**Purpose.** To investigate the relationship between size of demyelinated lesion, extent of axonal loss, and degree of latency delay of visual evoked potentials (VEPs) in a rat model of experimental demyelination.

**Methods.** Lysolecithin 1% (0.4 or 0.8 μL) was microinjected into an optic nerve of each of 14 rats 2 mm posterior to the globe. Standard flash VEPs were recorded with skull-implanted electrodes before and 2, 4, and 6 days after the microinjection. The optic nerves were stained with Luxol-fast blue and Bielschowsky’s silver to assess demyelination and axonal pathology, respectively. Demyelinated areas were measured on serial sections, and lesion volumes were deduced by three-dimensional reconstruction.

**Results.** Focal lesions of demyelination and variable axonal loss were observed. The mean volume of the lesion was 3.2 ± 1.1 × 10⁻³ mm³. The injected eye showed a significant latency delay and amplitude decrease. Regression analysis demonstrated a strong correlation between N1 latency delay and lesion volume (r = 0.863, P < 0.0001), which remained significant after adjustment for axonal loss (r = 0.829, P < 0.001). N1 latency delay also showed a correlation with axonal loss (r = 0.552, P = 0.041), but the correlation became nonsignificant when controlling for demyelination (r = 0.387, P = 0.191). A linear association between N1-P2 amplitude decrease and axonal loss (r = 0.681, P = 0.007) was also observed.

**Conclusions.** The latency of the VEP accurately reflected the amount of demyelination in the visual pathway, whereas the amplitude correlated with axonal damage. This study supports the concept that the VEP provides a highly sensitive tool with which to measure demyelination in optic neuritis. (Invest Ophthal Mol Vis Sci. 2011;52:6911–6918) DOI:10.1167/iovs.11-7434

Optic neuritis (ON) is a frequent initial manifestation of multiple sclerosis (MS), defined histologically as inflammatory demyelination, axonal injury, astrocytosis, and varying degrees of remyelination. In contrast to most brain lesions, the effects of MS lesions on the optic nerve are clinically apparent and potentially measurable and therefore present an opportunity to examine the processes of myelin destruction and axonal degeneration. Experimental models of MS have been based on immune-mediated demyelination or the use of gliotoxins. The most commonly used immune-mediated animal model for MS is experimental autoimmune encephalomyelitis (EAE), but this model is limited because it induces mainly an inflammatory reaction and is not representative of the primary process of MS. It is also unpredictable, with different animals developing varying severities of optic neuritis. In addition, the optic nerve lesions in EAE are always associated with the presence of inflammation and demyelination of the brain and spinal cord.

Lysolecithin is a major component of oxidized low-density lipoproteins and has a detrimental effect on myelin and myelinating cells. It has been used to induce focal demyelination in the peripheral and central nervous system. In contrast to other gliotoxins, lysolecithin targets the myelin, leaving some of the myelin-forming oligodendrocytes/Schwann cells and other cellular components unaffected, thus allowing remyelination to proceed. It has a well-characterized time course and subsequent remyelination onsets at the end of first week of injection. Furthermore, lysolecithin treatment has only a minor effect on the structural integrity of the axonal membrane. Lysolecithin has been successfully used to induce focal optic neuritis in the primate.

The visual evoked potential (VEP) is a noninvasive tool for investigating the function of the visual system; it has been proposed to assess the integrity of the visual pathway in ON. The amplitude of the VEPs is believed to reflect the number of functional optic nerve fibers. Our previous work revealed a strong correlation between retinal nerve fiber layer thickness and VEPs in ON patients. We also demonstrated that initial lesion size was the single most important factor in determining the outcome of remyelination and consequent axonal loss. It has been suggested that the effect of conduction change can be qualitatively measured by the latency delay of the VEPs. This has also been assumed that initial latency prolongation of the VEP corresponds to the size of the demyelinated area of the optic nerve, whereas subsequent shortening of latency has been thought to represent the process of optic nerve remyelination. This assumption, however, has never been tested. VEPs have been used to investigate the visual pathway function in the EAE models. In these studies, a correlation between VEPs and histopathologic results was observed. However, as mentioned, it is difficult to reproduce and to quantify demyelination in the visual pathway in an EAE model. This study was designed to examine the relationship between the amount of demyelination and the VEP latency in a rat model.

