogeneity of MS lesions,41 in contrast to the notion of gradual evolution of MS lesions (with OLG loss representing a very early stage in formation of all lesions).30 Identifying distinct subtypes may also partly explain the differences in results reported by other groups using full-field VEP.

The limitations of this study are its relatively small sample size and its short duration of follow-up. Recruitment of more patients and further follow-up of patients in this ongoing study may help us to better understand the processes of this complex disease.

References