A focal lesion of demyelination in the rat optic nerve was produced using a lysolecithin microinjection. We examined the correlation between VEP parameters and the level of myelination and axonal loss each animal over a time course of 1 week. This study aimed to provide a reproducible approach to quantitative measurement of demyelination in vivo, which would support the use of the VEP in clinical diagnosis and prognosis evaluation of ON patients.

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METHODS

Animals

Eighteen male Sprague-Dawley rats (weight range, 300–350 g; age range, 10–12 weeks; Animal Research Centre, Perth, Australia) were used. All animals were maintained in an air-conditioned room with controlled temperature (21°C ± 2°C) and fixed daily 12-hour light/12-hour dark cycles. All procedures involving animals were conducted in accordance with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes and the guidelines of the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Each animal was anesthetized with an intraperitoneal injection of ketamine (75 mg/kg) and medetomidine (0.5 mg/kg) for both surgery and electrophysiology recording.

Electrophysiology Recording Procedures

Stainless steel skull screws (Micro Fasteners, Thomastown, Australia) were implanted through the skull into the visual cortex (7 mm behind the bregma and 3 mm lateral of the midline) as the positive electrodes, penetrating the cortex to approximately 0.5 mm.23,24 A reference screw electrode was placed on the midline 3 mm anterior to the bregma. Dental cement (Rapid Repair; DeguDent GmbH, Hanau, Germany) was used to fix the screws. The skin of the head was closed, and at least 1 week was allowed for the animals to recover from the surgery.

For VEP recording, the animals were anesthetized, placed in a dark room, and allowed to adapt to darkness for 5 minutes. VEP recording with short adaptation or without dark adaptation has been established.23,25–27 In addition, our flash protocol, described here, rapidly light-adapts the rat eye. The body temperature was maintained at 37°C by the homeothermic blanket system with a rectal thermometer probe (Harvard Apparatus, Holliston, MA). The pupils were dilated using 1.0% tropicamide eyedrops (Alcon Laboratories, Forth Worth, TX). The skin over the skull was opened. The positive screw over the contralateral visual cortex of the stimulated eye and the reference screw were connected to the amplifier. A needle electrode (F-E3M-72; Grass Instruments, Quincy, MA) was inserted into the tail as the ground. The electrode impedance was measured (F-EZM5 Impedance Meter; Grass Instruments) and was maintained below 5 kΩ. Visual stimuli were generated by a mini-Ganzfeld stimulator (3 cd · s/m²). Photic stimulation was delivered 100 times at a frequency of 1 Hz. The time of measurement was 200 ms, and the sample rate was 5 kHz. Responses were amplified 20,000 times with low and high band-pass filter settings of 1 and 100 Hz (BMA-400 Bioamplifier; CWE, Inc., Ardmore, PA). After the recording, the wound was closed and antibiotics were administered. The VEP recordings were performed before and on days 2, 4, and 6 after the lysolecithin microinjection, using the same protocol at each session. Five rats also had ERGs recorded (on days 0 and 6) to exclude damage in the retina. An ERG (single 3 cd · s/m² flash) was recorded before VEP recording in partially dark-adapted (1-hour) animals to establish retinal integrity. We were not trying to perform full ERG profiles (otherwise 12-hour adaptation would have been needed). For ERG recording, a gold wire ring electrode (Roland Consult, Branden- burg, Germany) was placed on the center of the cornea to serve as the positive lead, and the reference electrode was provided by a stainless steel needle inserted into the skin over the forehead. The filter was set.
TABLE 1. Comparison of P1-N1-P2 Parameters before and 6 Days after Optic Nerve Microinjection

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 6</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td><strong>0.8 μL (n = 9)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>27.2 ± 1.0</td>
<td>34.9 ± 2.3*</td>
<td>0.008†</td>
</tr>
<tr>
<td>N1</td>
<td>39.9 ± 1.4</td>
<td>52.8 ± 3.7**</td>
<td>0.007†</td>
</tr>
<tr>
<td>P2</td>
<td>58.6 ± 2.1</td>
<td>80.0 ± 5.2*</td>
<td>0.003†</td>
</tr>
<tr>
<td>Amplitude, μV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1-N1</td>
<td>26.4 ± 4.0</td>
<td>10.1 ± 2.3</td>
<td>0.002†</td>
</tr>
<tr>
<td>N1-P2</td>
<td>48.5 ± 8.5</td>
<td>26.1 ± 6.7</td>
<td>0.034†</td>
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<tr>
<td><strong>0.4 μL (n = 5)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Latency, ms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>25.8 ± 0.5</td>
<td>28.8 ± 1.2*</td>
<td>0.047†</td>
</tr>
<tr>
<td>N1</td>
<td>58.4 ± 2.7</td>
<td>45.4 ± 4.4*</td>
<td>0.031†</td>
</tr>
<tr>
<td>P2</td>
<td>56.3 ± 4.1</td>
<td>69.6 ± 5.1*</td>
<td>0.005†</td>
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<tr>
<td>Amplitude, μV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1-N1</td>
<td>25.7 ± 6.1</td>
<td>13.9 ± 4.7</td>
<td>0.037†</td>
</tr>
<tr>
<td>N1-P2</td>
<td>55.2 ± 7.3</td>
<td>41.9 ± 10.6</td>
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<tr>
<td><strong>Sham (n = 4)</strong></td>
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<tr>
<td>Latency, ms</td>
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<td></td>
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<tr>
<td>P1</td>
<td>28.4 ± 1.5</td>
<td>28.9 ± 1.5</td>
<td>0.416</td>
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<tr>
<td>N1</td>
<td>42.0 ± 4.8</td>
<td>40.3 ± 3.5</td>
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<tr>
<td>P2</td>
<td>60.6 ± 7.1</td>
<td>60.6 ± 6.8</td>
<td>0.984</td>
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<tr>
<td>Amplitude, μV</td>
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<tr>
<td>P1-N1</td>
<td>27.6 ± 10.3</td>
<td>28.3 ± 15.4</td>
<td>0.932</td>
</tr>
<tr>
<td>N1-P2</td>
<td>55.8 ± 4.2</td>
<td>36.7 ± 8.0</td>
<td>0.908</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

* P < 0.05, latency delay was significantly longer in the 0.8-μL group than in the 0.4-μL group (unpaired t-test).

† P < 0.05, significantly different between day 0 and day 6 (paired t-test).

to the frequency ranges of 0.1 to 1000 Hz, and the amplification was 10,000 times.

Microinjection of Lysolecithin

After anesthesia, the head was shaved and the skin was disinfected with 75% ethanol. A 1- to 1.5 cm incision was made in the skin above the orbit of a randomly selected eye. The lacrimal glands and extracocular muscles were resected to expose 3 mm of the optic nerve under an operating microscope. The dura and arachnoid matter around the optic nerve were opened longitudinally. Microinjection was performed with a glass micropipette attached to a Hamilton syringe. The pipette was inserted into the optic nerve as superficially as possible at 2 mm posterior to the globe, and 0.8 μL (n = 9) or 0.4 μL (n = 5) 1% lysolecithin (with 0.02% Evan’s Blue; Sigma, St. Louis, MO) was slowly pressure-injected into the nerve over approximately 30 seconds. Another four rats were injected with 0.02% Evan’s Blue in 0.4 μL saline as sham injection controls. After the injection, the skin incision was sutured, and antibiotic was administered to prevent infection. Fellow eyes served as internal controls for electrophysiology recording. The animals were allowed to recover from anesthesia on a warming pad.

Histopathology

On day 6, after VEP recordings, the animals were euthanized with an overdose of anesthetic and then perfused transcardially with 4% paraformaldehyde. Optic nerves (from globe to chiasm) were removed and fixed in 1% paraformaldehyde overnight, processed in an automatic tissue processor (Leica, Wetzlar, Germany), and embedded in paraffin. Five-micrometer-thick transversal sections were made using a rotary microtome (Carl Zeiss, Oberkochen, Germany). The slides were stained with hematoxylin and eosin (HE), Luxol fast blue, and Bielschowsky’s silver impregnation. Luxol fast blue and Bielschowsky’s silver staining were used to assess demyelination and axonal pathology, respectively.22,25 Demyelinated areas were measured on Luxol fast blue–stained sections using image analysis software (Vision; Carl Zeiss). Axonal density was determined for each nerve on Bielschowsky’s staining by counting the number of axons in nine standardized microscopic fields of 400 μm².2

Statistical Analysis

Peak latency and amplitude of individual components of the VEP waveform were measured for each eye. Mean latency and amplitude before and after microinjection were compared by paired Student’s t-test. The real latency delay and amplitude decrease of the injected eye were rectified by comparison with the fellow eye, which provided the baseline.

Axonal loss was determined on Bielschowsky’s staining by comparing the axonal density of the injected nerve with the control. The demyelinated area was measured on each consecutive cross-section of the nerve (every 250 μm). The lesion volume was then deduced by three-dimensional reconstruction.

The correlation between the volume of demyelinated lesion and the VEP parameters was assessed by regression analysis. The relationship between axonal loss and amplitude decrease was also examined by linear regression. The correlation between VEPs and histopathology was then adjusted by partial correlation analysis controlling for demyelination and axonal loss, respectively (SPSS software). Statistical significance was defined as P < 0.05 in all the data analysis.

RESULTS

Latency Delay and Amplitude Decrease in VEPs

Figure 1A shows the representative VEP traces in an individual rat before and 6 days after microinjection with lysolecithin. The rat VEP waveform presents positive and negative deflections that were designated as P1, N1, P2, N2, P3, and N3 components by Creel et al.25 The early components are more consistent and principally affected by excitation by retinogeniculate fibers.23,29-40 Therefore, we used the parameters of the P1-N1-P2 complex for our VEP analysis. Table 1 summarizes the latency and amplitude values on day 0 and day 6. Prolongation of the latency and decrease of the amplitude were observed in all the components in the lysolecithin-injected eyes. The 0.8-μL group showed a longer latency delay than the 0.4-μL group. There was no significant difference in amplitude decrease between these two dose groups. By contrast, no latency delay or amplitude decrease was observed in the sham injection group (Fig. 1C, Table 1).

Figure 1B and Table 2 present the averaged waveform of ERGs from five rats and the mean parameters of a-b waves. There was no significant difference in the latency or the amplitude of ERGs before and after microinjection (P = 0.641–0.979). Figure 2 shows the VEP parameters during the follow-up period from day 0 to day 6. The latency delay and amplitude decrease were observed on day 2 and did not change between day 2 and day 6.

Lesions in the Optic Nerves

Focal lesions of demyelination were observed involving several optic nerve bundles after lysolecithin injection. The mean volume of the lesion was 3.2 ± 1.1 × 10⁻² mm³. The length of

TABLE 2. Comparison of a-Wave and b-Wave Parameters before and 6 Days after Microinjection

<table>
<thead>
<tr>
<th></th>
<th>Day 0</th>
<th>Day 6</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>a-Wave latency, ms</td>
<td>18.2 ± 1.4</td>
<td>18.1 ± 2.4</td>
<td>0.979</td>
</tr>
<tr>
<td>b-Wave latency, ms</td>
<td>48.8 ± 4.8</td>
<td>47.0 ± 1.6</td>
<td>0.641</td>
</tr>
<tr>
<td>a-Wave amplitude, μV</td>
<td>32.9 ± 9.8</td>
<td>29.7 ± 10.5</td>
<td>0.798</td>
</tr>
<tr>
<td>b-Wave amplitude, μV</td>
<td>120.9 ± 15.9</td>
<td>114.7 ± 18.5</td>
<td>0.740</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. n = 5 (2 with 0.4 μL and 3 with 0.8 μL lysolecithin microinjection).
the lesion varied between 0.25 mm and 2.25 mm. Figure 3A is a diagram showing a representative partial lesion in the optic nerve of a rat on Luxol-fast blue–stained serial sections. The lesion appeared on four consecutive sections with a varying cross-sectional area (10.2%–52.0%). The volume of the lesion was significantly affected by the dose of lysolecithin (Fig. 3B). Only slight demyelination was observed in the nerves with sham injection (mean lesion volume, 0.18 ± 0.12 × 10^{-2} mm^3). Figure 4 shows the histopathology of the optic nerves with a comparison of HE, Luxol-fast blue, and Bielschowsky’s silver staining. The lesions were found to be highly cellular on HE staining. Bielschowsky’s staining demonstrated axonal loss in the injected nerves. The mean axonal density was 21.3 ± 5.5 × 10^4/mm^2 for the lysolecithin-injected nerve and 35.1 ± 3.6 × 10^4/mm^2 for the control (P < 0.0001). There was also a minor axonal loss observed in the sham injection group (34.3 ± 3.20 × 10^4/mm^2 for the fellow eye vs. 31.20 ± 4.2 × 10^4/mm^2 for the injected eye; P = 0.017). Axonal loss was statistically more severe in the lysolecithin groups than in the sham injection group. However, there was no significant difference in axonal loss between the 0.8- and 0.4-µL groups (Fig. 4).

**Correlation between Electrophysiology and Histopathology**

Linear regression analysis demonstrated a strong correlation between the latency delay and the lesion volume (r = 0.735–0.863, P = 0.0001–0.002 for different peaks; Fig. 5). The latency of the first negative peak (N1) showed the highest correlation with the lesion (r = 0.863, P < 0.0001). Each 2.8 × 10^{-2} mm^3 lesion of demyelination contributed...
approximately 1 ms of N1 latency delay. The linear relationship remained significant after adjustment for axonal loss by partial correlation analysis. The adjusted $r$ values of P1, N1, and P2 with lesion volume were 0.815 ($P = 0.001$), 0.829 ($P < 0.001$), and 0.657 ($P = 0.015$), respectively. There was also a significant correlation between the lesion volume and the N1-P2 amplitude decrease ($r = 0.712$, $P = 0.004$; Fig. 6). However, there was no correlation between the P1-N1 am-
between axonal loss and N1-P2 amplitude decrease (the VEP parameters. We observed a linear relationship between axonal loss and the lesion volume (n = 14). Figure 6 presents the regression analysis between axonal loss and the VEP parameters. We observed a linear relationship between axonal loss and N1-P2 amplitude decrease (r = 0.712, P = 0.004). Figure 7 presents the regression analysis between axonal loss and the lesion volume (r = 0.429, P = 0.126). Figure 7 presents the regression analysis between axonal loss and the lesion volume (r = 0.429, P = 0.126).

DISCUSSION
In this study, we investigated the relationship between VEP parameters and demyelination in the optic nerve. We found that the latency delay of VEPs (especially N1 latency) closely reflected the amount of demyelination in the visual pathway. Our data supported the assumption that latency prolongation of the VEP corresponds to the size of the demyelinated area in the visual pathway. VEPs have been used to study the visual pathway function in rodent models of EAE, and a correlation between VEP and histopathology was observed. However, in the EAE model, demyelination and inflammation distribute widely in the nervous system, and it is difficult to quantify the effect of demyelination on signal conduction. McDonald and Sears revealed that the action potential remained fast while conducting along the normally myelinated portions of the axolemma. It was markedly slowed along the demyelinated region, and each 1 mm of demyelination contributed approximately 1 ms of conduction delay. However, to our knowledge, no study has been published that examines the relationship between demyelination and latency delay in vivo. Our present study showed that each 2.8 × 10⁻³ mm³ of demyelination in the rat optic nerve corresponded to 1 ms of N1 delay. In the sham injection group, the mean lesion volume was only 1.8 × 10⁻³ mm³, and there was no significant delay. Our results support the potential of the VEP as a noninvasive tool to quantify demyelination in vivo and to evaluate the prognosis of optic neuritis in the clinic. VEPs may also be useful in assessing the effects of therapeutic agents in both animal models and in humans with optic neuritis.

It has been suggested that the magnitude of the VEP reflects the number of functional afferent fibers reaching the striate cortex and the degree of synaptic activity in V1. Our previous study demonstrated a strong correlation between the mfVEP amplitude and a measurement of optic nerve axonal damage (retinal nerve fiber layer thickness) in optic neuritis patients. In the present study, we also observed that the amplitude decrease of flash VEPs correlated with axonal loss in rats. The correlation between axonal damage and amplitude, however, was weaker than that between demyelination and latency. This might have been because of the effect of acute inflammation in the optic nerve. In optic neuritis, the number of functional afferent fibers is determined by a combination of two factors: the severity of the inflammation and the axonal degeneration. Therefore, VEP amplitude decrease indicated axonal atrophy or focal inflammation, or a combination of both. Furthermore, VEP amplitude in the rat is more variable (coefficient of variation, CoV ≈ 20%) than latency (CoV ≈ 5%). In fact, we only observed a significant correlation of axonal loss with N1-P2, but not with P1-N1, amplitude, which again may be due to the higher variability of the latter compared with the former (unpublished data, 2011). In this model, demyelination affected only a portion of the axons over the whole cross-sectional area. Therefore, the signals from the retinal ganglion cells reached the primary visual cortex at different time points through the healthy and demyelinated axons, respectively, which resulted in the widening of the VEP waveform and the flattening of the peaks. This might also have contributed to the amplitude decrease. Finally, the function of the lost axons may be partially compensated in the visual system at the cortical level, which might also have led to a weaker correlation between axonal damage and amplitude decrease.

By contrast, no strong correlation was observed between N1 delay and axonal loss (Fig. 7). The correlation between N1 delay and axonal loss became nonsignificant after adjustment for demyelination. Heiduschka et al. also showed that retinal ganglion cell loss led to a decrease of VEP amplitude without any changes in latency. This indicates that the weak correlation between N1 delay and axonal loss in this model might have resulted from the correlation between axonal loss and demyelination.
In the rat model, we demonstrated a moderate association between the N1-P2 amplitude and lesion size (Fig. 6). Demyelination impairs axonal function, and conduction block can occur in freshly segmentally demyelinated axons.\textsuperscript{32} This might result in a decrease in VEP amplitude. In addition, it is believed that demyelination is intimately related to axonal loss in MS.\textsuperscript{34} Lack of trophic support from myelin or myelin-forming cells may also cause the degeneration of demyelinated axons.\textsuperscript{35-36}

The interrelationship between amplitude decrease and demyelination in rats supported this conclusion from our previous study\textsuperscript{17} and implied that demyelination may play an important role in axonal degeneration.

Several demyelinating agents have been used to induce demyelination in the central nervous system by local administration, including lyssolecithin, ethidium bromide, antibodies to oligodendrocyte-related molecules, and bacterial endotoxin.\textsuperscript{3}

The choice of toxin influences the speed of postexposure demyelination and the synchronicity of subsequent remyelination. The reported locations of toxin-induced focal demyelination included the spinal cord, the caudal cerebellar peduncle, the corpus callosum, and the optic nerve.\textsuperscript{4} Carroll et al.\textsuperscript{37-39} first reported the model of focal optic nerve demyelination in rats and cats using galactocerebroside (Gal-C) antibodies. The rat ON model with Gal-C injection was later repeated by Zhu et al.\textsuperscript{40} Lyssolecithin was also injected into the chiasm of the rats, and changes in VEP amplitude and latency were observed.\textsuperscript{27} However, no histologic result was shown in that study. Lyssolecithin was recently demonstrated to induce focal demyelination in the macaque optic nerve.\textsuperscript{9} In the present study, we induced a focal lesion of demyelination in the rat optic nerve as an analogous model of the macaque using lyssolecithin. It has been suggested that in lyssolecithin-induced demyelination, spontaneous remyelination begins at the end of the first week and the remyelination process takes 5 to 6 weeks to complete.\textsuperscript{9,10} Therefore, we used day 6 as the end point to avoid the interference of remyelination. Our VEP data indicated that maximum demyelination and axonal loss were observable on day 2 in the rat model; there was no remyelination in the first week after the injection (Fig. 2). The histopathology of our model was in good agreement with the optic nerve lesion in macaque. The lesions were highly cellular. According to the macaque model, these areas contained primarily macrophages.\textsuperscript{7} However, remyelination in the optic nerve was not as successful as that in the spinal cord in the macaque model. Mozafari et al.\textsuperscript{41} recently showed that remyelination in the optic chiasm and nerves took place primarily in the caudal part of demyelination area, which is closer to the third ventricle. Therefore, remyelination in the intraorbital optic nerve may occur very late because the place is relatively far from the brain parenchyma.

The limitation of this study was its relatively short duration of follow-up. We have no information about remyelination in this animal model and have begun a long-term study to investigate the process of remyelination and potentially to validate the use of VEP latency as a tool to monitor remyelination in vivo. Another advantage of longer follow-up is to exclude the influences of acute inflammation and conduction block on the VEPs.

In summary, we demonstrated a strong correlation between VEP latency and the amount of demyelination in a rat ON model. This confirmed that VEP is a sensitive tool to monitor and to quantify demyelination in vivo. In addition, the amplitude decrease reflected the axonal damage in the optic nerve to a certain extent. Our study supports the notion that VEPs can play a role in assessing the disease process, in monitoring optic neuritis, and potentially in evaluating the effects of new therapeutics.

Acknowledgments

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References


